D15
16/9/09

Reference
Only

Ref label

41 0640358 0

ProQuest Number: 10183381

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 10183381

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

324339

THE NOTTINGHAM TRENT UNIVERSITY LLR

Short Loan | PHD/BNS/05 WIL

THE IMPACT OF MINING ACTIVITIES ON THE PEDOSPHERE AND A CRITICAL EVALUATION OF BIOACCUMULATION.

Robert (Bob) Wilson

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

August 2005

This thesis is dedicated to my sons, Ben and Dan, who provided the inspiration, to my daughter Ruth who helped in so many ways, to my wife Ruth who gave indefatigable support both in the field and at home, and to my mother Ruth, who would have been very proud.

Acknowledgements

My immense gratitude goes to my supervisors Prof. Brian Pyatt and Dr. Alan Braithwaite for their support, advice and good humour – it was essential. Thanks also to Dr. Tim Reynolds for archaeological guidance and reading of some of the early scripts.

Many thanks to Dr. David Crabb and Dr. Chris Terrell-Nield for advice on the use and presentation of statistics. Thanks too to Dr. Susan Smith for help with the vagaries of Excel and Steve Bursland for much needed guidance on the use of the scanning electron microscope. Dr Mike Coffey was always available to aid understanding of some of the processes involved and to make helpful suggestions especially relating to the repetitiveness of some of the data collection.

Many people at Nottingham Trent University gave advice and always had time for a discussion on whatever topic was causing me a problem at the time. To the academic staff concerned thanks a million – I owe you a drink.

The lab technicians were as always disparaging but withstood my panic and impatience with great fortitude. Grateful thanks to Rob Bentham, Nigel Mould, Kev Birch and Terry Campion.

A few people outside of the university added depth to my knowledge and require special mention. Dave 'the bum' Berkshire for making me aware of the unusual effects of copper on sheep, the staff in the library at the University of Arizona, Tucson, for help and use of their facilities and Brennan Lang of the University of British Columbia, Vancouver, for providing information on the workings at Britannia Mine. Prof. Mark Seaward from the University of Bradford gave guidance with the identification of lichen species.

I am indebted to Navigation Technologies Inc. for permission to download their maps in conjunction with Yahoo Inc. and to the Ordnance Survey Get-a-map service. Images reproduced with kind permission of Ordnance Survey and Ordnance Survey of Northern Ireland. Thanks also to Farrah Rooney, Curator of Education and Collections at the BC Museum of Mining for permission to use images of Britannia Mine.

Special thanks to Lynn and Kevin McLane who put me up in Squamish, B.C. and to Zof Watts who put up with me Golden, B.C.

Finally, I am indebted to the Higher Education Funding Council for England for their financial support, without which it is unlikely that the research would have been carried out.

The impacts of mining activities on the pedosphere and a critical evaluation of bioaccumulation.

a.)	Dedication	i
b.)	Acknowledgements	ii
c.)	Table of contents	iii
	Abstract	1
1.	**Introduction**	3
2.	**Aims and Objectives**	8
3.	**Materials and Methods**	10
3.1	Reagents and reference materials	10
3.2	Standard operating procedures	11
3.3	Sample collection and preparation for aqueous analysis	11
3.3.1	Soil	
3.3.2	Foliage	
3.3.3	Bark	
3.3.4	Flowers and florets	
3.3.5	Stems and twigs ('woody' tissue)	
3.3.6	Cones, fruits and berries	
3.3.7	Lichens	
3.3.8	Faeces	
3.3.9	Invertebrates	
3.4	pH determination	15
3.4.1	Soil	
3.4.2	Water	
3.5	Conductivity determination	16
3.5.1	Soil	
3.5.2	Water	
3.6	Analytical Techniques	16
3.6.1	Flame atomic absorption spectrometry (FAAS)	
3.6.2	Inductively coupled plasma – atomic emission spectrometry (ICP-AES)	
3.6.3	Energy dispersive X-ray analysis (EDAX)	

3.6.4 Ion Chromatographic Analysis

3.7 Statistical Techniques 23

3.7.1 Limits of detection (LOD) and Limits of Quantitation (LOQ)
3.7.2 Relative standard deviation or standard error (RSD)
3.7.3 Accuracy
3.7.4 Correlation coefficients
3.7.5 Analysis of variance (ANOVA)
3.7.6 95% Confidence Interval determination

4.1 Determination of most suitable extraction methods for subsequent analysis of concentrations of Cd,Cu, Pb and Zn in samples associated with old mining operations. 29

4.2 Standard operating procedure for extraction for subsequent aqueous analysis using FAAS or ICP.AES techniques.

4.2.1 Soil

4.2.2 Vegetation

5. Evaluation of a novel method for the preparation of spoil samples for subsequent acid digestion and analysis for heavy metals. 56

6. Critical evaluation of a pressurised microwave extraction system for the determination of selected heavy metals concentrations in soil and vegetation. 63

7. Evaluation of analytical procedures for the determination of concentrations of Cd, Cu, Pb and Zn in various samples associated with mining operations 72

8. Heavy metal dispersion, dilution, bioaccumulation and health implications in an area surrounding an ancient copper/zinc mine situated in Anglesey, North Wales, UK 87

9. The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada. 100

10. Heavy metal concentrations in an arid environment around Lavender Pit, Bisbee, Arizona, USA. 115

11. Heavy metal concentrations in the vicinity of a relatively new mine (Mission Mine), near Tucson, Arizona, USA. 128

12. Heavy metal bioaccumulatuion by *Olea europaea* and *Citrus sinensis* in the vicinity of ancient mine workings at the Limni mine, near Polis, Cyprus 137

13. An examination of the possible mobility of heavy metals in selected soil samples from areas surrounding metalliferous mine workings using sequential extraction and a single extraction procedure. 145

14. Examination of data from metalliferous mine workings relating to bioaccumulation. 161

15. Outcome and conclusion 167

15.1 Standard Operating Procedures
15.2 Evaluation of digestion procedures
15.3a Use of microniser as agrinding method for soil samples
15.3b Evaluation of pressurised microwave extraction procedure
15.4 Assessment of analytical techniques
15.5a Heavy metal dispersion, dilution and bioaccumulation at Parys Mountain, Anglesey, UK.
15.5b The dispersion of heavy metals in the vicinity of Britannia Mine British Colombia, Canada.
15.5c Heavy metal concentrations in the vicinity of Lavender Pit. Arizona, USA.
15.5d Mission Mine, Arizona, USA.
15.5e Cyprus olives and oranges.
15.6 Effects of pH etc on mobility of heavy metals
15.7 Examination of data from metalliferous mine workings relating to bioaccumulation.
15.8 Conclusion

16. Future work 179

References 183

Appendix Published work. 200

The impacts of mining activities on the pedosphere and a critical evaluation of bioaccumulation.

Abstract

The research has demonstrated the dispersion and dilution of copper, zinc, lead and cadmium from open pit metalliferous mine workings in UK, British Columbia and Arizona during and since the working lives of the mines concerned. Standard operating procedures were developed for the collection and preparation of soil and varying vegetation tissues. Five processes for the digestion of such environmental samples including the use of microwaves were critically evaluated and the use of aqua regia on a hot plate was found to be the most precise and accurate method for subsequent analysis of copper, zinc, lead and cadmium by flame atomic absorption spectrophotometry. Limits of detection and quantitation were also determined. Similar experiments were carried out to determine the most precise method of analysing the resulting extracts from environmental samples and the similarity of FAAS and ICP-AES data demonstrated. Analysis using ion chromatography, energy dispersive x-ray analysis and ICP-MS were found to be less satisfactory.

Mine workings of varying ages were chosen in different climatic areas for examination. Cessation of dispersal from older mine workings was demonstrated by the use of epiphytic lichens which are capable of accumulating heavy metals emitted by atmospheric dispersion. The effects of sheet and gulley erosion were considered and other factors affecting atmospheric pollution from mine workings were examined. Temporal effects on the presence of heavy metals in the pedosphere were considered and the persistence and abundance of such pollution adequately demonstrated. Land in the vicinity of all of the mines was found to be heavily polluted and in many cases exceeded the heavy metal concentrations the soil contamination limits of the European Union and Canada especially where the sample sites were in domestic housing and park areas in adjacent towns.

Evidence of bioaccumulation in different plant tissues is presented together with substantiation of partitioning within various plant tissues. Indications of suitable tissues of the plants under examination for biomonitoring of copper, zinc and lead are proposed. Heavy metal speciation related to bioavailability was closely examined

using sequential and EDTA extraction procedures and the possible occurrence of homeostatic processes in plants is discussed together with indications of movement through trophic levels to animals including humans. It was also projected that metal tolerant plant tissues preferentially accumulate heavy metals in order to protect seeds. A novel way of calculating an accumulation factor of metals in plants is proposed and its relevance to metal speciation in the soil investigated. Where appropriate international and national standards relating to heavy metal concentrations in soil were demonstrated and their relevance to the current research is indicated.

Further work on metal speciation, leaching mechanisms and plant accumulation processes is suggested together with investigations into subsequent accumulation of the target metals by mammals including humans.

Chapter 1.

Introduction.

This research aims to examine the effects of ancient and more recent (ie. from circa 25 years to 2000 years BP) metalliferous exploitation on the surrounding biosphere with particular reference to accumulation through succeeding trophic levels. Previous workers (Pyatt *et al.*, 2000) have carried out investigations into the presence, location and bioaccumulation of heavy metals from an ancient metalliferous mining site in Jordan, where the workings are believed to date back to the early Bronze Age and were subsequently exploited during the Roman and Byzantine periods. The heavy metal content of the spoil, plants and various body parts of animals were examined. Despite many centuries of erosion, high concentrations (>1000 mg kg^{-1}) of heavy metals were found in the spoil, plants and animals examined. Work carried out by Clark *et al.* (2001), in Australia at sites whose workings date from the mid 19^{th} century illustrated that some heavy metals, particularly cadmium and lead were mobilised and concentrated particularly in the soil drainage systems by oxidation. Although the downstream effects were not evaluated it can be assumed that the metals concerned would be accumulated in plants in the watershed. Work by Shotyk (1996), West *et al* (1997) and Pyatt and Grattan (2003), demonstrated that peat bogs in different parts of Europe can be used for the determination of the temporal distribution of heavy metals by analysing cores taken from the bogs in conjunction with carbon dating. The contamination of soil and plants by heavy metals as a result of copper mining activities was shown to contain excessive concentrations of arsenic cadmium, lead and zinc as well as copper (Bech *et al*, 1997). All of the research examined relating to copper, zinc, lead and cadmium pollution of soil, demonstrated concentrations above background in the surrounding soil, although the extent of current dispersion from disused mining sites does not seem to have been addressed.

Since copper and lead mining has been carried out in various parts of the world for many centuries it was decided that a selection of these sites would be examined in this project. Additionally, open pit mines were chosen for the research in order that some consistency of possible dispersion mechanisms is maintained. The concentrations of copper and lead would be determined in a series of trophic levels together with the concentrations of cadmium and zinc which regularly occur in

conjunction with the mined metals. It is also of interest that copper and zinc are essential metals for plant growth certainly in trace quantities (Sparks, 1995). Cadmium and lead are not essential and might be considered to be toxic to organisms.

Exposure to metals can occur through a variety of methods. Metals may be inhaled as dust or fume and may also be ingested involuntarily through food and drink (Hu, 2002). The toxic effects of copper, zinc, lead and cadmium on plants and animals are well documented and national regulations are enforced in most countries in terms of metal concentration values as a function of land use (DEFRA, 2002) with a view to protecting the health of their citizens. Forstner (1995) stated "The 'passage poisons', Zn, Cu and Ni in the food chain are more toxic to the plants themselves than to humans and animals which may eventually eat these plants. The 'accumulation poisons' Pb, Cd and Ti on the other hand are usually tolerated by plants in greater amounts than is recommended for their utilisation as food and feed." Zinc and copper accumulation in animals are controlled by homeostatic mechanisms (Miller and Neathery, 1980) however sheep are particularly affected by chronic copper poisoning (Ilha *et al* 2001) since they absorb copper in proportion to the amount ingested, not to the body's need as with the absorption of other minerals (Martin, 1998). Several workers have shown that heavy metal accumulation in plants is partitioned regardless of the source of the contamination. Pyatt (2001) demonstrated the qualitative differences in concentrations of different metals in species of *Acacia retinoides* and *Eucalyptus torquata* growing on ancient copper mine sites in Cyprus. In general leaves and pods accumulated higher levels of contaminants than the seeds. Ernst in 1993 stated that generally the outer bark of a tree trunk is more enriched by heavy metals than the inner bark. The least accumulation is found in the trunk wood. Kruckeberg and Wu (1992) demonstrated differences in copper concentrations in different tissues in three plant species growing in nutrient solutions containing copper. Transfer factors (ratio of metal concentration in plant to metal concentration in soil) for metals in plants have been explored and found to be as high as 3.7 in the case of cadmium in *Chamaemelum fuscatum* (Del Rio *et al*, 2002) and 3.3 in the case of lead in *Vesbascum blattaria* (Shallari *et al*, 1998). These authors and others (De Kol, 1994, Ma *et al* 2001) have demonstrated that different plant species accumulate heavy metals over a range of pollutant concentrations in soils and it is essential to use the same species within a site investigation and at relevant control sites.

Factors affecting metal uptake by vegetation are decisively influenced by the seasonal alternating increases in biomass, difficulties in the balance of content of substances due to age and classic abiotic factors such as soil, light, precipitation and wind conditions (Markert, 1993). The bioaccumulation of cadmium, copper, lead and zinc by plants from soil either directly contaminated by ore, for example, spoil heaps and soil further away from the spoil heaps, contaminated by either leaching or atmospheric transport, will vary. The direction and distance from the source together with climatic conditions will affect the concentration of metals in the soil in which plants grow.

Movement of heavy metals from the soil to higher trophic levels is generally influenced by the consumption of vegetation by grazing animals and invertebrates. Species which 'process' soil such as earthworms (Heikens *et al.*, 2001) can accumulate large concentrations of heavy metals which can then be passed on to birds and mammals (Scharenberg and Ebeling, 1996). This same work illustrated the increase in transfer factor values for cadmium, copper lead and zinc from soil to plants and from plants to primary consumers. The transfer factors reduced as secondary consumers such as small mammals trapped on the site were examined.

The movement of heavy metals from mining sites through trophic levels to humans is less well documented although some work has been done on industrial exposure of humans to lead emissions (Brito *et al*, 2000). Pyatt and Grattan (2001) postulated that humans working in the ancient copper mines at Wadi Faynan, Jordan, would have suffered from heavy metal poisoning.

The research therefore requires a structured approach and the initial consideration is the various pathways by which heavy metals can mobilise from old mining sites, essentially spoil heaps, through increasing trophic levels. Figure1.1 illustrates some of the various pathways through which this can occur. Speciation of the metals in soil, together with soil type, pH, geochemistry and climatic factors affect their mobility, lability and consequently bioavailability therefore an investigation of these

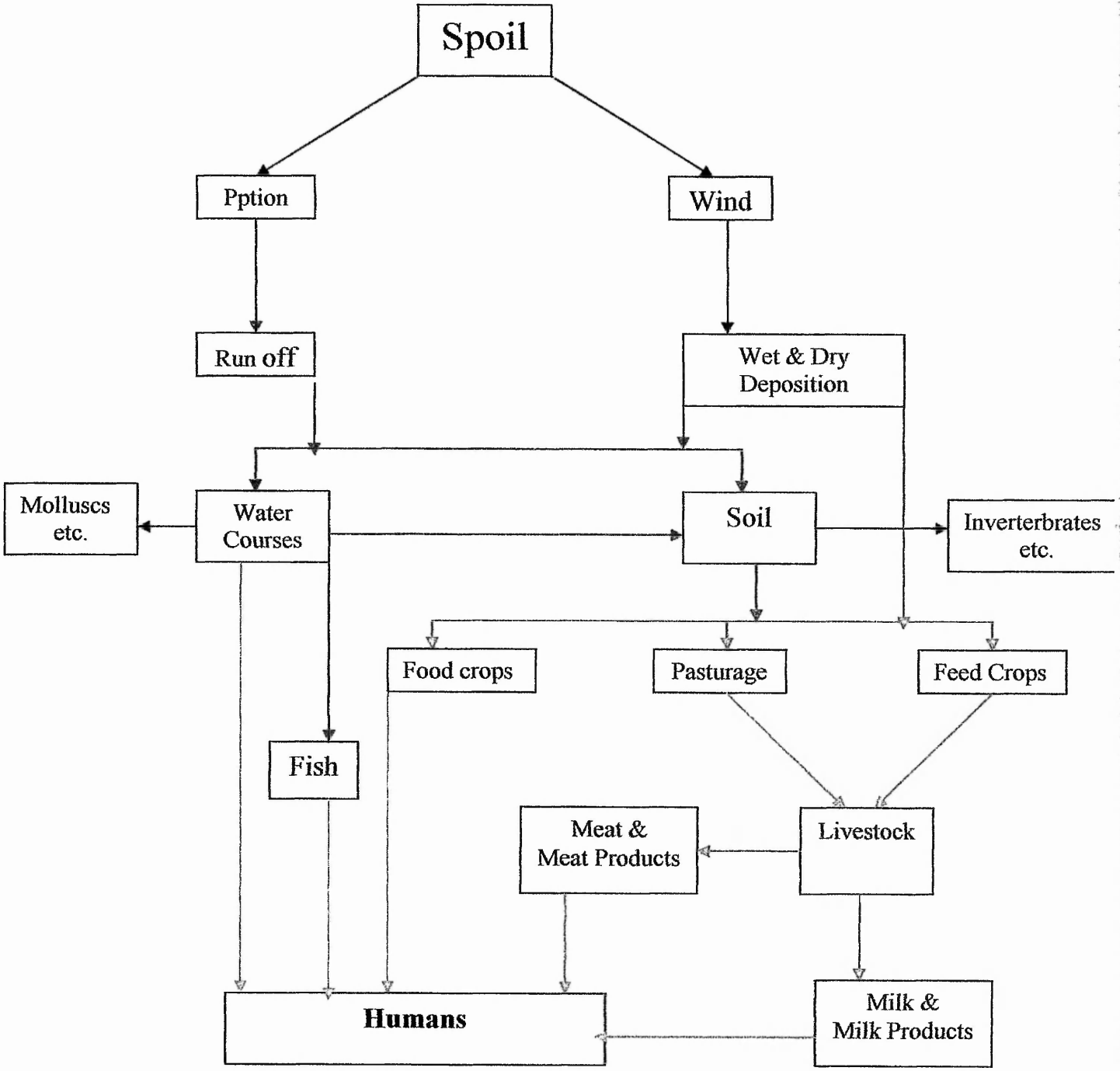


Figure 1.1 Pathways available for the accumulation of heavy metals through trophic levels from metalliferous mine workings to humans

phenomena will be necessary to fully understand the processes which ultimately effect and control bioaccumulation.

The dispersion of heavy metals takes place predominantly downwind of mine workings and as a result of leaching, due to precipitation, into water courses. Evidence of bioaccumulation in vegetation and possible movement through the food chain will be investigated by the most practical laboratory techniques subject to precision and accuracy assessment. Sample sites, approximately in line with the prevailing winds, will be selected and it is expected that metal concentrations would decrease with distance from the workings. It is considered that open cast mine workings will produce more definitive boundaries of metal concentrations as a result of atmospheric transportation of particulates. Control samples will be collected in the vicinity of the study areas to ensure that background levels of pollution are taken into consideration.

Animal and human monitoring will present additional problems since records of ancient mine workings and their environs are limited to skeletal remains or apocryphal writings and modern sampling will be subject to bioethical considerations. Contact with local historians and institutions such as newspapers, town councils and hospitals are envisaged in the latter stages of the research.

The thesis structure will be in the form of chapters reporting each stage of the research, which will include an introduction, materials and method section, results and a discussion of the results obtained. The initial chapters will outline the aims of the research, followed by the development of standard operating procedures (SOP) which will than be used in subsequent chapters to illustrate the collection, preparation, extraction, analytical procedures and statistical processes used. Each method development and location investigated is assigned a chapter and conclusions drawn from each part of the research are outlined in the penultimate chapter. The last chapter summarises further work which could be carried out to further increase knowledge of factors affecting bioaccumulation and ecotoxicological mechanisms relating to selected heavy metals in the environment surrounding metalliferous mine workings.

Chapter 2.

Aims and Objectives

The primary aim of the research is to determine the temporal and other abiotic effects on the dispersion, dilution and persistence of heavy metals from metalliferous mine workings and subsequent bioaccumulation through trophic levels. A structured approach was carried out to achieve the following:-

1). Develop standard operating procedures (SOP) for collection, preparation, digestion and analysis of soil and varying vegetation samples

2). Determine the most efficient general laboratory method of obtaining suitable analyte samples from soils and vegetation for subsequent analysis for Cd, Cu, Pb and Zn. Many papers have been produced addressing the issues of extracting suitable analyte from soil, vegetation and animal tissue samples, the most popular techniques are referred to as Wet Digestion (Tingi, *et al*, 1997) or Wet Ashing (Radojevic and Bashkin. 1999). All of the methods however involve extraction procedures utilising an individual acid or a combination of acids or a combination of acids and oxidizing agents.

3). Evaluate alternative methods for the preparation and analysis of samples which may occur as the project progresses.

4). Determine the most precise and accurate analytical procedure readily available for the determination of cadmium, copper, lead and zinc in the resulting sample extracts. The accuracy and precision of flame atomic absorption spectrophotometry (FAAS), inductively coupled plasma – atomic emission spectrophotometry (ICP-AES) and inductively coupled plasma-mass spectrophotometry (ICP-MS) will be compared for the analysis of soils and vegetation digested as determined in a.) above. Ion chromatography had been used by one team (Bruno *et al*, 2000) claiming detection limits of 10-15 ppb for Cu and Zn, 30 ppb for Cd and 60 ppb for Pb. Since these authors had used ion chromatography successfully and the technique allows simultaneous determination of Cd, Cu, Pb and Zn, it was decided to include this analytical

method in the investigation. The use of energy dispersive X-ray microanalysis would also be carried out directly on the samples after drying and grinding.

5). Evaluate possible sites for inclusion in the overall aims of the project in terms of heavy metal dispersion from workings and spoil tips. The effects of precipitation and wind are of particular interest, therefore a number of climate zones and temporal variations will be studied. Sites selected should reflect temporal different operating dates, different climatic conditions and different geological features. Having determined the suitability of mine workings for inclusion in the project, data will be obtained to illustrate dispersion, dilution, bioaccumulation and health implications both in during and after the cessation of operations. Sampling procedures should, if possible, be consistent at each site. Samples of soil, vegetation and animal tissue will be collected from areas influenced by prevailing winds and from similar habitat types. In order to equalise the effects of dispersion during and after mining operations it was decided that all sites should be copper mines of the open pit variety. Control samples will be collected from sites in the same geological area, similar habitats, unaffected by prevailing winds and in the case of vegetation and animal tissue, from comparable species.

6). The relationship between total metal content of soil and the proportion of which is readily bioavailable will be investigated. The effects of pH and other chemical processes within the soil will be evaluated in terms of possible change in metal speciation and hence further bioaccumulation.

7). Examine the collected data from each site to compare bioaccumulation patterns and determine whether partitioning of heavy metals in plant tissues varies from species to species and to establish whether accumulation mechanisms vary.

Chapter 3

Materials and Experimental Methods

3.1 Reagents and reference materials.

70% Nitric acid. Certified Grade.	Fischer Scientific UK Ltd Bishop Meadow Road Loughborough Leics LE11 5RG UK
32% Hydrochloric acid, Certified Grade.	Fischer Scientific UK Ltd
70% Perchloric acid, Certified Grade.	Fischer Scientific UK Ltd
30% Hydrogen peroxide, Certified Grade.	Fischer Scientific UK Ltd
1000 μg mL^{-1} FAAS and ICP calibration solutions (Cd, Cu, Pb and Zn)	Reagecon Shannon Free Zone Shannon Co Clare Ireland
European Community Bureau of Reference Certified reference materials (CRM)	Sigma Aldrich Company Ltd The Old Brickyard New Road Gillingham Dorset SP8 4XT UK

Deionised, distilled water	Purite Ltd
(maximum conductivity 0.2μScm^{-1})	Bandet Way
Produced using a Still Plus fitted with	Thame
D130 Deioniser column	Oxon
C340 Carbon polisher column.	OX9 3SJ UK.

All glassware, HDPE bottles and other equipment were cleaned by immersion in 10% HNO_3 for 16 hours, thoroughly rinsed with deionised distilled water and dried for a minimum of 4 hours at 60^{0}C. Contamination was avoided by always using new polythene bags and disposable nitrile examination gloves (powder free).

3.2 Standard Operating Procedures

Procedures for sampling and sample preparation were taken from various governmental organisations, standard text books on other research papers as acknowledged in the relevant text, however the extraction procedure adopted was determined as part of the research and is reported fully in Chapter 4.

3.3 Sample collection and preparation for aqueous analysis.

3.3.1 Soil

Spoil samples were collected using a clean stainless steel trowel. The surface 5mm of soil in an approximately 400cm^2 area was scraped off and discarded. Approximately 200g of soil was then collected to a maximum depth of 50mm and placed in a polythene bag. Each sample was individually triple bagged to prevent transit damage and transported to the laboratory. The spoil samples were air dried for seven days in a drying cabinet at 25^{0}C and placed in an oven for 3 hours at 60^{0}C (USEPA, 1998). After cooling the samples were passed through a 2mm sieve and ground for 5 minutes in a clean pestle and mortar before transfer to HDPE storage bottles.

3.3.2 Foliage

Leaves were collected using examination gloves and placed in polythene bags. The samples were triple bagged for transport to the laboratory. When comparisons of heavy metal dispersion from a specific source are being carried out, leaf samples are taken from the same species of tree, at similar height and from the same aspect. Leaf samples were washed thoroughly with deionised distilled water and dried for 24 hours at 25^0C in a positive pressure drying cabinet. The leaves were then dried for 24 hours at 70^0C (Radojevic and Bashkin, 1999). Approximately 10g of the dried needles were ground for 5 minutes in an agate pestle and mortar before transfer to HDPE storage bottles.

3.3.3 Tree Bark

Bark samples were collected using a clean stainless steel scalpel or chisel and the segments of bark placed in a polythene bag. All samples were triple bagged before transport to the laboratory. When comparison of heavy metal dispersion from a specific source were being carried out, bark samples were taken from the same species of tree of approximately the same age, at a similar height similar height and from the same aspect.

In all cases bark was separated from woody sections and cut into maximum $2mm^2$ segments using a clean stainless steel scalpel and dried for 24 hours at 25^0C in a positive pressure drying cabinet. Material from the edges of field samples were rejected in case contamination had occurred. The bark segments were then dried for 24 hours at 70^0C cooled and placed in a HDPE storage bottle.

3.3.4 Flowers and florets.

Flower or florets from plants were collected using clean vinyl examination gloves and placed in polythene bags. The samples were triple bagged for transport to the laboratory. Flower or floret samples were washed thoroughly with deionised distilled water and dried for 24 hours at 25^0C in a positive pressure drying cabinet. The flower or florets were then dried for 24 hours at 70^0C (Radojevic and Bashkin, 1999). Approximately 5 g of the dried flowers were ground for 5 minutes in an agate pestle and mortar before transfer to HDPE storage bottles.

3.3.5 Woody sections of plants and trees (twigs and stems)

Approximately 15g of the woody sections of plants or trees were collected within 100mm of the base of floret structures using clean stainless steel secateurs and the samples placed in a polythene bag. Samples were then triple bagged and transported to the laboratory.

The sections were cut into approximately 25mm lengths and washed thoroughly with demineralised distilled water and dried for 24 hours at 25^0C in a positive pressure drying cabinet. The woody sections were then dried for 24 hours at 70^0C (Radofevic and Bashkin, 1999). Approximately 10 g of dried woody sections were placed in a clean Waring Commercial Blender and blended at low speed for 2 minutes followed by 3 minutes at high speed. The resultant blend was transferred to a HDPE storage bottles.

3.3.6 Cones and fruits

Approximately 15g of cones, fruits or berries (in season) were collected using clean stainless steel secateurs and the samples placed in a polythene bag. Samples were then triple bagged and transported to the laboratory. Cones, fruits or berries were washed thoroughly with demineralised distilled water and dried for 24 hours at 25^0C in a positive pressure drying cabinet. They were then dried for 24 hours at 70^0C (Radofevic and Bashkin, 1999). Approximately 10 g of dried cones, fruits or berries were placed in a Waring Commercial Blender and blended at low speed for 2 minutes followed by 3 minutes at high speed. The resultant blend was transferred to a clean HDPE storage bottles. Where appropriate, seeds were removed from the cones, fruits or berries after drying, washed with acetone followed by demineralised distilled water and the two components processed separately as above.

3.3.7 Lichens

Approximately 15g of each lichen thallus was collected whilst wearing vinyl examination gloves and the samples placed in a polythene bag. Samples were then

triple bagged and transported to the laboratory. When comparison of heavy metal dispersion from a specific source are being carried out using epiphytic lichens on trees, samples are taken from the same species of tree of approximately the same age, at a similar height similar height and from the same aspect. Samples of lichen occurring on the ground were collected from the same slope aspect.

Lichen samples were thoroughly washed with demineralised distilled water and dried for 24 hours at 25^{0}C in a positive pressure drying cabinet then dried for 24 hours at 70^{0}C. After drying the lichen was separated from any attached vegetation. Where possible lichen samples were ground in a mortar and pestle for 5 minutes and transferred to a clean HDPE storage bottle. In some cases, particularly fruticose lichens eg. *Usnea* spp., the tissue is not suitable for grinding and was cut into maximum 2mm lengths using clean stainless steel scissors and transferred to a HDPE storage bottle.

3.3.8 Faeces

Faeces samples were collected using a suitable clean implement eg. stainless steel spatula, trowel or vinyl examination gloves and placed in a polythene bag. All samples were triple bagged for transport to the laboratory.

Faeces samples were dried for 24 hours at 25^{0}C in a positive pressure drying cabinet then heated for 24 hours at 70^{0}C. After cooling approximately 6g of sample were ground for 5 minutes in an agate pestle and mortar then transferred to HDPE storage bottles.

3.3.9 Invertebrates

In each case a minimum of 10 invertebrates of the same species were collected wearing vinyl examination gloves either directly from the soil or vegetation surface or from pit fall traps and placed immediately in a clean sample tube. The animals were covered with 30% ethyl alcohol and transported back to the laboratory. The alcohol was decanted from the sample tube in the laboratory and the invertebrates were washed thoroughly with demineralised distilled water. Invertebrate samples

were dried for 24 hours at 25^{0}C in a positive pressure drying cabinet then heated for 24 hours at 70^{0}C. After cooling the dried invertebrates were stored in a HDPE storage bottle.

3.3.10 Water

Approximately 500mL of water was collected in a HDPE sample bottle and placed in a polystyrene case for transport back to the laboratory.
On return to the laboratory, pH and conductivity were checked as in SOP 3.4.2 and 3.5.2. After completing pH and conductivity testing the water was filtered through Whatman 541 filter paper into a clean HDPE sample bottle and 0.5% of 70% Certified Nitric Acid was added to stabilise the sample for storage (USEPA, 1998) in a refrigerator at 4^{0}C.

3.4 pH determination.

3.4.1 Soil

On return to the laboratory 10 g of fresh soil was placed in a sample tube and 20mL of demineralised distilled water added (Radojevic and Bashkin, 1999). The tube was shaken vigorously for 5 minutes and allowed to stand for 1 hour then centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was tested using a Hanna pH 210 Microprocessor pH meter fitted with a HI 1230B probe. The instrument was calibrated using pH 10.01, 7.01 and 4.01 buffer solutions immediately before each determination cycle.

3.4.2 Water

Approximately 60mL of water was transferred to a HDPE sample bottle
and pH was checked using a previously calibrated Hanna pH 210 microprocessor pH meter fitted with a HI 1230B probe.

3.5 Conductivity determination

3.5.1 Soil

On return to the laboratory 10 g of fresh soil was placed in a sample tube and 20mL of demineralised distilled water added (Radojevic and Bashkin, 1999). The tube was shaken vigorously for 5 minutes and allowed to stand for 1 hour then centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was tested using a Corning model 220 conductivity meter calibrated using 0.1M potassium chloride at the determined room temperature. Results were recorded as microseimens per cm ($\mu s\ cm^{-1}$)

3.5.2 Water

Approximately 60mL of water was transferred to a HDPE sample bottle
And conductivity was determined using previously calibrated Corning model 220 conductivity meter. Results were recorded as microseimens per cm ($\mu s\ cm^{-1}$)

3.6 Analytical techniques

3.6.1 Flame atomic absorption spectrophotometry (FAAS)

Analysis was carried out using a Perkin Elmer Flame Atomic Absorption Spectrometer Model 1100 fitted with an impact bead and appropriate hollow cathode lamps. The wavelength for each target metal and linear range for the instrument are shown in Table 3.1.

Element	λ nm	Linear Range mgL^{-1}
Cd	228.8	LOD – 0.5
Cu	324.8	LOD - 2.0
Pb	217.1	LOD - 5.0
Zn	213.9	LOD - 1.0

Table 3. 1 Analytical data relative to Perkin Elmer 1100 AAS instrument fitted with an impact bead.

Five calibration solutions were made up from 1000 mgL^{-1} certified standards and within the linear ranges in Table 3.1.

Dilution factors for the extracts were determined by trial and error; final dilution ratios allowed determination of absorption unit values obtained within the linear range for each metal. An example of a calibration equation (Figure 3.1) and calculations to determine heavy metal concentrations in dried samples of soil, spoil and leaves etc. are also shown below.

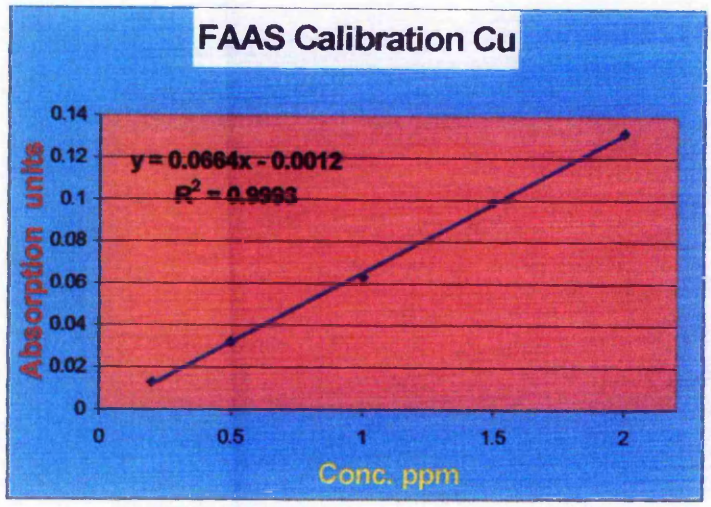


Figure 3.1 Calibration graph and Equation obtained for copper, 12 February 2003.

$$\text{Concentration of Cu} = \frac{\text{absorption}}{0.0664} + 0.0012$$

$$\text{eg for NG6/25} \quad \text{Cu} = \frac{0.107}{0.0664} + 0.0012$$

$$= \mathbf{1.6126}\ \text{mg L}^{-1}$$

Dilution factor used on NP6/25 = 25

Therefore concentration in extract = 1.6216 x 25

$$= \mathbf{40.54}\ \text{mg L}^{-1}$$

Metal concentration in dry soil = $C \times V/M$

Where C is the concentration of the metal in the sample extract as determined by AAS (mg L^{-1}), V is the volume of the extract and M is the weight (g)of the dry sample (Radojevik, M .and Bashkin, V.N. 1999).

For sample NP6 metal Cu concentration $= \frac{40.54 \times 25}{0.5078}$

$= \underline{\mathbf{1995.8}\ \text{mg kg}^{-1}\ \text{in dry soil.}}$

3.6.2 Inductively coupled plasma – optical emission spectrophotometry (ICP-OES)

Analysis was carried out using a Perkin Elmer Optima 2100 ICP-OES fitted with a AS-90 plus autosampler. The instrument was calibrated before every run which consisted of a maximum of 98 samples including calibration samples. The instrument was calibrated using mixed Cu, Zn, Pb and Cd standards of 0.5 mgL^{-1} and 1.0 mgL^{-1} prepared from 1000mgL^{-1} certified standards diluted with processed extractant which was also used as a blank during calibration. Three replicates of each sample were analysed by the instrument to obtain a mean metal concentration. Standard operating parameters of the instrument used are as shown in Tables 3.2 and 3.3.

Figures 3.2 – 3.5 illustrate typical calibration curves produced by the instrument in order to calculate metal concentrations of samples under investigation.

Element	λ nm	Survey Low nm	Survey Up nm	Peak Algorithm	Points per peak
Copper	327.393	327.244	327.542	Area	7
Zinc	206.2	206.108	206.192	Area	7
Lead	220.353	220.255	220.451	Area	7
Cadmium	228.802	228.669	228.905	Area	7

Table 3.2 Standard wavelength and survey range for analysing target metals on Perkin Elmer 2100 ICP-OES

Plasma L min^{-1}	Auxiliary L min^{-1}	Nebuliser L min^{-1}	View dist. mm	Plasma view	Pump flow rate L min^{-1}	Autosampler Flow Rate	Time
15.0	0.2	0.8	15.0	axial	1.5	1.5 L min^{-1}	30 sec

Table 3.3 Standard processing data used for analysis of target metals using Perkin Elmer Optima 2100 ICP-OES.

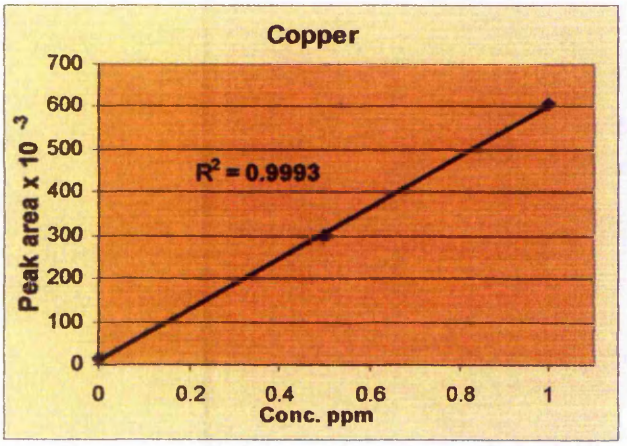


Figure 3.2 Calibration graph for copper produced by ICP-OES instrument

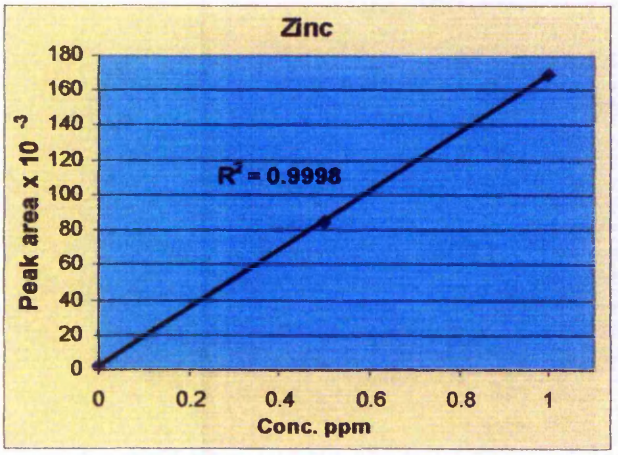


Figure 3.3 Calibration graph for zinc produced by ICP-OES instrument.

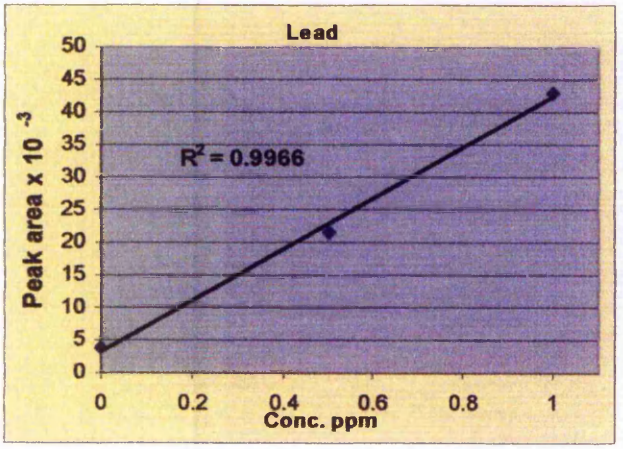


Figure 3.4 Calibration graph for lead produced by ICP-OES instrument.

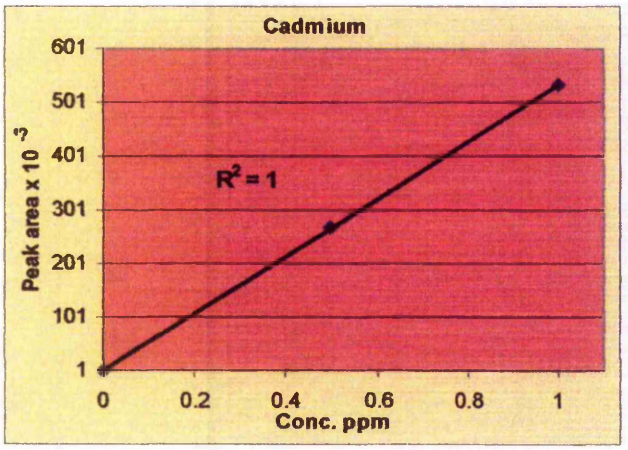


Figure 3.5 Calibration graph for cadmium produced by ICP-OES instrument.

Readings from the Perkin Elmer Optima 2100 were produced as a mean of 3 replicates taken from each sample and were expressed in ppm.

Metal concentrations in the various substrates are obtained by using the formula

$$Conc = \frac{CxV}{M} \text{ mg kg}^{-1}$$

where C = concentration of metal in extract ppm

V = volume of extract produced mL

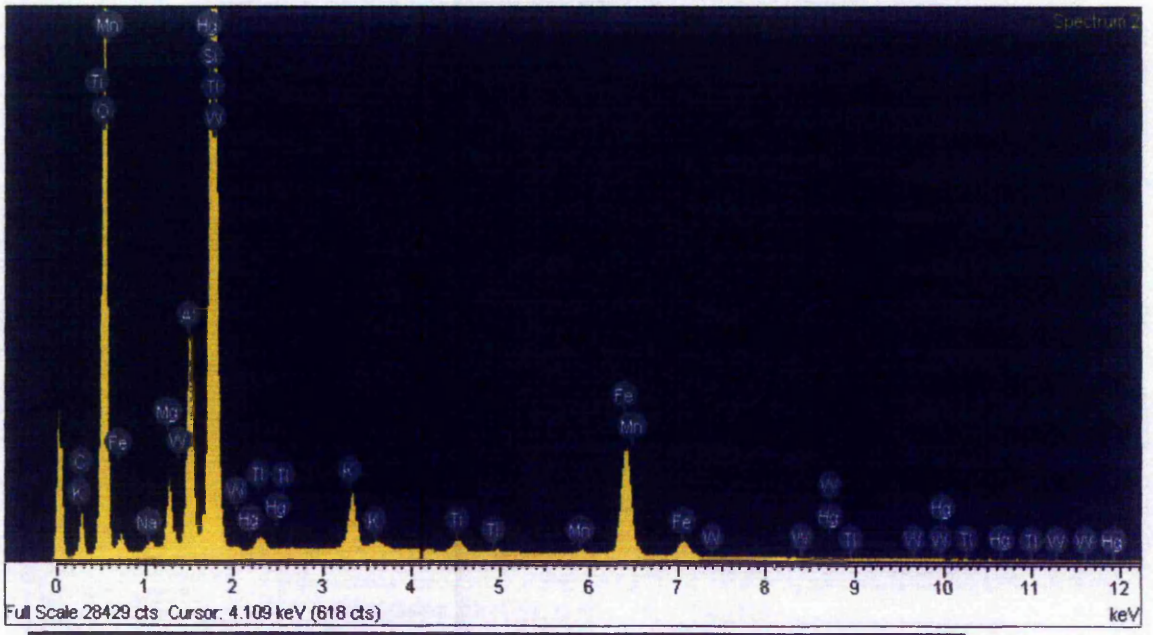
M = mass of substrate extracted g

3.6.3 Energy Dispersion X-ray Analysis (EDAX)

This technique allows multi element analysis without destructive extraction of the substrate under examination. In this case, substrate was mounted on 12.5 mm stainless steel stubs using adhesive pads and coated with carbon in an Edwards Sputter Coater using argon as the flushing gas and a vacuum reading of less than 2 mbars.

Coated stubs were analysed using a Jeol JSM 840A scanning electron microscope to which was attached an Oxford Analytical EDS X-ray micro analysis system with Inca software, using an acquisition time of 60 seconds. This instrument array detects and

quantifies all elements above carbon in the periodic table. Figure 3.6 demonstrates the type of spectrum and report generated after analysis by this technique.



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C	25.28	0.3161	13.34	0.47	21.29
O	219.88	0.7849	46.73	0.32	55.98
Na	1.41	0.6698	0.35	0.03	0.29
Mg	6.92	0.6521	1.77	0.04	1.39
Al	20.08	0.7538	4.44	0.05	3.16
Si	94.49	0.8015	19.67	0.14	13.42
K	10.00	0.9871	1.69	0.03	0.83
Ti	2.44	0.8214	0.50	0.03	0.20
Mn	1.35	0.8130	0.28	0.03	0.10
Fe	45.52	0.8299	9.15	0.09	3.14
W	2.58	0.7184	0.60	0.16	0.06
Hg	1.53	0.7295	0.35	0.07	0.03
Tl	4.64	0.6804	1.14	0.07	0.11
Totals			100.00		

Figure 3.6 Report generated by EDAX instrumentation when analysing shore soil from Britannia Mine, B.C. Canada

3.6.4 Ion Chromatography

Ion chromatography was carried out using Dionex DX 600 instrumentation fitted with an Ionpac CS5A column and post column reaction chamber. Eluent used was Metpac oxalic acid and the post column reagent was 0.12g of 4-(2-pyridylazo) resorcinol, monosodium salt, monohydrate (PAR) dissolved in 1000mL of post column diluent consisting of 1.0 M 2-dimethylaminoethanol, 0.5M ammonium hydroxide amd 0.3M sodium bicarbonate.. Detection was achieved by absorbance measurement at 530 nm using an AD25 UV/Visible detector. Eluent flow rate was 1.2mL min^{-1}, post column

diluent flow rate was 0.6 mL min^{-1} and the injection volume was 50µL. A manual injection approach was used and data collection was undertaken over 10 minutes. The instrument was calibrated using five mixed calibration solutions of 1,2,3,4, and 5 ppm which were prepared from 1000ppm certified ion chromatography standards. Peak area values were used as indicators of concentration and Table 3.4 illustrates typical data obtained from such analysis and Figure 3.7 illustrates a typical graph obtained indicating elution times for each metal.

|Data File : C:\PEAKNET\DATA\INVES002.DXD Report Date: 5/12/105 1:58:46 PM |
|Sample Name: 1PPM Collected : 2/3/104 12:06:16 PM |
|Inject # : 2 Vial # : |
|Method File: PEAKNET\METHOD.ACI\METAUTO.MET
|System Name: DX 600 T METALS Detector : UV/Vis |
|Column Type: Operator : |
|Data Points: 3000 Rate : 5.00 Hz |

Calibration Volume Dilution Start Stop Area Reject Pk. Width Threshold

External 1 1 0.00 10.00 1000 10.00 0.50

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl
3	1.90	LEAD	1.0	19660	104964	
4	2.22	COPPER	1.0	123811	899260	
5	3.18	CADMIUM	1.0	29679	497267	
6	7.28	ZINC	1.0	47784	1028062	

Table 3.4 Typical report obtained for peak area from Dionex DX600 Ion chromatography instrument.

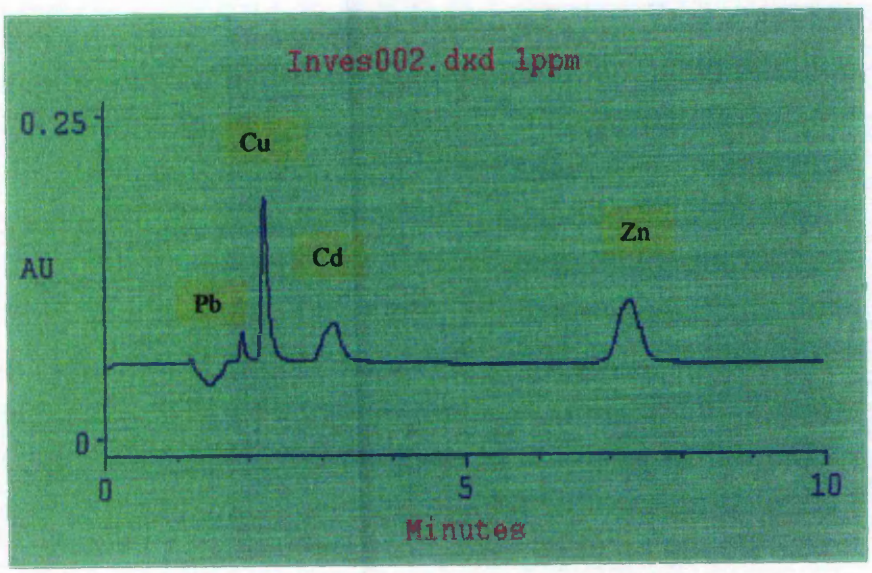


Figure 3.7 Typical plot obtained from Dionex DX600 Ion chromatography instrument.

Calibration graphs were constructed as in Figure 3.8 for all target metals and the equation used to calculate the concentration of metals in the extract with subsequent calculation of metal concentration in the dries soil or vegetation exactly as shown in 3.4.1 FAAS above.

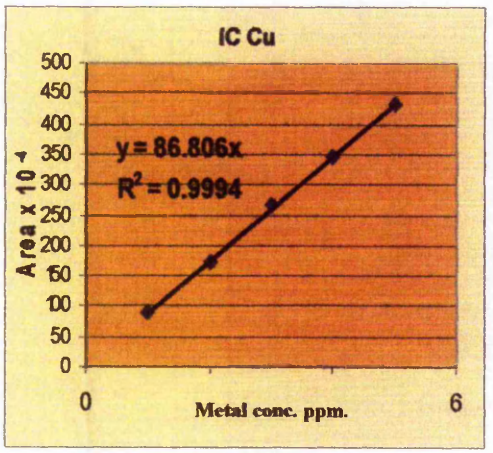


Figure 3.8 Calibration graph for copper using Dionex DX600 I.C. instrument.

3.7 Statistical analysis techniques

3.7.1 Limit of Detection and Limit of Quantitation.

Since five standards were used to calibrate the FAAS and Ion chromatography instruments, for each target metal, a limit of detection (LOD) and limit of quantitation (LOQ) could be calculated for each calibration graph. LOD of an analyte is described as that concentration which gives an instrument signal significantly different from the blank or 'background' signal. LOQ has been suggested as the lowest limit of precise quantitative measurements as opposed to qualitative detection. There is an increasing trend to define LOD as the analyte concentration giving a signal equal to the blank signal y_B, plus three standard deviations of the blank s_B and LOQ is suggested as y_B plus ten x s_B (Miller and Miller, 2000). It must be stressed that the resultant signal strength and corresponding concentration values are purely arbitrary but are becoming increasingly accepted as useful indicators of the precision of the instrument being used. Since the values for these parameters are calculated from current calibration data, the age and condition of the instrument being used is an integral factor influencing the results obtained.. For calculation purposes y_B is determined as

the intercept on the y axis obtained from a regression equation applied to a best fit line on the calibration graph and s_B is equal to the slope of the calibration graph $s_{y/x}$.

$$\text{LOD} = \text{intercept} + 3\ (s_{y/x})$$

$$\text{LOQ} = \text{intercept} + 10\ (s_{y/x})$$

LOD and LOQ values obtained during this study were expressed in absorption units. These were subsequently converted into metal concentrations and an example of results obtained is shown in Table 3.

Metal	LOD absorption	LOD mgL^{-1}	% of linear range	LOQ absorption	LOQ mgL^{-1}	% of linear range
Copper	0.0041	0.09	4.53	0.0135	0.31	15.4
Zinc	0.0067	0.04	3.83	0.0189	0.11	11.0
Lead	0.0017	0.19	3.80	0.0053	0.57	11.5
Cadmium	0.0072	0.05	9.15	0.0224	0.14	27.3

Table 3.5 Mean LOD and LOQ concentrations calculated from calibration graphs produced over a period of 3 weeks using flame atomic absorption spectrophotometry. Percentage of the total calibration linearity is also indicated.

The LOD and LOQ for copper, zinc and lead are similar as a percentage of the linear range however cadmium exhibits a higher percentage of linearity.

3.7.2 Relative Standard Deviation.

Relative standard deviation is a measure of the relative error encountered and can be used as a measure of precision when determining concentrations of varying magnitude (Miller and Miller, 2000).

Relative standard deviation (RSD) was calculated as follows:-

$$RSD = \frac{SD}{Mean} x100\%$$

Where *RSD* = Relative standard deviation
SD = Standard deviation for respective metal concentration
Mean = Mean of replicate metal concentration

In order to achieve meaningful results a minimum of 6 replicates of each sample were required and as the research progressed ten replicates of each sample were used where ever possible. RSD values of 4% or less were considered good for this type of environmental analysis, where, particularly in the case of soils and related materials, lack of homogeneity is a considerable problem and some efforts were made within the research to try and alleviate variations due to this property. Pérez and Valiente (2005) considered RSD values of <12% satisfactory and noted that higher RSD values for some of their samples were due either to very low metal concentrations or heterogeneity of soil samples. As the research progressed such values became increasingly prevalent, especially where higher metal concentrations ie. >30 mg kg^{-1} where encountered.

3.7.3 Accuracy

Quality assurance standards used throughout the research were certified materials obtained from the European Community Bureau of Reference, Brussels and were used consistently to assess extraction and analytical processes.
Accuracy values were calculated as follows

$$AccuracyA\ \% = 100 - \left[\frac{abs(x_{QA} - x_A)}{x_{QA}}\right]100$$

where *abs* is the absolute value of bracketed calculation

x_{QA} is the certified concentration of the reference material

x_A is the concentration obtained after extraction and analysis of the certified material.

3.7.4 Correlation Coefficients

This technique was used to determine relationships between the composition of point source pollution dispersion pattern at varying distances from the source, normally down wind. Rawlins *et al.*, (2003) used the technique extensively to demonstrate the relationship between the composition of parent material to the composition of surrounding soils and Maisto *et al.*, (2004) used the same statistical process to illustrate the relationship between bioaccumulation of copper and lead and the concentration of these metals in soil. Data collected relating to soil concentration at varying distance from the source were tabulated in Microsoft Excel and this software was used to obtain correlation graphs and coefficients. Figure 3.10 illustrates a typical graph and regression constant obtained at Lavender Pit, Arizona.

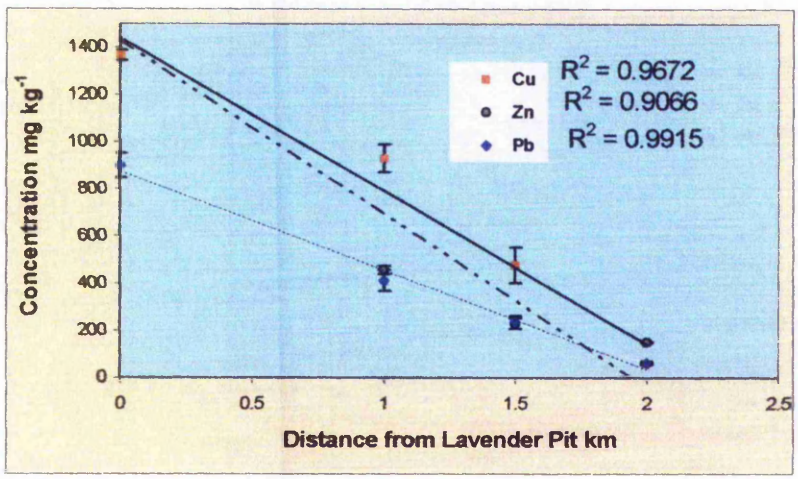


Figure 3.9 Relationship between the metal concentration in dried soil collected at sites around Bisbee and the distance from Lavender Pit. Error bars represent the standard deviation obtained from 10 replicates.

3.7.5 Analysis of Variance

Analysis of variance (ANOVA) used in this research employed a statistical package, Minitab version 14, to generate two way ANOVA analysis of two variables within a sequence of analytical processes. The variance is partitioned into components:

Total variation = $SS_{within} + SS_{betweenA} + SS_{between\ B} + SS_{interaction}$ where SS is the sum of squares for each component (Pentecost, 1999). The technique explores the null hypothesis that there is no statistically significant difference between the variables concerned using *F* distribution tables in this case at the 5% probability level. The additional benefit of using Minitab software is that interactive graphs illustrating significant differences or otherwise in processes can be produced. Figure 3.11 shows graphical representation of data produced to compare procedures used to analyse extract from 3 samples of contaminated soil and 3 samples of vegetation. The metal concentration represents the mean of copper, zinc, lead and cadmium concentrations determined using three analytical techniques.

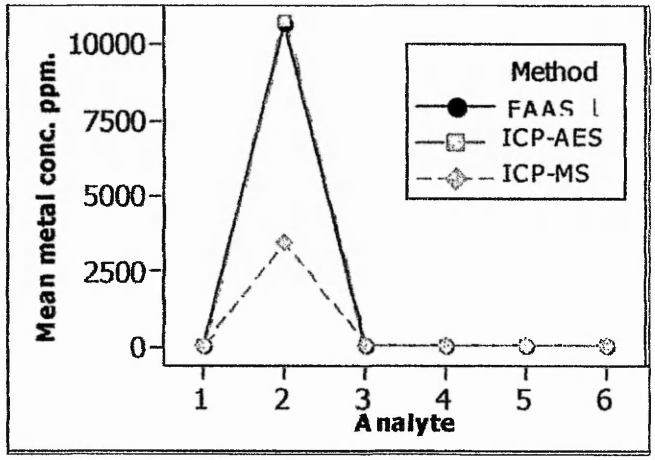


Figure 3.10 ANOVA interaction plot for comparison of Cu, Zn Pb and Cd in 6 samples of soil and vegetation by 3 analytical methods concentrations using aqua regia extraction.

3.7.6 95% confidence interval determination.

During the early work carried in this research, a 95% confidence interval was used to apply error bars to charts as a means of comparing the variation of results about the mean and is defined as a range in which we may reasonably assume includes the true value. This value is particularly useful when a small number of samples are being evaluated (Miller and Miller, 2000).

It was calculated as follows:-

$$\mathrm{CI} = \frac{\bar{x} \pm t_{n-1} s}{\sqrt{n}}$$

Where t_{n-1} = value obtained from tables using the factor n-1 to determine the number of degrees of freedom under which the t value is obtained. In practice for small sample numbers it reduces the range which would be obtained if $\pm$ s (standard deviation) was used to apply error bars.

Chapter 4

4.1 Determination of most suitable extraction method for subsequent analysis of concentrations of Cd,Cu, Pb and Zn in samples from old mining operations.

Many extraction or digestion methods have been used to obtain analyte for analysis. This chapter evaluates five different methods in an attempt to determine the most precise and pragmatic method requiring as little specialise equipment as possible.

Introduction:

This investigation seeks to determine the most efficient method of digesting vegetable and mineral samples for subsequent analysis. The use of readily available laboratory equipment was a primary consideration. One of the most common and reliable analytical techniques for the determination of heavy metal concentrations in environmental analysis is Flame Atomic Absorption Spectrometry although it can be time consuming.

Many papers have been produced addressing the issues of extracting suitable analytes from soil, vegetation or animal tissue samples, the most popular being referred to as Wet Digestion (Tingi, *et al.*, 1997) or Wet Ashing (Radofevic and Bashkin. 1999). All of the methods however use an individual acid or a combination of acids or a combination of acids and oxidizing agents. Additionally open digestion on a hot plate (Bellis, *et al.*, 2001), reflux extraction at atmospheric pressure (Pueyo, *et al.*, 2001) and microwave aided digestion in either open (McGrath. 1998) or closed vessels (Martinez, *et al.*,1999) or simply closed vessels such as the Parr bomb (Moeller, *et al.*, 2001.) have been extensively used.

An examination of 72 research papers concerned with heavy metal determinations in soil (and sediments), vegetation and animals produced the following data relating to extraction methods:-

Open digestion in a conical flask or similar on a hot plate – 56%.

Microwave assisted closed pressure vessel – 31%.

Analytical methods not requiring extraction – 13%.

One method utilizing reflux digestion using aqua regia (Pueyo, M. *et al.*, 2001).

The combination of reagents employed within each of these methods varied, however nitric acid was used in each procedure. The effectiveness of concentrated nitric acid has been reported by Wasilewska, *et al.*, (2002) when digesting organic materials in pressurized equipment and by Bellis, *et al.*, (2001) for open digestion of tree bark.

Combinations of acids range from nitric acid only, aqua regia, nitric acid/hydrogen peroxide, nitric acid/sulphuric acid, nitric acid/perchloric acid and more complex combinations such as nitric/hydrochloric/perchloric/hydrofluoric acids. When open digestion was used the most frequent combinations were:

HNO_3 – 36%

HNO_3/H_2O_2 – 18%

Aqua Regia (HNO_3/HCl) – 31%

$HNO_3/HClO_4$ – 10%

Other acids or combinations of acids – 5%

When microwave assisted pressure digestion was used the most frequent combinations were:-

HNO_3 – 59%

Aqua regia – 27%

HNO_3/H_2O_2 – 11%

Other acids or combinations of acids – 13%

It can be argued that the use of hydrofluoric acid in extraction procedures is not essential unless total metal concentrations are required including metals complexed within the silica matrices of soil particles and to a lesser extent in plant tissue. The concentrations of heavy metals, incorporated in such a way, are considered to be relatively small and are not available for bioaccumulation. It was therefore decided to

use only methods which would be suitable for use in general laboratory fume cupboards for this exercise and to use only the open method of digestion.

Additionally, Ipolyi, *et al.*, 2002, carried out an investigation into accelerating the extraction processes used in a three step sequential extraction method. One of the ideas investigated was the use of a normal laboratory microwave oven. Drastic reductions in extraction time for particularly the acid phase were reported and it was suggested that microwaves had a quantifiable influence on the efficiency of the process. It was decided that the use of microwave digestion would be investigated in conjunction with nitric acid only for this study.

The reagents chosen for open digestion in a fume cupboard were:

1. Nitric acid only.

2. Nitric acid/hydrogen peroxide

3. Aqua regia

4. Nitric acid/perchloric acid

5. Nitric acid with microwave assistance.

For the purposes of this initial investigation the following materials would be extracted using each method together with certified standards obtained from the Commission of European Communities, Community Bureau of Reference (BCR), as control materials.

1. Soil from a spoil tip at Parys Mountain, Anglesey, U.K.

2. Soil from a spoil tip at Snail Beach, Shropshire, U.K.

3. Leaves from a hazel (*Corylus avellana*) bush at Snail Beach, Shropshire, U.K.

4. Needles from a Scots Pine (*Pinus sylvestris*) tree at Black Rock, Cromford, Derbyshire, U.K.

5. CRM-101. Spruce (*Picea abies*) needles with certified analysis.

6. CRM-142R Light sandy soil with certified analysis.

Experimental Procedures.

Site locations

1. Parys Mountain is an abandoned copper mine on the Isle of Anglesey in North Wales UK. (Figure 4.1) Copper has been mined on the site for 4000 years initially by prehistoric populations and subsequently by the Romans (Curtis, 2004). By the mid eighteenth century the area was extensively developed and copper from the mines dominated the world's markets in the 1780's.

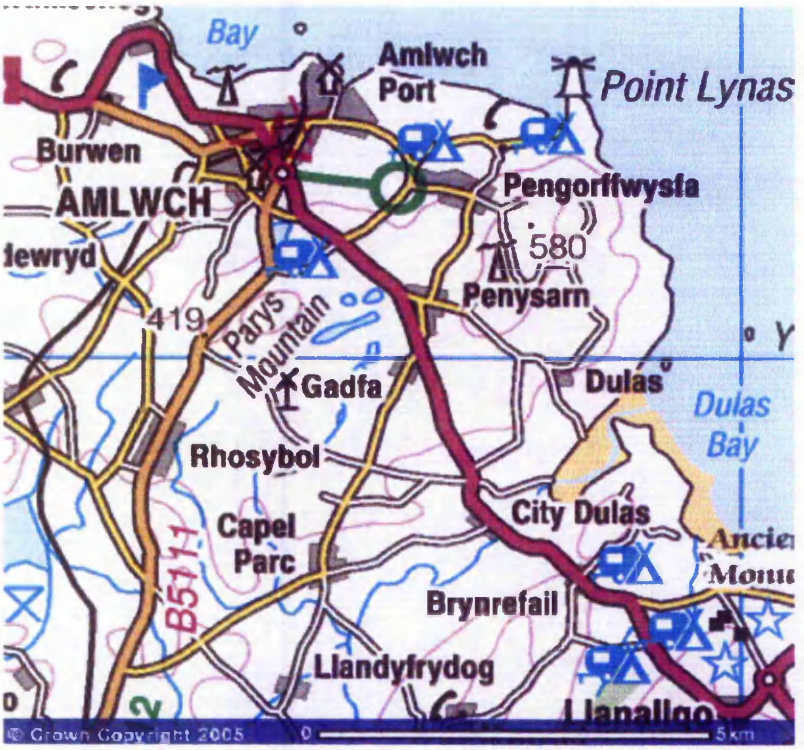


Figure 4.1 Location of Parys Mountain, Anglesey, North Wales

Zalasiewicz in 2000 stated that Parys Mountain was once the largest copper mine in the world but by the 1880's competition from cheaper ores from abroad led to the decline of the mines. In 1984 mining once again commenced and 2000 tonnes of ore was extracted. The area is now an industrial heritage site (Amlwch Industrial Heritage Trust, 1998).

A spoil sample was taken from the base of the spoil heap created by the latest mining operations (Grid Reference SH443906) located downwind from the workings.

2. Snailbeach is an old lead mine located near the town of Minsterley, about 16 km SW of Shrewsbury, UK. (Figure 4.2). It was reputed to have yielded the greatest volume of lead per hectare of any mine in Europe and operations date back to Roman times. Small quantities of silver and zinc were also mined and operations ceased here in 1955. (www[1])

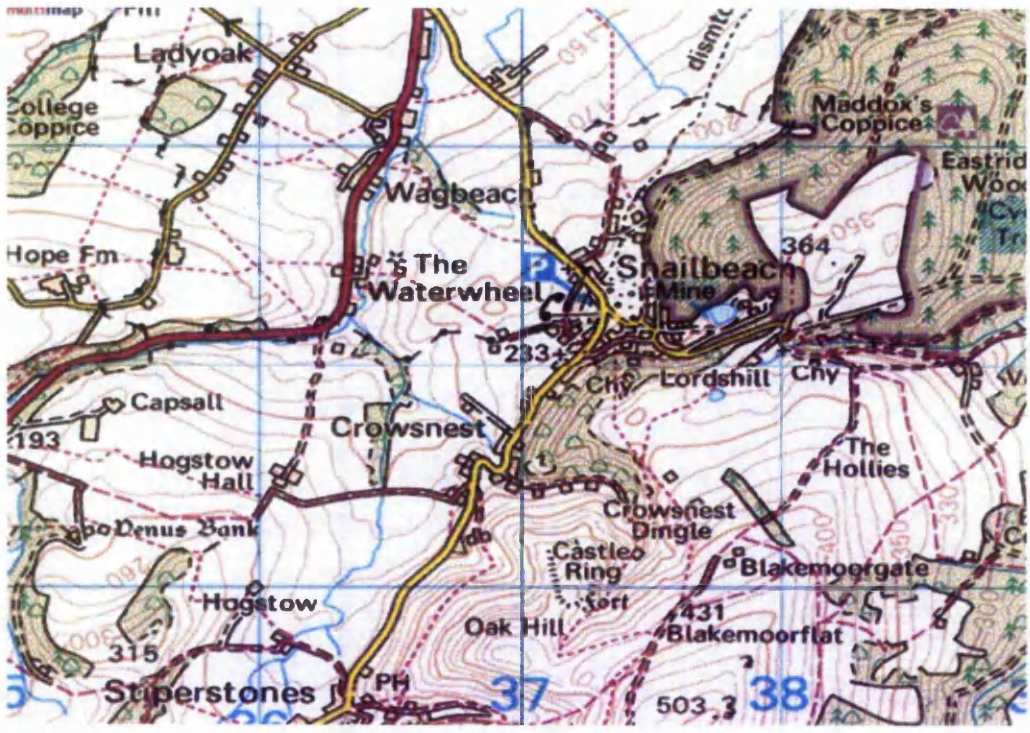


Figure 4.2 Location of Snailbeach lead mine

A spoil sample was taken from the base of a spoil heap, lacking vegetation, located to the NW of the main buildings (Grid Reference SJ376022).

To the north of the road running through the main site is a flat rocky area with approximately 5% vegetation cover (Grid Reference SJ374023). Stunted hazel (*Corylus avellana*) bushes approximately 1.5 meters high are the only trees available and leaves were taken from three bushes growing together.

3. Black Rock in Derbyshire lies to the south west of Matlock above the village of Cromford (Figure 4.3)

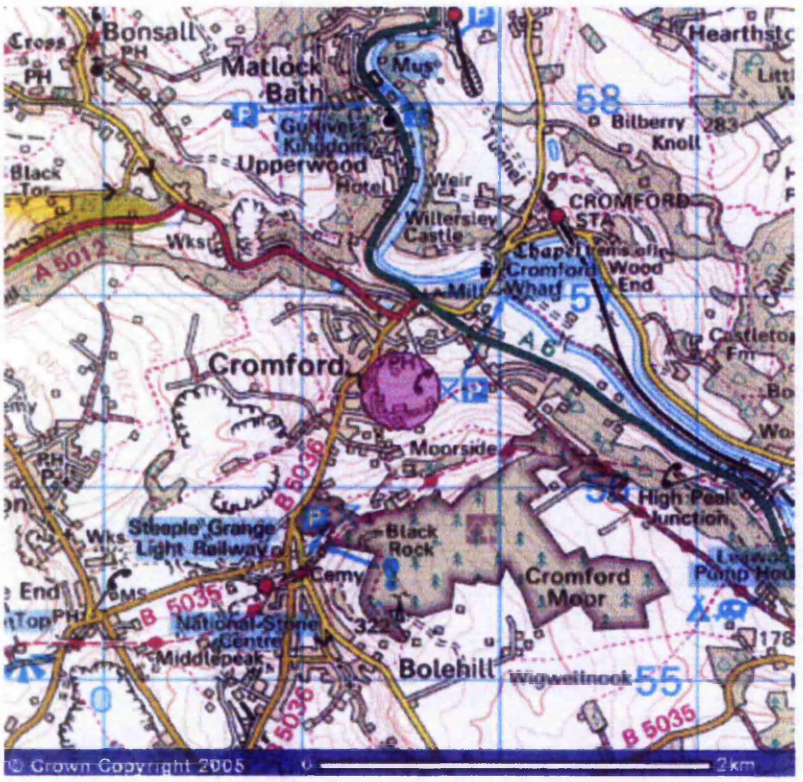


Figure 4.3 Location of Black Rock, Cromford, Derbyshire.

It is the site of an old lead mine and has a large spoil heap below which is a small mixed forest (Grid Reference SK292558). A sample of needles from a pine tree (*Pinus sylvestris*) was collected from this site.

All samples of soil and leaves were collected and prepared in accordance with Standard Operating Procedure

Digestion and extraction procedure.

Five procedures were investigated endeavouring to keep each extraction as straight forward as possible. 0.5g ± 0.025g of dried, ground soil and 0.25g ± 0.012g of dried ground vegetation were used and the final solution, from each extraction, was made up to 25mL for analysis. The increased sample size for soils was chosen because several workers including Scharenberg and Ebeling, 1995 and Pyatt, 2001 had demonstrated that plant leaves can accumulate higher concentrations of heavy metals than the soil in which the trees grow by a factor of at least two. The higher bulk density of dried vegetative material relative to that of soil was also a factor.

1. Extraction with concentrated nitric acid (after Radojevic and Bashkin, 1999 and Pederson, *et al.*, 2000).

Soil and leaves were accurately weighed into a 50mL beaker and 7..0 mL of 70% v/v nitric acid was added. The flask was carefully swirled to mix the reactants and simmered gently for 90 minutes. After cooling a further 2.5mL of 70% v/v nitric acid added and the beaker gently simmered for a further 30 minutes. After cooling the mixture was filtered through a Whatman 451 filter paper. The beaker and the filter paper were washed thoroughly with 0.1M nitric acid into a 25mL volumetric flask and the solution made up to the mark with 0.1M nitric acid. After mixing the extractant was transferred to an HDPE sample bottle for storage and subsequent analysis.

2. Extraction with aqua regia (Radojevic and Bashkin, 1999)

Aqua regia was made by carefully adding 150mL of 32% v/v hydrochloric acid to 50mL of 70% v/v nitric acid in a 500mL conical flask.

The extraction process was carried out exactly as method 1. above, substituting aqua regia for 70%v/v nitric acid

3. Concentrated nitric acid/hydrogen peroxide combination. (Kozlov *et al.*,1998)

Soil and leaves were accurately weighed into a 50mL conical flask and 7mL of 70% v/v nitric acid was added. The flask was carefully swirled and the mixture boiled gently for 90 minutes. After cooling lmL of 30% v/v hydrogen peroxide was added and the mixture gently heated. After the effervescence had subsided a further 1mL of hydrogen peroxide was added and the mixture heated gently for 30 minutes. After cooling the extractant was filtered through a Whatman 451 filter paper and the equipment washed with 0.1M nitric acid into a 25 mL graduated flask and the solution made up to the mark with 0.1M nitric acid, mixed thoroughly and transferred to an HDPE bottle for storage and subsequent analysis.

4. Extraction with nitric acid/perchloric acid. (Reinecke, *et al.*, 1999)

Soil and leaves were accurately weighed into a 50 mL conical flask. 7 ml of 70% v/v nitric acid was added and heated for 1 hour at 100/110^{0}C. After cooling, 2.0mL of 70% v/v perchloric acid was added and the mixture reheated at 110^{0}C for hour or until brown fumes were emitted. After cooling 5ml of 0.1M nitric acid was added and the mixture filtered through a Whatman 451 filter paper into a 25mL graduated flask and made up with 0.1M nitric acid. After thorough mixing the solution was transferred to an HDPE bottle for storage and suitable analysis.

5. Extraction with nitric acid digestion assisted by microwaves. (Ipolyi *et al*, 2002).

Soil and leaves were weighed accurately into a 50mL conical flask and 7 mL of 70% v/v nitric acid was added. The flask was placed in a Sharp R254 microwave oven and exposed to 270W for 20 minutes. 5 mL of 0.1M nitric acid was added after cooling and the mixture filtered through a Whatman 451 filter paper. All equipment was washed through with 0.1M nitric acid into a 25mL graduated flask and made up with 0.1M nitric acid. After thorough mixing the solution was transferred to an HDPE bottle for storage and subsequent analysis.

Seven replicates of each sample were processed and all extractions were stored in a refrigerator at 4^0C until required for analysis.

Analysis.

Analysis was carried out using FAAS as in Standard Procedure 3.6.1.

Results:

The figures below indicate the average concentration obtained from each extraction process. The error bars indicate upper and lower 95% confidence intervals determined using a student *t*-test. Generally seven replicates survived the processing and dilution processes however some were contaminated etc. and where this was the case comment has been made.

Key to prefixes used in all chart presentations:-

N Nitric acid only.

P Nitric acid/perchloric acid combination.

A Aqua regia.

O Nitric acid/hydrogen peroxide combination

M Microwave assisted nitric acid

Evaluation of open microwave/nitric acid digestion was carried out on samples of Snail Beach spoil and hazel leaves since the emission of fumes presented problems in the microwave oven being used. It was considered sufficient to evaluate these extractions for indications of improvements in extraction of the metals in question before proceeding with other samples.

The second letter on the chart, Method axis, refers to the substrate being extracted i.e.

S Snail Beach spoil.

P Parys Mountain spoil.

L CRM 142 Light sandy soil (certified reference material).

B Scots Pine needles collected at Black Rock.

H Hazel Leaves collected at Snail Beach.

N CRM 101 Spruce Needles (certified reference material)

Hence NS = nitric acid digestion of Snail Beach spoil

PS = nitric acid/perchloric acid digestion of Snail Beach spoil

AS = aqua regia digestion of Snail Beach spoil

OS = nitric acid/hydrogen peroxide digestion of Snail Beach spoil

MS = microwave assisted nitric acid digestion of Snail Beach spoil

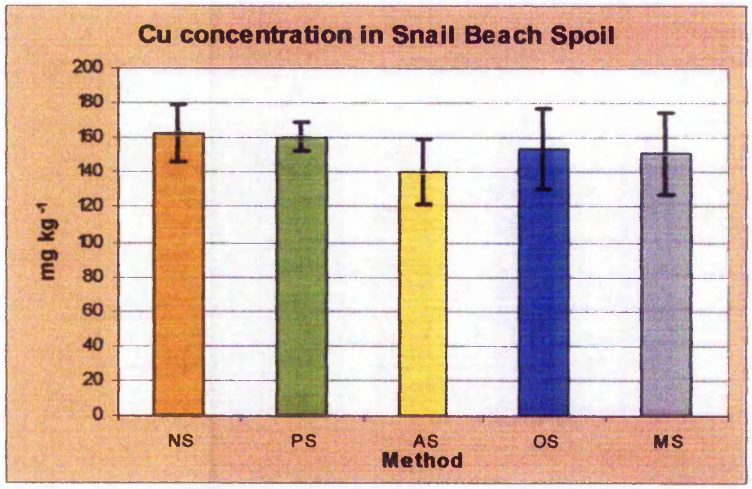


Figure 4.4 Comparison of copper concentration obtained by five extraction methods followed by analysis using FAAS on seven replicates from a sample of spoil collected at Snail Beach, Shropshire, UK.

Figure 4.4 indicates that nitric acid digestion is more efficient at extracting copper than the other processes but has a higher variance at a 95% C.I. (±10.38%) than nitric acid/perchloric acid digestion (±5.17%) when applied to Snail Beach spoil.

Figure 4.5 illustrates that nitric acid digestion yields the highest zinc concentration (9144.2 mg kg^{-1}) with the lowest variance in 95% C.I. (±2.4%) for Snail Beach spoil samples followed by nitric acid/hydrogen peroxide digestion with a zinc concentration of 9107.5 mg kg^{-1} and a variance in 95% C.I. of 4.75%.

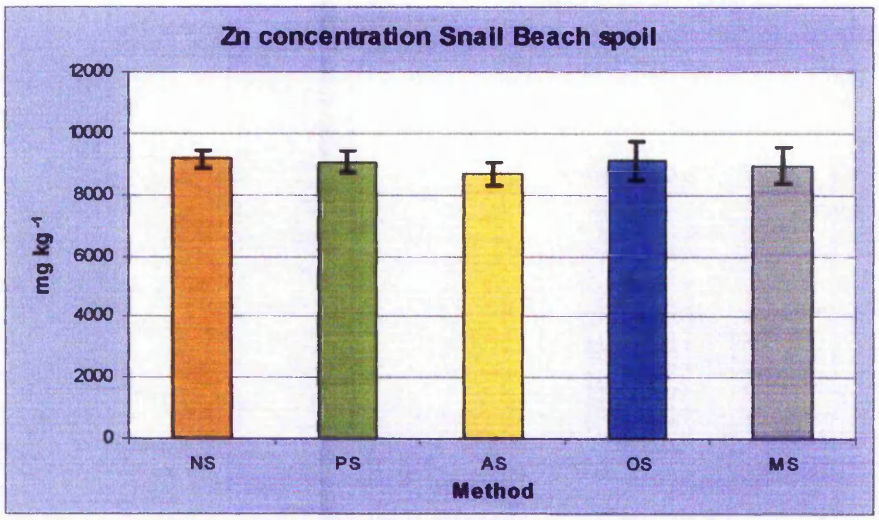


Figure 4.5 Comparison of zinc concentration obtained using five extraction methods followed by analysis using FAAS on seven replicates from a sample of spoil collected at Snail Beach, Shropshire, UK.

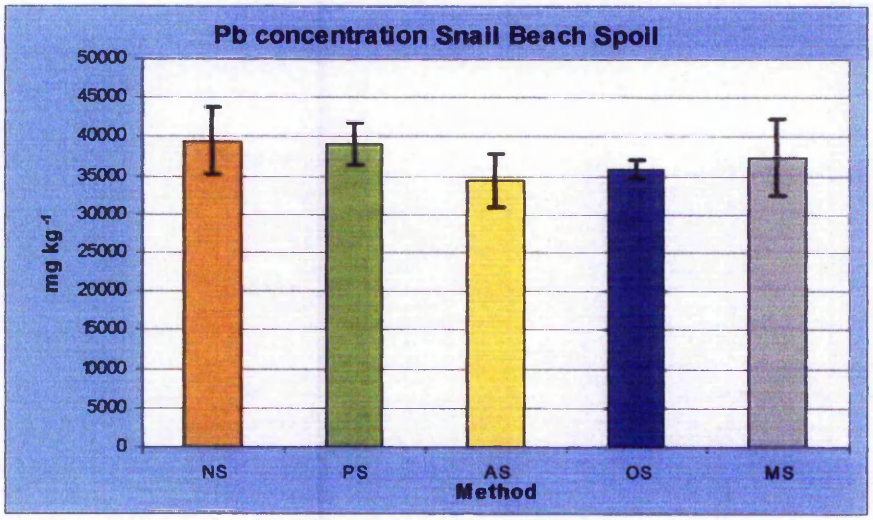


Figure 4.6 Comparison of lead concentration obtained using five extraction methods followed by analysis using FAAS on five replicates from a sample of spoil collected at Snail Beach, Shropshire, UK

Figure 4.6 shows that nitric acid digestion yields the highest concentration of lead (39460.8 mg kg^{-1}) and a 95% C.I. of 7.19%. Nitric acid/perchloric acid digestion gave

a marginally lower lead concentration (39121 mg kg^{-1}) but an improved variance in 95% C.I. of 4.59%.

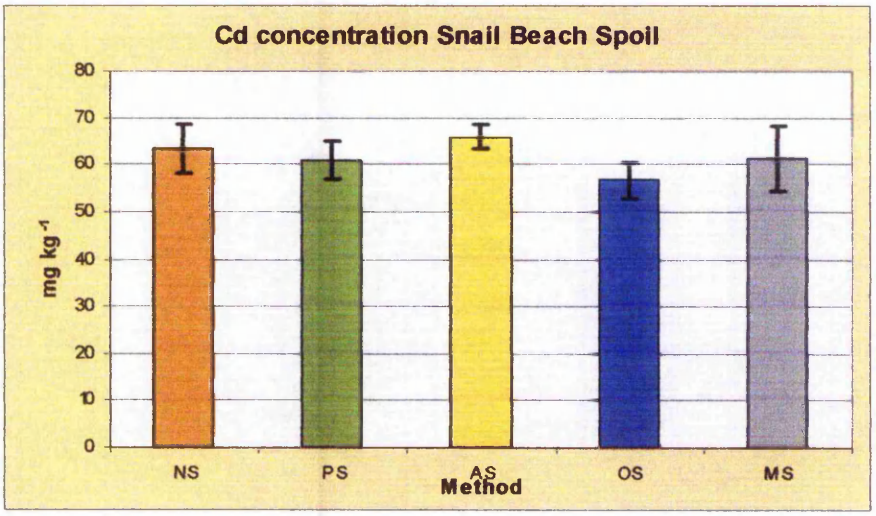


Figure 4.7 Comparison of cadmium concentration obtained using five extraction methods followed by analysis with FAAS on seven replicates from a sample of spoil collected at Snail Beach, Shropshire, UK.

Figure 4.7 indicates that aqua regia digestion produces the highest average concentration of cadmium with average 65.86mg kg^{-1} and 95% confidence interval variance of ± 9.1%. Nitric acid digestion produced a lower cadmium concentration but an improved 95% C.I. variance of ±6.7%. In all of the above cases results from 7 sample replicates were used for each digestion except that of nitric acid where only 6 replicates were finally analyzed.

Figure 4.8 illustrates that aqua regia digestion produced the highest concentration of copper (2045.4 mg kg^{-1}) in Parys Mountain spoil with the smallest 95% C.I. variance.(± 1.25%) Nitric acid/perchloric digestion produced slightly lower average concentration (1948.0 mg kg^{-1}) and a C.I. variance of ± 2.25%. Nitric acid digestion produced the lowest concentration.

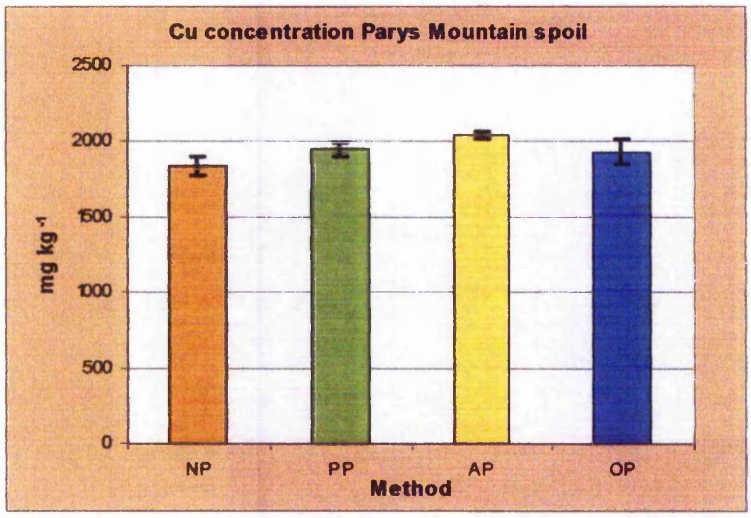


Figure 4.8 Comparison of copper concentration using four extraction methods followed by analysis with FAAS on seven replicates from a sample of spoil collected at Parys Mountain, Anglesey, UK.

Figure 4.9 shows that perchloric acid/nitric acid digestion yields the highest concentration (658.7 mg kg^{-1}) of zinc in Parys Mountain spoil. The result for aqua regia, 645.7 mg kg^{-1} concentration is slightly lower however this extraction method exhibited the lowest 95% C.I. variance of $\pm$ 3.8%.

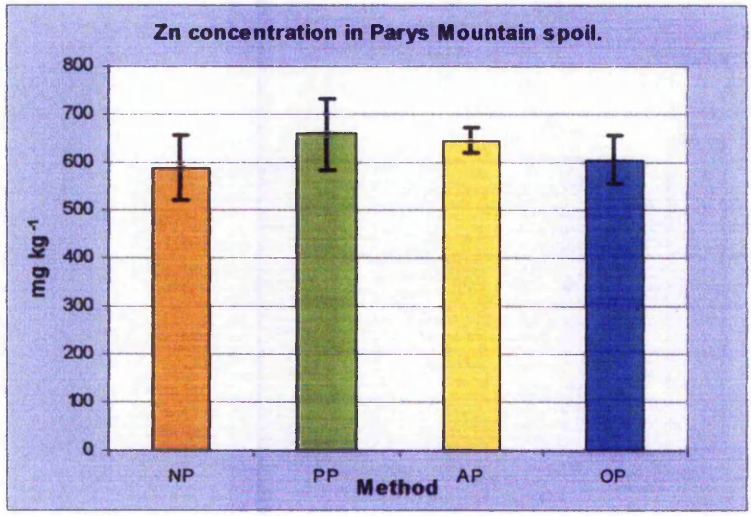


Figure 4.9 Comparison of zinc concentration using four extraction methods followed Anglesey, UK by analysis with FAAS on five replicates from a sample of spoil collected at Parys Mountain,.

Figure 4.10 demonstrates that the average lead concentration in Parys Mountain spoil obtained using all digestion methods give similar average lead concentration (7042.9 -7161.5 mg kg^{-1}). The 95% C.I. variance of 4.7% for nitric acid/hydrogen peroxide is better than the other methods.

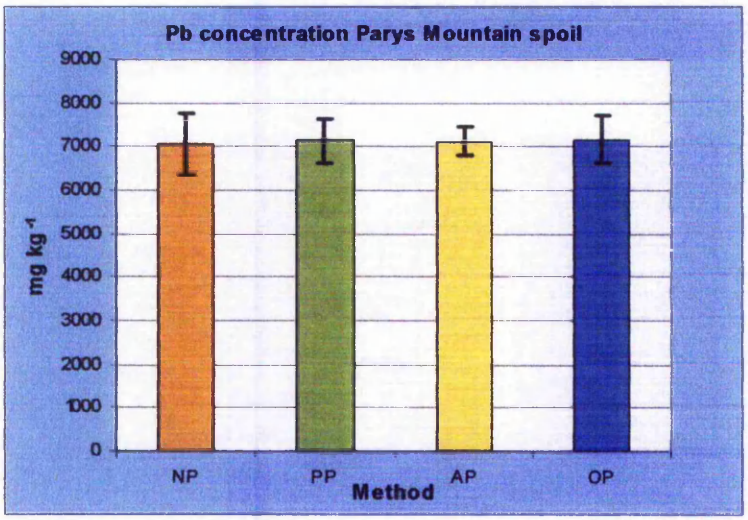


Figure 4.10 Comparison of lead concentration using four extraction methods followed by analysis with FAAS on seven replicates from a sample of spoil collected at Parys Mountain, Anglesey, UK.

Six replicates of nitric acid digestion and nitric acid/perchloric acid digestion were analysed one sample in each series having been spilt. Cadmium concentration in Parys Mountain spoil extracts was below the LOD for the instrument in use.

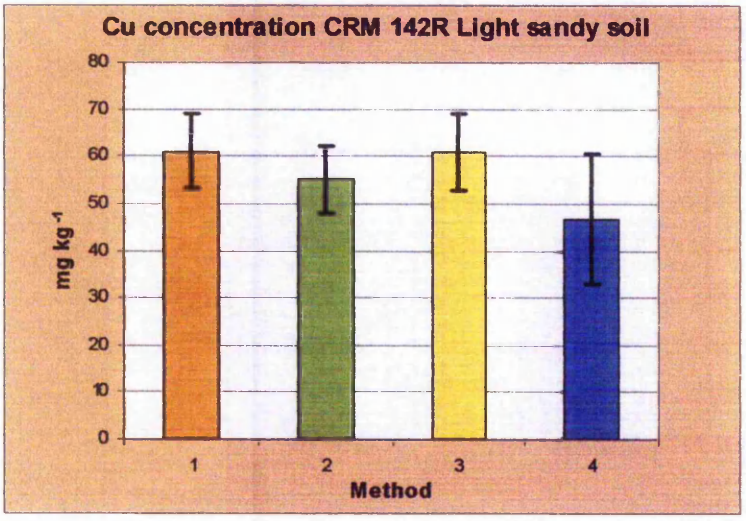


Figure 4.11 Comparison of copper concentration using four extraction methods followed by analysis with FAAS on seven replicates of CRM 142R Light sandy soil certified standard.

Figure 4.11 illustrates that the highest average copper concentration for CRM 142R light sandy soil was obtained using nitric acid digestion (61.09 mg kg^{-1}) closely followed by aqua regia digestion (61.01 mg kg^{-1}). All digestion methods produced

high 95% confidence interval variances suggesting lack of homogeneity in this aliquot of spoil.

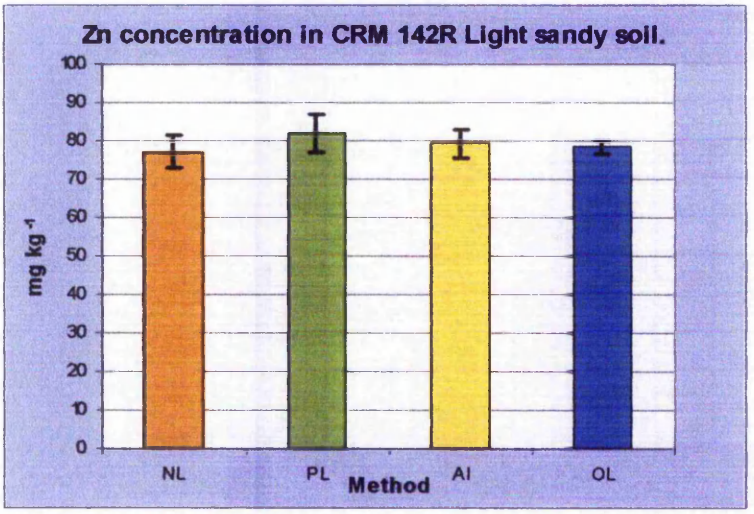


Figure 4.12 Comparison of zinc concentration using four extraction methods followed by analysis with FAAS on seven replicates of CRM 142R Light sandy soil certified standard.

Figure 4.12 indicates that the highest average zinc concentration in CRM 142R Light sandy soil was obtained using nitric acid/perchloric acid digestion (81.9 mg kg^{-1}) with a 95% C.I. variance of $\pm$ 6.2%. However the other methods are all close to this average concentration. Aqua regia 79.3 mg kg^{-1}, nitric acid 77.1 mg kg^{-1} and nitric acid/hydrogen peroxide 78.3 mg kg^{-1}. The 95%C.I. variance for nitric acid/hydrogen peroxide digestion of zinc in CRM 142R light sandy soil is lowest at $\pm$ 4.6%.

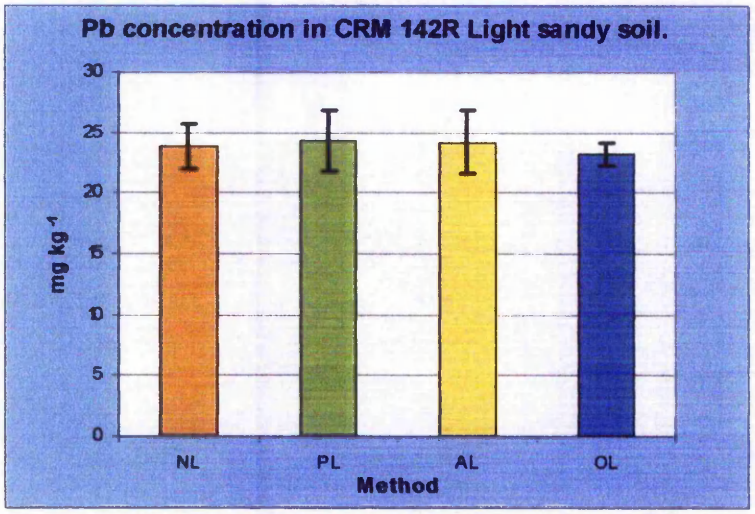


Figure 4.13 Comparison of lead concentration using four extraction methods followed by analysis with FAAS on seven replicates of a sample of CRM 142R Light sandy soil certified standard.

Figure 4.13 shows that the highest average lead concentration in CRM 142R Light sandy soil is obtained using nitric acid/perchloric acid digestion (24.9 mg kg^{-1}) very closely followed by aqua regia acid digestion (24.3 mg kg^{-1}). 95% CI value is also similar.

Cadmium was not detected in any of the extracts prepared with CRN 142R Light sandy soil.

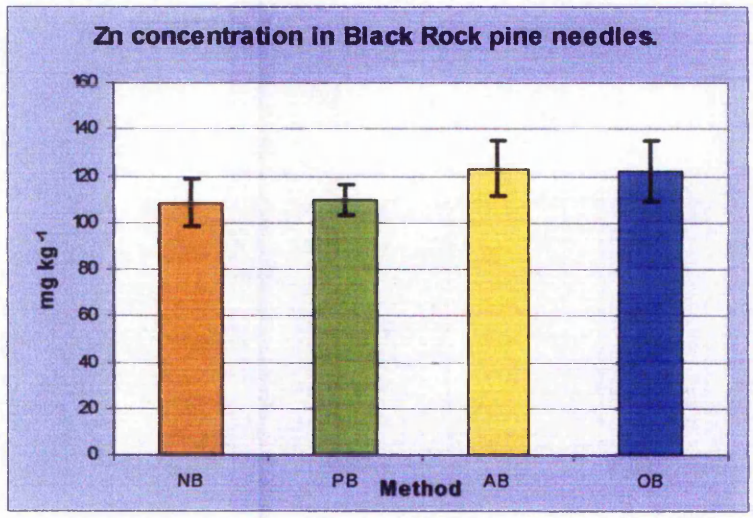


Figure 4.14 Comparison of zinc concentration using four extraction methods followed by analysis with FAAS on seven replicates of a sample of pine needles collected at Black Rocks, Derbyshire, UK

Figure 4.14 illustrates that aqua regia digestion extracts the highest average zinc concentration in Scots pine needles and a C.I. variance of 9.5%

The results of seven replicate samples of Scots pine needles were used for each determination of metal concentration except for the nitric acid/perchloric acid digestion where six replicates were successively completed without method error.

Figure 4.15 demonstrates that lead was present in the pine needles collected at Black Rocks but in very low concentrations. Old lead mines surround the area, however, and the results are included for completeness. Aqua regia digestion exhibits the highest lead concentration (13.5 mg kg^{-1}) but all 95% CI values are high (2.2 – 6.1) due to the low concentration values in the extract.

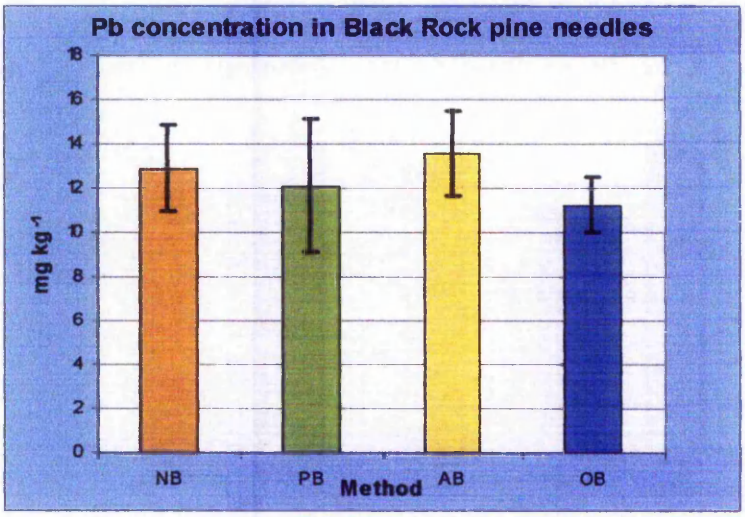


Figure 4.15 Comparison of lead concentration using four extraction methods followed by analysis with FAAS on seven replicates of a sample of pine needles collected at Black Rock, Derbyshire, UK.

Figure 4.16 shows the highest zinc concentration (1966.3mg kg^{-1}) in hazel leaves from Snail Beach is obtained using nitric acid/perchloric acid digestion and a 95% C.I. variance of ± 4.04%. Aqua regia although producing the lowest zinc concentration (1814.0mg kg^{-1}), resulted in the lowest 95% C.I. variance of ±2.81%.

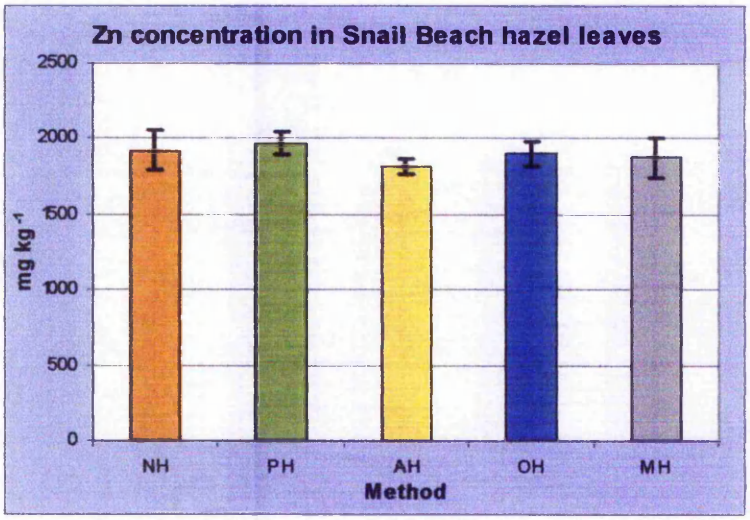


Figure 4.16 Comparison of zinc concentration using five extraction methods followed by analysis with FAAS on seven replicates of a sample of hazel leaves collected at Snail Beach, Shropshire, UK.

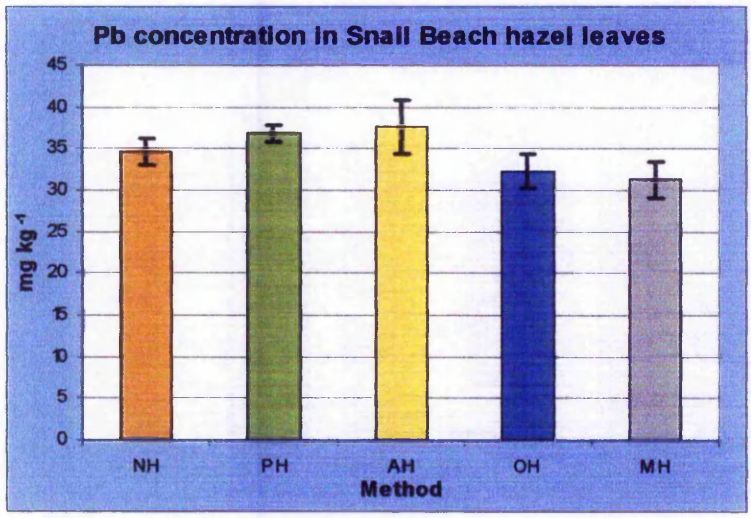


Figure 4.17 Comparison of lead concentration using five extraction methods followed by analysis with FAAS on seven replicates of a sample of hazel leaves collected at Snail Beach, Shropshire, UK.

Figure 4.17 illustrates that the highest lead concentration in hazel leaves from Snail Beach is obtained using aqua regia extraction.

Figure 4.18 indicates that the highest average concentration of zinc in CRM 101 Spruce needles reference material is produced by aqua regia digestion (31.9mg kg^{-1}) with a 95% C.I. variance of ±8.2%. Nitric acid digestion has the lowest average zinc concentration and the highest 95% C.I. variation.

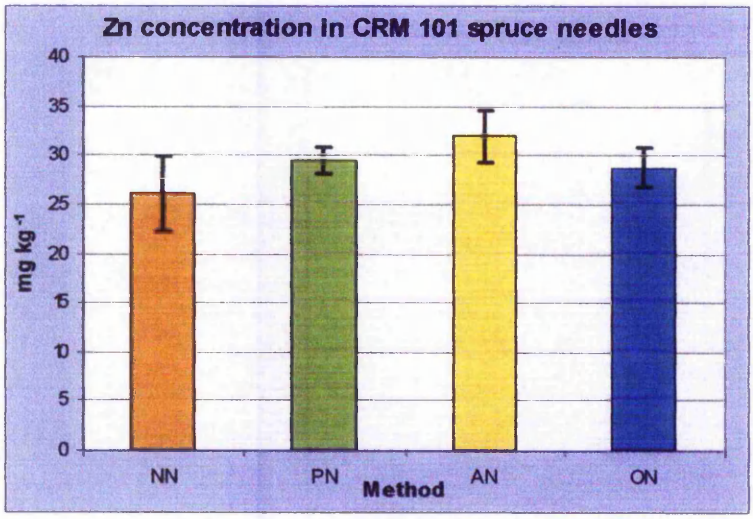


Figure 4.18 Comparison of zinc concentration using four extraction methods followed by analysis with FAAS on seven replicates of a sample of CRM 101 Spruce needles.

Limit of detection and limit of quantitation.

LOD and LOQ values obtained during this experiment calculated as outlined in SOP 3.7.1 were expressed in absorption units. These were subsequently converted into metal concentrations and are shown in Table 4.1

Metal	LOD absorption	LOD mgL^{-1}	% of linear range	LOQ absorption	LOQ mgL^{-1}	% of linear range
Copper	0.0041	0.09	4.53	0.0135	0.31	15.4
Zinc	0.0067	0.04	3.83	0.0189	0.11	11.0
Lead	0.0017	0.19	3.80	0.0053	0.57	11.5
Cadmium	0.0072	0.05	9.15	0.0224	0.14	27.3

Table 4.1 Mean LOD and LOQ concentrations calculated from calibration curves produced over a period of 3 weeks using flame atomic absorption spectrophotometry. Percentage of the total calibration linearity is also indicated.

The LOD and LOQ for copper, zinc and lead are similar as a percentage of the linear range however cadmium exhibits a higher percentage of linearity.

Absorption values equivalent to the LOD concentrations in Table 4.1 were considered to be undetectable using this particular FAAS instrument and those below LOQ concentrations are considered to be unquantifiable but indicate a presence which may be quantifiable by other analytical methods.

Relative standard deviation

Table 4.2 shows the mean relative standard deviations, determined as in SOP 3.7.2 obtained for each target metal from each extraction method and each analyte.

Extraction method	**Mean relative standard deviation %**			
	Copper	**Zinc**	**Lead**	**Cadmium**
Nitric acid	10.8	8.4	9.1	20.1
Nitric/perchloric acids	7.2	5.4	9.9	14.9
Aqua Regia	10.2	6.3	9.4	11.8
Nitric acid/hydrogen peroxide	17.5	7.0	10.2	11.3

Table 4.2 Mean relative standard deviation for each target element using different extraction reagents

Accuracy

The Q.A. standards for extraction analysis were CRM 142R Light Sandy Soil and CRM 101 Spruce needles and accuracy values, determined as in SOP 3.7.3, are shown in Table 4.3

Sample	Cu mean mg kg^{-1}	Cu A%	Zn mean mg kg^{-1}	Zn A%	Pb mean mg kg^{-1}	Pb A%
CRM 142R	69.8	-	93.3	-	25.7	-
NL	61.1	87.52	77.1	82.7	23.9	92.96
PL	55.0	78.87	81.9	87.8	24.3	94.47
AL	61.0	87.41	79.3	85.0	24.2	94.12
OL	46.7	66.86	78.3	84.0	23.2	90.12
CRM 101	5.00*	-	35.3	-	2.8**	-
NN	<LOD	-	26.1	73.8	<LOQ	-
PN	<LOD	-	29.5	83.6	<LOQ	-
AN	<LOD	-	31.9	90.40	<LOQ	-
ON	<LOD	-	28.7	81.4	<LOQ	-

Table 4.3 Accuracy % for determination of metal concentrations in extracts as a result of four different digestion methods.

* Indicative values obtained by participating laboratories but not certified

** Additional value recorded by some of the participating laboratories but not certified

Analysis of variation (ANOVA)

Two-way ANOVA was carried out as described in SOP 3.5.5, comparing mean metal concentration with extraction method with each metal detected for each of the six substrates using Minitab release 13.30 (19). The difference between the four acid digestion methods was not statistically significant at the 5% level of probability for each of the six substrates analysed however microwave assisted digestion was significantly different in the case of hazel collected at Snailbeach.

Discussion:

The analysis of soil and vegetation samples is difficult due to the variability of the substrate under examination, homogeneity of the prepared samples being the most critical factor. The Community Bureau of Reference (1994) make much of this point

in the preparation and supply of there certified samples of soil and also in 1990 with reference to their certified samples of spruce (*Picea abies*) needles.

The generally high level of relative standard deviation (Table 4.2) obtained from each series of replicates is a result of several processing factors not least of which is the heterogeneity of the samples under examination and the processing of the samples before digestion. US EPA(1998) method used for soil and sediment preparation requires separation large stones etc from the original sample by passing through a 2mm sieve however particles of inert base rock will still pass through to the grinding process and may or may not be ground as finely as other metal bearing components in the matrix.

The potential errors when processing mineral and biological samples can be represented as:-

$$P_T = P_S + P_{SD} + P_H + P_I + P_C$$

where $_T$ = Total

$_S$ = Sample variation

$_{SD}$ = Sample digestion

$_H$ = Extraction handling

$_I$ = Instrument

$_C$ = Calculation

In the case of acid digestion the error factor should ideally not exceed 4% and the error due to instrumentation, in this case Flame Atomic Absorption Spectrometry, is unlikely to exceed 1%. Statistically significant errors arising from calculation would only arise when multiplication factors of 10^6 are involved, which in these determinations will be irrelevant. Therefore the major potential in this experiment are indeed as a result of sample variation.

Similarly the sum of standard deviation can be represented as:-

$$S_T = \sqrt{S_S^{\,2} + S_{SD}^{\,2} + S_H^{\,2} + S_I^{\,2} + S_C^{\,2}}$$

SD was used to calculate 95% confidence intervals using Student *t*-test, which in turn, was used to assign the error bars on the bar charts used in the result section. Smaller deviations would have resulted in smaller variances in 95% confidence intervals. The use of more replicates could have reduced the value of SD and also reduced the 95% C.I. values since the Student *t* test formula used uses $\sqrt{n}$ as the quotient.

The main factor affecting this investigation is that of errors arising from sample preparation and subsequent handling. In order to facilitate handling and storage facilities only 500mg of soil and 250 mg of vegetation was used in each replicate. Clearly larger quantities of sample used together with subsequent larger volumes of extract would reduce the digestion and handling errors.

Since two-way ANOVA examination showed there was no significant difference between each extraction method, it was decided that the criteria used for determining the most efficient extraction method would be a combination of maximum mean concentration of metal and minimum 95% C.I. in conjunction with accuracy determinations carried out on the reference materials.

Despite the variation in 95% C.I. and relative standard deviation within each extraction method and each substrate it is proposed that meaningful observations can be made. All results for metal concentrations obtained by each extraction method are of similar orders of magnitude and the variation in standard deviation obtained is in the order of those obtained by other workers. Indeed Pueyo *et al.*, in 2001, highlighted the problems encountered when interlaboratory studies were undertaken in an endeavor to certify polluted river sediment. As well as standard deviation variation between laboratories, some 40% of results obtained were unusable. Analysis by X-ray fluorescence on control samples of copper mine wastes (Kruckeberg and Wu, 1992) illustrates large variations in concentrations observed for copper and zinc. Pederson *et al.*(2000) obtained large standard deviations (±55 mg kg^{-1} on means of 105 mg kg^{-1}) using 10 replicates for copper concentrations in *Fallopia convolvulus* shoots grown in copper contaminated soils.

The results obtained for each sample are satisfactory, however the lead concentration of Parys Mountain spoil is surprising since extraction of lead is not recorded during the active period of mining at this site (Rowlands, 1966). All extraction methods and replicates, however, produced lead concentrations of the same order of magnitude.

It was decided that the criteria used for determining the most efficient extraction method would be a combination of maximum mean concentration of metal and minimum 95% C.I. in conjunction with accuracy determinations carried out on the reference materials.

The use of microwaves with the various digestion methods on Snail Beach spoil and hazel leaves collected at Snail Beach (see Figures 4.1 – 4.4 and Figure.4.13 – 4.14) did not improve the extraction process with the exception of cadmium in Snail Beach spoil. In all cases the standard deviation and 95% C.I. was one of the highest in that particular extraction.

The most efficient extraction method was aqua regia as shown in Figure 4.16. Aqua regia was particularly effective when used to digest vegetation. When used to digest spoil from Snail Beach it was least effective except in the case of cadmium extraction.

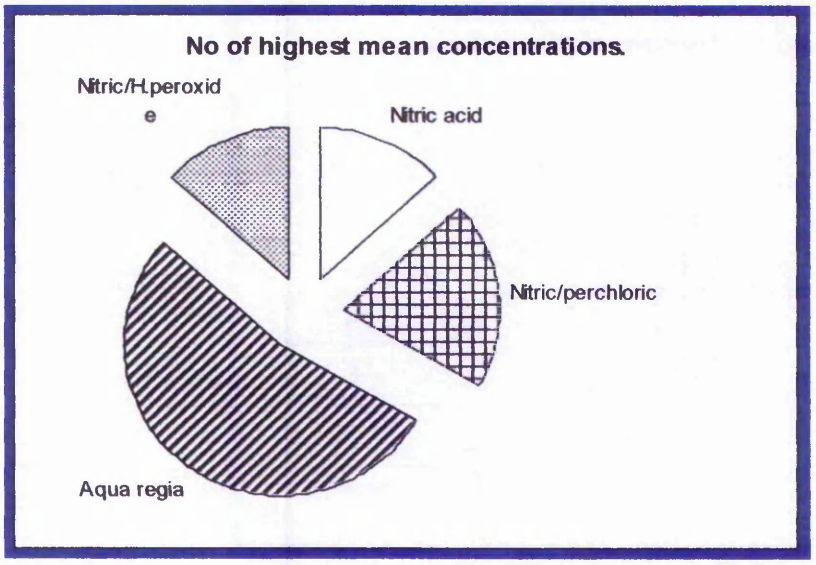


Figure 4.16 Proportion of most efficient means obtained with each digestion method on each substrate

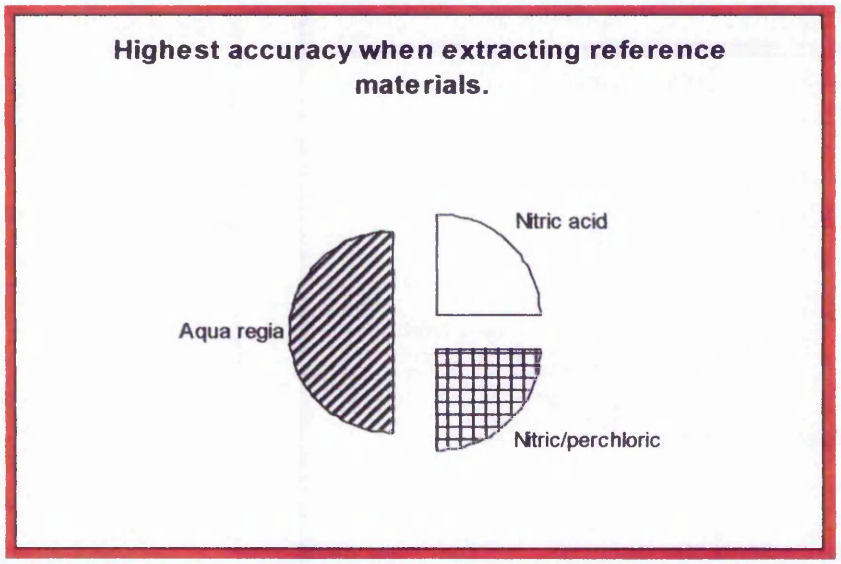


Figure 4.17 Proportion of most accurate (A%) recorded by the various extraction methods used with CRM 142R Light sandy soil and CRM 101 Spruce needles reference materials.

Figure 4.17 illustrates that aqua regia digestion exhibits more accurate results when compared with other methods used. Accuracy data is not included relative to copper, lead and cadmium extractions by all reagents on CRM 101 Spruce needles since the absorption values obtained for copper were below the calculated LOD and those for lead were marginally above the LOD but below the LOQ. No signal was detected for cadmium in CRM101.

Reviewing the above data demonstrates that the suitability of individual digestion methods vary according to the metal under examination and the substrate being analyzed. In the case of soils metal distribution and the degree of binding to individual phases in the soil matrix varies as does the speciation of the metals within the matrix. Lead is predominantly bound to carbonates, oxides and residual components and to a lesser extent to the organic fraction. Copper is bound mainly to the residual fraction and zinc is bound to oxides and to a lesser extent carbonates and residual. Cadmium shows an almost even split between oxides and residual fractions. (Murphy *et al.*, 2000). The origin of the soil and the type of ore deposits in the area will affect the extractability of the metals under examination; however the most successful general extractant was aqua regia.

Pyatt *et al.*, (2000), showed that metals can be accumulated in vegetation by factors of 12 times greater for lead and 5.5 times greater for copper compared with surrounding soil concentrations; additionally root and leaf concentrations can vary considerably and by different concentrations from species to species. This feature of bioaccumulation processes can affect the selection of the amount of material to be digested; however, this study has shown that aqua regia is the most successful extractant for vegetation.

Conclusion

Despite the problems associated with sample collection, preparation and extraction discussed above, the author recommends the use of the aqua regia digestion method outlined as a general purpose process for the digestion of soil and vegetation samples. Standard operating procedures for aqua regia open extraction are appended to end of this chapter.

Sample collection and transportation procedures adopted in this study were satisfactory, however sample preparation and subsequent handling could be improved. The drying procedures employed appear to be sufficient but the grinding process needs to be improved for both soil and vegetation samples. The use of a micronizer, similar to those use for the preparation of pigments and extenders for the paint industry will be investigated in an extension of this work. Particle sizes <50 microns would be produced and this should improve homogeneity in the handling, storage and digestion processes.

The quantity of sample used for each determination should be increased to 1.0g of soil and 0.5g of vegetation as a minimum requirement and the quantity of reagents increased accordingly. The final volume of extractant produced would also be increased to 50mL improving accuracy by reducing processing losses.

The number of replicates of each sample used in the process should be increased to a minimum of ten to produce more meaningful statistical data as well as improving precision.

The use of microwave assistance for digestion in open conditions had no time saving effect when used with nitric acid and was considered to possess no advantage in this type of digestion process. Further work using pressurised microwave extraction vessels will be investigated later in this research.

4.2 Standard operating procedure for extraction for subsequent aqueous analysis using FAAS or ICP.AES techniques.

4.2.1 Soil

1.0 g ± 0.05 g of soil, prepared according to SOP 3.3.1, was weighed into a 50mL conical flask. 10 mL of aqua regia was added and the flask simmered for 90 minutes on a hotplate in a fume cupboard. The flask was removed, cooled and a further 4 mL of aqua regia were added. The flask was simmered for a further 30 minutes before cooling and filtering through Whatman 541 filter paper into a clean 50mL graduated flask. The 50 ml conical flask, filter paper and funnel were washed through thoroughly and the final volume made up with 0.5M HNO_3. The extract was transferred to a HDPE bottle and stored in a refrigerator at 5^0C until required for analysis.

4.2.3 Vegetation

0.5 g ± 0.025g of vegetation prepared as outlined in SOP 3.3.2 to 3.3.7 was weighed into a 50 mL conical flask. mL of aqua regia was added and the flask simmered for 90 minutes on a hotplate in a fume cupboard. The flask was removed, cooled and a further 4 mL of aqua regia were added. The flask was simmered for a further 30 minutes before cooling and filtering through Whatman 541 filter paper into a clean 50mL graduated flask. The 50 mL conical flask, filter paper and funnel were washed through thoroughly and the final volume made up with 0.5M HNO_3. The extract was

transferred to a HDPE bottle and stored in a refrigerator at 5^0C until required for analysis.

As a result of this evaluation, it was decided that overall aqua regia extraction over a period of two hours on a hot plate produced the most precise and accurate results and will be used throughout this research.

Chapter 5.

Evaluation of a novel method for the preparation of soil samples for subsequent acid digestion and analysis for heavy metals.

The use of a pestle and mortar is time consuming, tiring and a possibly inconsistent method of grinding dried and sieved soil an before extraction and subsequent analysis for heavy metals. An evaluation of an alternative mechanised method of preparation was undertaken.

Introduction.

The preparation of sediment, soil and spoil samples is detailed in many research papers and text books. Radojevic and Bashkin, 1999, suggest drying, grinding and sieving through 2mm sieve as do Koptsik *et al.*, 2003. Drying and sieving through a 2mm mesh on an 'as received' basis has been used (De Gregori *et al.*, 2003, Bech, *et al.*, 1997, Kruckerberg and Wu, 1991, Pederson *et al.*, 2000). Other workers have dried soil, sediment and spoil samples and sieved them to produce particle sizes of <107μm (Veinott, *et al.*, 2003). Some researchers have passed the dried samples through a 2mm sieve, ground the resultant material in various ways then sieved again through sub 74μm sieves (Arias *et al*, 1998. Del Rio *et al.*, 2002. Cid *et al.*, 2001. Tokalioglu *et al.*, 2003). Achievement of some degree of homogeneity is the essential objective of all of these processes, however the use of sieves <2mm appears to defeat this objective since some of the sample will be rejected. The USEPA in 1998, indicated that sieving grinding and mixing should be undertaken to ensure homogeneity of soil and sediment samples. The Community Bureau of Reference (1994) and Pueyo *et al.* (2001) followed sieving through a 2mm mesh with grinding in a hammer mill to produce a particle size of less than 90μm, followed by intensive mixing to produce homogeneity.

The reduction of particle size without rejecting constituent material would lead to more consistent samples for analysis and would possibly improve the extraction of target heavy metals in a standardised acid digestion procedure.

Earlier work by the author produced a procedure for the extraction of heavy metals from soil and spoil samples (Wilson *et al.*, 2005A) using the internationally recognised process of using a 2mm sieve after drying (Radojevic and Bashkin, 1999) followed by grinding for 5 minutes in a clean agate pestle and mortar. The resultant samples were stored in HDPE bottles and these were shaken for 5 minutes before aliquots were withdrawn for acid digestion.

The aim of this investigation was to compare the efficiency and precision of this process with a novel approach to grinding the <2mm particles in a stainless steel micronising mill powered by a supply of dry air or gas such as nitrogen, a procedure which could be used in most general environmental or analytical laboratories.

The spoil under examination was collected on the site of an old lead mine, adjacent to Black Rock, Cromford, Derbyshire, U.K.. Black Rock is composed of Ashover Grit (Brown, 1981) but the area immediately west of the outcrop consists primarily of limestone together with the spoil consisting mainly of galena and associated minerals mined from the Dovegang vein (Slack, 2000). The location of Black Rock is shown in Figure 4.3

Materials and Methods.

Sample Collection and Preparation.

Spoil was collected in accordance with SOP 3.3.1 as far as sieving through a 2 mm sieve. Sample reference BR2P was ground in a clean pestle agate and mortar for 5 minutes and the resultant powder transferred to a clean HDPE bottle.

Sample reference BR2M was passed through a clean stainless steel Atritor Micronising Mill Model M2 fitted with a 4 holed 36 TCD (Atritor Ltd, PO Box 101, Edgwick Park Industrial Estate, Canal Road, Coventry, CV6 5RD, UK). The

microniser can be operated using any dry gas at a pressure of 7 bar and supply rate of 0.6 m^3min^{-1}. The processing time for 200g was approximately 4 minutes. Table 1 indicates the particle size distribution obtained using both methods measured on a Malvern Instruments Ltd, laser analyser fitted with a MS-64 Dry Powder Feeder

10 replicates of each sample were extracted as outlined in SOP 4.2.1 and analysed using FAAS as detailed in SOP 3.6.1.

Soil solutions used for the measurement of pH vary from 5g of dried sieved soil and 96.5mL demineralised water (Yukselen and Alpaslan, 2001) to 1:2 soil weight:water volume (De Gregori *et al*, 2003). The method used for this investigation was SOP 3.4.1 and the average of 3 replicates was determined.

Results

The micronised sample has a much lower particle size than the sample produced with a pestle and mortar (Table 5.1 and Figure 5.1). Spoil ground using the microniser consisted of 100 % below 100 μm and 95% below 50 μm. The product produced using a pestle and mortar consisted of only 53% below 100 μm and 39% below 50 μm. This sample also contained 2% of particles in excess of 500 μm.

Particle Diameter μm	Percentage under Particle Diameter	
	BR2P	BR2M
125.0	58.95	100
100.0	53.01	99.96
75.0	47.04	98.62
50.0	39.14	95.18
25.0	28.59	92.66
9.0	17.16	80.39
4.5	9.53	51.7
2.0	2.65	15.59

Table 5.1 Particle size distribution obtained using pestle and mortar (BR2P) and Atritor M2 Micronising Mill (BR2M)

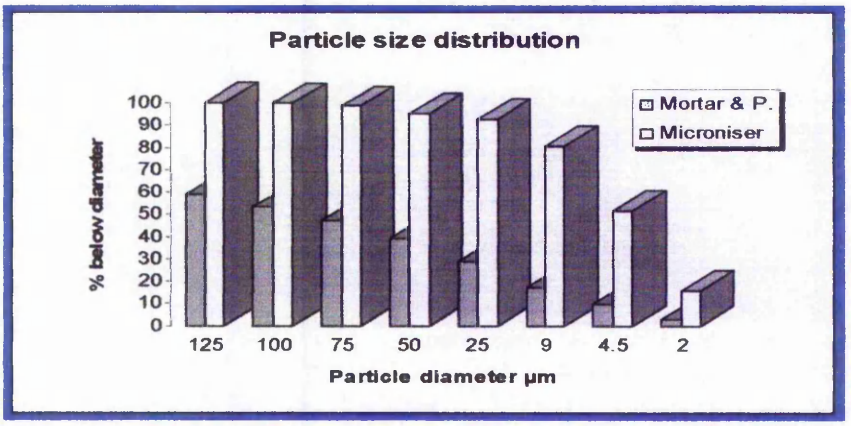


Figure 5.1. Particle size distribution of Black Rock spoil samples ground using a pestle and mortar and an Atritor Microniser

Microniser Evaluation Samples	Conc Cu mg kg^{-1}	Conc Zn mg kg^{-1}	Conc. Pb mg kg^{-1}	Conc Cd mg kg^{-1}
Micronised replicates				
BR2M/1	202.7	42743	38187	297.3
BR2M/2	151.9	43768	70577	325.8
BR2M/3	151.6	43674	55197	344.2
BR2M/4	194.0	43959	42013	312.0
BR2M/5	197.1	44737	59488	317.5
BR2M/6	175.3	40083	58428	340.8
BR2M/7	149.1	41915	71149	310.3
BR2M/8	178.9	42032	77029	328.9
BR2M/9	203.4	42887	40231	346.5
BR2M/10	196.1	44451	66496	306.2
Mean	180.0	43025	57880	323.0
Standard Deviation	22.11	1405.71	13901.70	17.03837
Relative S.D. %	12.28	3.27	24.02	5.275404
95% C.I. ±	15.81	1005	9943	12.19
Mortar and Pestle replicates				
BR2P/1	239.0	40674	63359	337.0
BR2P/2	195.8	38969	54972	359.0
BR2P/3	202.0	40205	37809	360.8
BR2P/4	249.3	42438	46664	410.2
BR2P/5	249.7	42494	29203	449.9
BR2P/6	244.1	39420	47593	334.7
BR2P/7	273.9	35855	47592	340.2
BR2P/8	319.7	38718	37396	338.1
BR2P/9	242.7	38126	56770	380.6
BR2P/10	202.2	38130	45420	380.2
Mean	241.9	39503	46678	369.0
Standard Deviation	37.26	2038.97	10123.26	37.47827
Relative S.D. %	15.40	5.16	21.69	10.15471
95% C.I. ±	26.65	1458	724.0	26.8

Table 5.2. Metal concentrations in replicates of alternatively prepared samples of dried spoil from Black Rock, Derbyshire

Table 5.2 shows the concentrations of Cu, Zn, Pb and Cd determined in 10 replicates of samples from each grinding process together with other statistical information. Figure 5.2 illustrates the mean metal concentrations obtained using each grinding method and 95% confidence intervals.

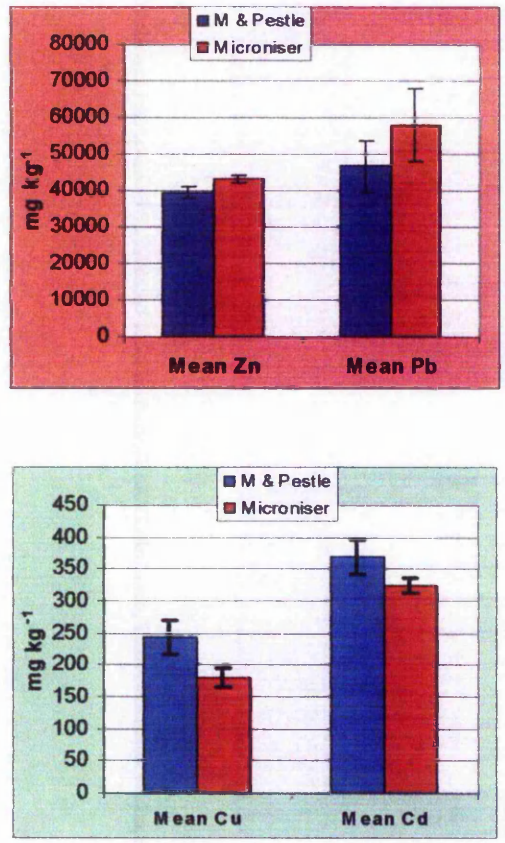


Figure 5.2 Metal concentrations in spoil samples prepared by micronising and grinding with a mortar and pestle. Error bars represent 95% confidence intervals using SOP 3.7.6.

Sample	pH	Conductivity μScm^{-1}
Micronised	7.54	701
Pestle & mortar	7.53	298

Table 5.3 Mean pH and conductivity readings obtained from samples of micronised and mortar and pestle ground spoil from Black Rocks

The pH and conductivity (average of 3 readings for each sample) are shown in Table 5.3. Values for pH are very similar but conductivity readings obtained for the micronised sample are higher than the pestle and mortar ground sample.

Discussion

The grinding of spoil samples in the Atritor Micronising Mill is much easier than the use of a mortar and pestle and the cleaning of the equipment is comparable in terms of ease and time.

Figure 5.2 indicates that micronised samples appear to contain higher concentrations of Zn and Pb but lower concentrations of Cu and Cd. The relative standard deviations obtained from all replicate analyses with the exception of Pb are reduced when micronised material is used. RSD (Table 5.2) is a comparator of the precision of results (Miller and Miller, 2000) and lower values indicate improved precision.

The use of a two tailed *F*-test to evaluate precision (Miller and Miller, 2000) showed that there was no significant difference, at the 5% probability level, between the two methods when Cu, Zn and Pb were determined. However, the variance of the mortar and pestle method was significantly greater than the micronised method at the 5% probability level i.e. the microniser method was more precise for Cd.

The heterogeneity of soils is a combination of several factors (Sparks, 1995, Yaron *et al*, 1996, Wilson *et al.*,2005). Differences in adsorptive properties of the many constituents of soil in vicinity (Streit and Stumm, 1993), topography and climate affecting the rate of leaching (Hewitt and Allot, 1997) will be the major cause of the variation in soil collected around Black Rock.

Mean pH values determined for micronised and pestle and mortar ground spoil were the same (Table 5.3). Not surprisingly the spoil is slightly alkaline due to the limestone present.

Mean conductivity readings of solutions obtained from the alternatively prepared samples were however notably different (Table 5.3). Since conductivity is a measure of the concentration of soluble salts (Rowell, 1994), the process of micronising spoil has resulted in an increase in this parameter. The production of finer particles appears to have released more soluble salts from the spoil constituents, in this case from the limestone aggregates and metal ores in the spoil.

It would appear that extended mixing of the ground material by rolling or continuous rotation of containers will be necessary to produce greater homogeneity in the ground spoil samples. Factors such as particle shape, bulk density and composition affect the homogeneity of soil and sediment samples especially in relation to heavy metal content (Community Bureau of Reference, 1994). Therefore, if homogenisation could be achieved then storage stability of the micronised material would be greater than the pestle and mortar ground material due to the lower particle size and much reduced size distribution.

This method requires further investigation but appears to facilitate the preparation of dried soils for acid digestion and subsequent analysis. However since the initiation of this evaluation, management changes at the company resulted in capital costs from £4500 to £11000 and a payment scheme for further trials, therefore it was decided to terminate any further trials.

Chapter 6

Critical evaluation of a pressurised microwave extraction system for the determination of selected heavy metal concentrations in soil and vegetation

Shortly after this research commenced, a pressurised microwave system became available and it was decided to carry out and compare the current USEPA microwave digestion process with the process evaluated and adopted as SOP 4.2.1 and 4.2.2.

Introduction

Pressurised microwave extraction of environmental samples is frequently used as preparation for analysis using FAAS, ICP-OES, ICP-MS and other techniques where an aqueous solution is introduced into the instrument. The extraction process is often referred to as a mineralisation procedure (Hoenig *et al.*, 1998, Polkowska *et al.*, 2000). Most environmental samples have been extracted using this technique particularly when heavy metal concentrations are being determined. A variety of materials have been extracted using pressurised microwave extraction including soil using nitric acid (Martinez *et al.*, 1999), aqua regia (Davidson *et al.*, 1998) and complex acid combinations including hydrofluoric acid (Mierzwa *et al.*, 1998). Heavy metals have also been extracted from plant tissue (Krachler *et al.*, 2002), particulates (Massadeh and Snook, 2002) and animal tissue (Wasilewska *et al.* 2002) using this method.

Processing times and pressures applied vary and in an attempt to standardise the technique initially for soil extraction, the USEPA (1998) developed a procedure, SW3051 Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils (www[2]) apparently in collaboration with CEM Corporation, Mathews, North Carolina. The procedure uses a specially designed microwave oven containing a total of 12 reaction vessels operating at a maximum pressure of 200 psi.

Many claims have been made in research papers that the use of microwave digestion either in open or closed systems have reduced extraction (or mineralization) times for soil in particular. Wilson *et al.* in 2005A demonstrated that microwaves applied to open extraction procedures, were less efficient than longer extraction times on a hot plate for both soil and vegetation samples. Of course the problem of heterogeneity in environmental samples such as soil and vegetation still remains and this process cannot overcome the problem, however any time saving procedure would be welcomed by researchers processing many samples. Additionally, claims have been made that the use of pressurised microwave extraction also increases the efficiency of metal extraction when compared with open or reflux extraction methods using the same acid or combination of acids.

This evaluation aims to determine the suitability of pressurised microwave digestion for soil and vegetation mineralization and to compare the procedure with an open digestion technique evaluated and recommended for soil and vegetation by Wilson *et al.* (2005A).

Materials and Methods

Seven soil samples from different locations and climatic zones were selected together with five varying vegetation samples. The soil samples used varied in metals concentration, pH and conductivity and were sourced in Arizona, British Columbia and Anglesey. Soil samples were collected and prepared in accordance with SOP 3.3.1. Vegetation samples used comprise of a mixture of leaves, needles and florets and were collected and prepared as in SOP 3.3.2 and 3.3.4.

Pressurised microwave extraction was carried out using a CEM model MDS 2000, capable of processing up to 12 PTFE extraction vessels simultaneously. USEPA method SW3051 is pre-programmed into the equipment and control is maintained by the software in the machine via a temperature and/or pressure probe fitted to one of the reaction vessels as illustrated in Figure 6.1. It is assumed using this technique that all other reaction vessels processed at the same time behave similarly. The equipment

used in this evaluation was fitted with the pressure probe only and this was considered satisfactory by the manufacturers when consulted.

0.5 g ± 0.012g of twelve soil and vegetation samples was weighed into each reaction vessel and 14 mL of Certified grade nitric acid SG 1.42 was added to the vessel. The vessels were sealed using pressure covers fitted with a 200 psi rupture membrane. Processing time outlined in USEPA method SW3051 was 10 minutes at a pressure setting of 110 psi., however, after initial digestions for this time period was undertaken, pressure readings on the machine did not exceed 50 psi therefore it was decided to process samples for 15 minutes for this evaluation. Pressure values for the soil samples used did not exceed 65 psi. however in the case of vegetation samples the pressure very quickly reached 110 psi. At this point the microwave generator switched off until pressure reduced to 100 psi at which point the microwave generator engaged once more. This cycling sequence continued throughout the 15 minute processing period. At the end of the processing period, vessels were left for a minimum of 2 hours or until pressure values returned to zero before opening. The extracts were filtered through Whatman 451 filter paper into a 50mL graduated flask. Vessels and filter paper were washed through with 0.1M nitric acid and the volume made up to 50mL with 0.1M nitric acid.

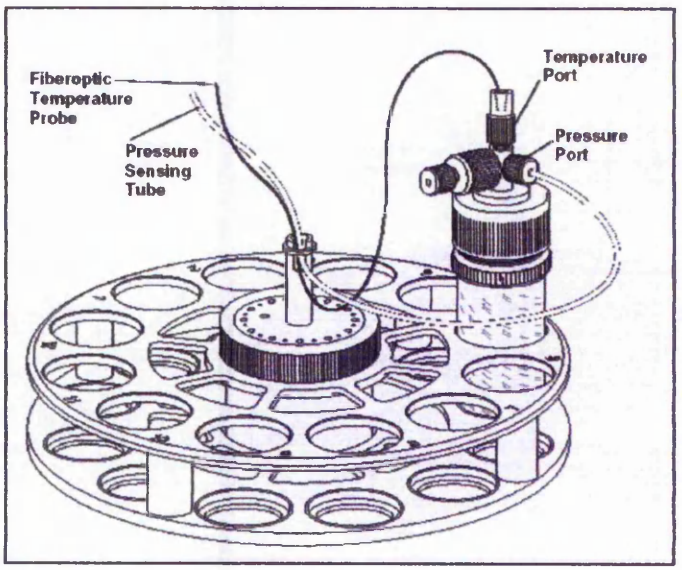


Figure 6.1 Pressure/temperature assembly on MS2000 microwave extraction vessel.

Eight replicates of each sample were processed in the oven simultaneously by placing reaction vessels in the spaces provide in the turntable (Figure 6.1). For comparison purposes eight replicates of each sample were processes in accordance with SOP 4.2.1 and all replicates were analysed using SOP 3.6.1 (FAAS).

Results

Table 6.1 indicates that, when analysed, open digestion produces higher concentrations of copper in all samples whereas lead concentrations in general are higher when pressurised microwave extraction is used. However copper was extracted from all vegetation samples by microwave extraction whilst open extraction yielded detectable copper concentrations in only two out of five of vegetation samples.

Sample	Type	Open Cu mg kg^{-1}	Micro Cu mg kg^{-1}	Open Zn mg kg^{-1}	Micro Zn mg kg^{-1}	Open Pb mg kg^{-1}	Micro Pb mg kg^{-1}
64A3	Soil	42.3	29.6	40.9	34.1	<LOD	11.96
64A5	Soil	26.6	21.8	34.1	31.1	<LOD	11.93
C2700	Soil	407	376	220	201	650	594
15A08	Soil	617	466	378	418.6	455	572
9PM1	Soil	1570	1367	2375	1863	1528	1241
15A01	Soil	47.9	36.1	67.4	72.7	16.7	32.9
SB1	Soil	130	117	9347	9401	33438	42707
9PM25	Vegetation	<LOQ	7.43	15.7	17.85	<LOD	<LOD
9PM06	Vegetation	<LOD	<LOQ	7.1	8.71	<LOD	<LOD
1103C 6	Vegetation	26.0	19.7	32.8	31.8	<LOD	<LOD
1103C 6A	Vegetation	26.0	21.0	41.0	44.7	<LOD	<LOD
BR 01	Vegetation	<LOD	3.27	10.8	135.74	12.9	<LOD

Table 6.1 Mean metal concentration determined using FAAS in selected samples after using open and pressurised microwave extraction techniques

Figures 6.2 and 6.3 illustrate higher copper concentrations and generally lower standard deviations (SD) when open digestion is used.

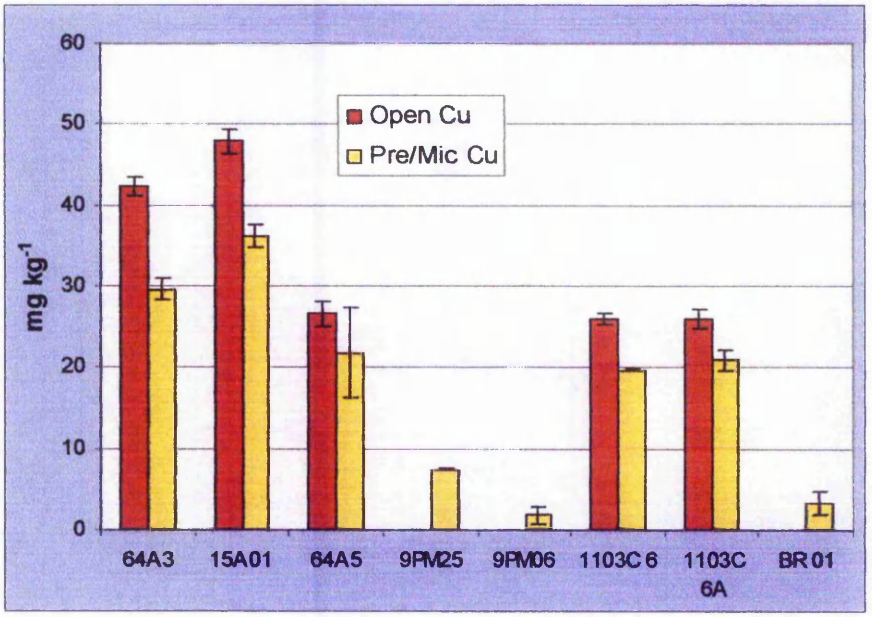


Figure 6.2 Copper concentration in lower value samples extracted using open and pressurised microwave techniques (error bars = SD)

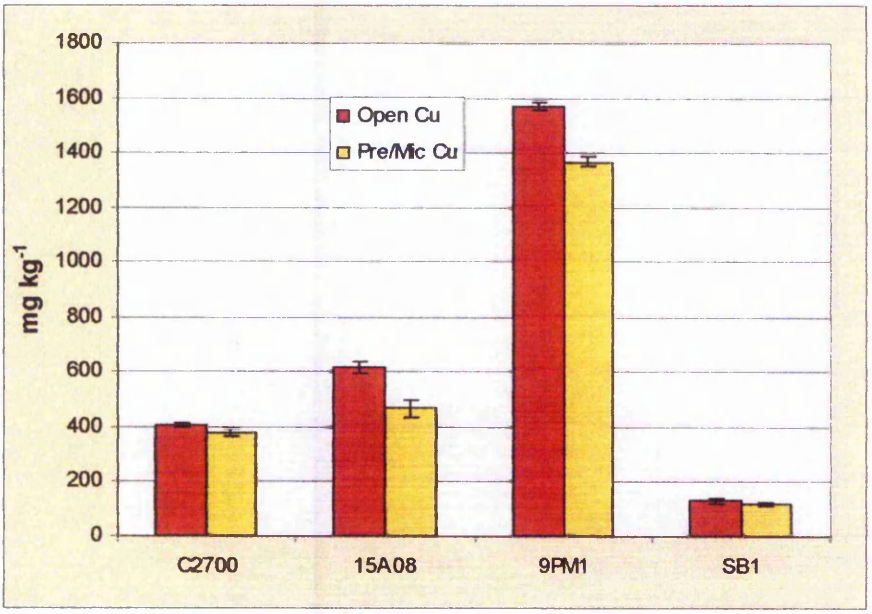


Figure 6.3 Copper concentration in higher value samples extracted using open and pressurised microwave techniques (error bars = SD)

Figures 6.4 and 6.5 show that seven out of twelve of the samples yielded higher zinc concentrations when pressurised microwave extraction was used. This extraction method in particularly yielded higher metal concentrations when used with vegetation samples.

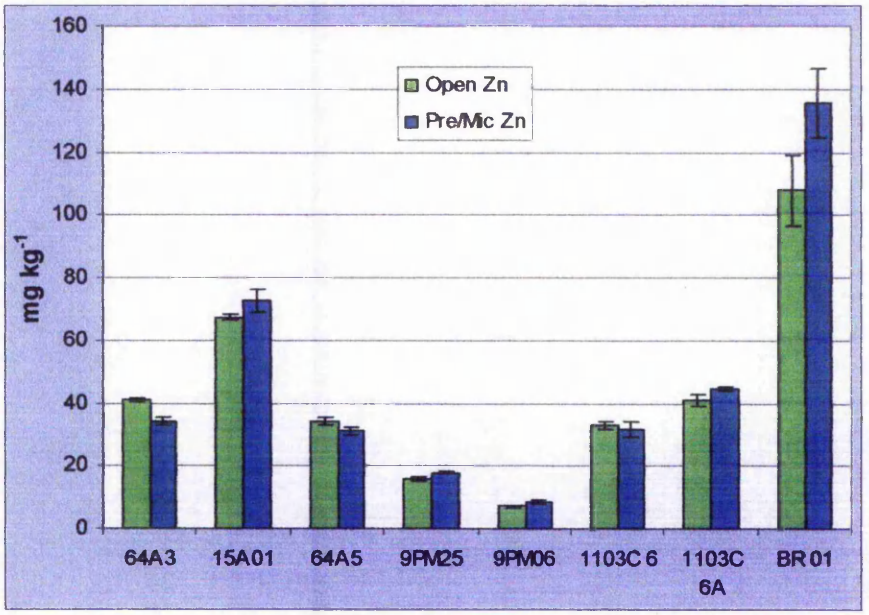


Figure 6.4 Zinc concentration in lower value samples extracted using open and pressurised microwave techniques (error bars = SD)

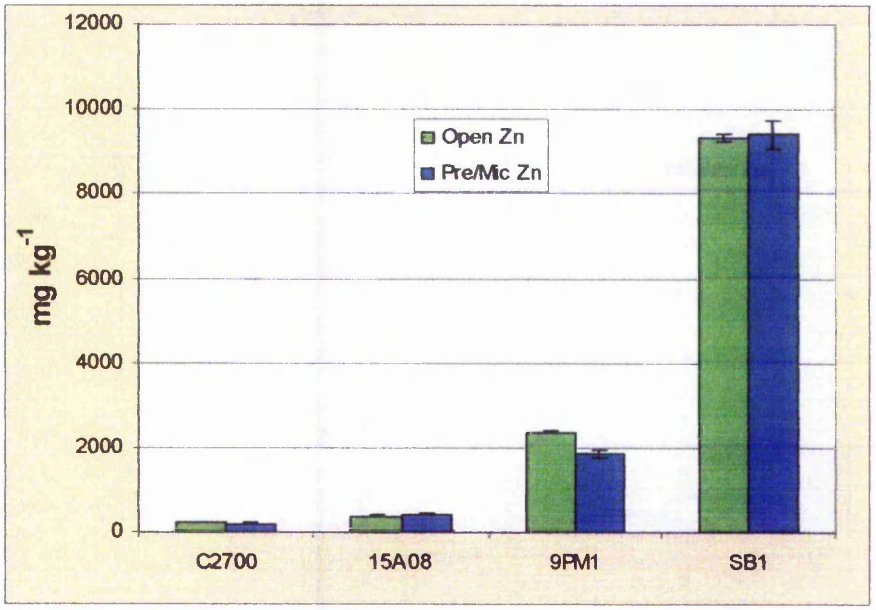


Figure 6.5 Zinc concentration in higher value samples extracted using open and pressurised microwave techniques (error bars = SD)

Tables 6.6 and 6.7 indicate that pressurised microwave extraction yields concentrations of lead in some samples where open extraction failed to produce detectable lead in most cases. SD values however were generally lower when open extraction is used.

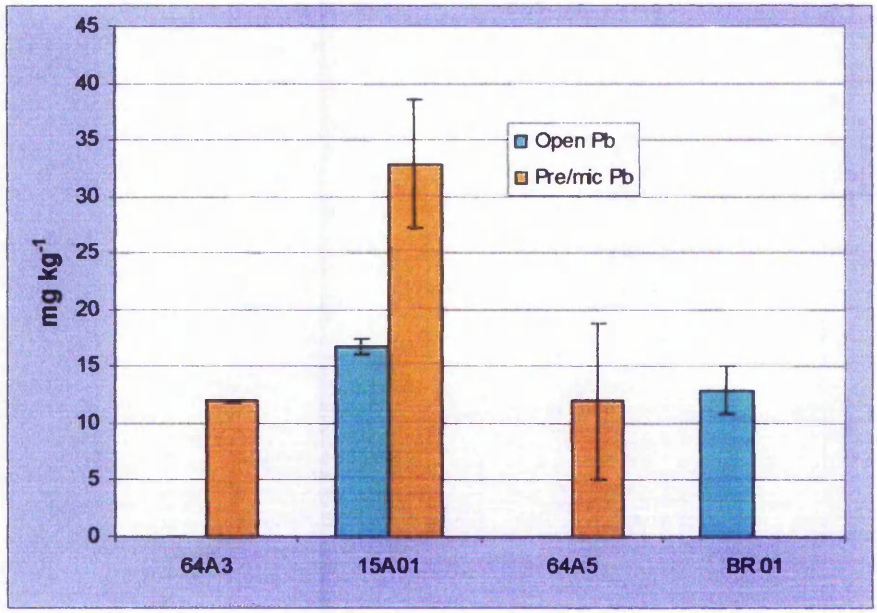


Figure 6.6 Lead concentration in lower value samples extracted using open and pressurised microwave techniques (error bars = SD)

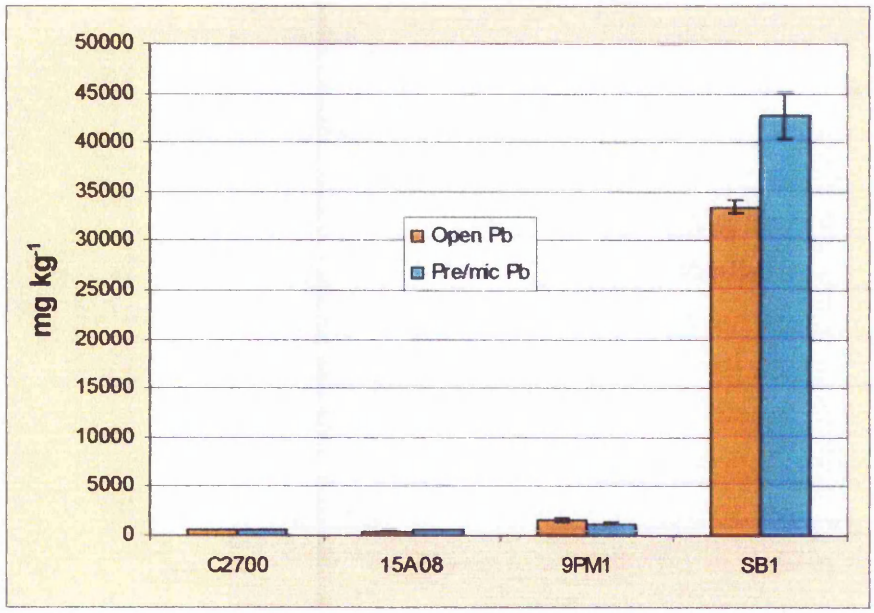


Figure 6.7 Lead concentration in higher value samples extracted using open and pressurised microwave techniques (error bars = SD)

Table 6.2 indicates that relative standard deviation values in the majority of cases were lower when open extraction is used.

Sample	Type	Open Cu RSD %	Micro Cu RSD %	Open Zn RSD %	Micro Zn RSD %	Open Pb RSD %	Micro Pb RSD %
64A3	Soil	2.0	4.4	1.0	3.1	2.0	0.74
64A5	Soil	6.2	25.8	3.5	4.0	-	57.72
C2700	Soil	1.5	3.99	2.4	5.7	1.1	1.92
15A08	Soil	3.8	6.15	2.7	3.73	11.2	7.87
9PM1	Soil	7.52	1.34	1.64	5.48	11.91	3.13
15A01	Soil	3.18	3.99	1.62	4.66	4.13	17.08
SB1	Soil	5.9	4.75	1.0	3.51	2.1	5.49
9PM25	Vegetation	-	0.26	4.4	2.11	-	-
9PM06	Vegetation	-	61.72	0.8	7.49	-	-
1103C 6	Vegetation	2.8	0.39	3.9	7.0	-	-
1103C 6A	Vegetation	4.1	6.18	6.2	1.21	-	-
BR 01	Vegetation	-	42.76	10.5	8.18	-	-

Table 6.2. Relative standard deviation values obtained when pressurised microwave extraction and open extraction was used prior to analysis by FAAS.

Discussion

Method 3051 recommended by USEPA has the apparent advantage that processing time is reduced to 10 minutes before cooling and eventual analysis whereas SOP 4.2.1 for soil and 4.2.2 for vegetation require over 2 hours processing time including a cooling period within the process. Additionally Wasilewska *et al.* in 2002 suggested that the use of pressurised microwaves increased the oxidation efficiency during wet digestion, thus releasing organically complexed trace metals and preventing organic interference during subsequent analysis using FAAS, ICP-OES etc. They also conclude that oxidation efficiency was related to higher temperature produced in the reaction vessels rather than the influence of microwaves *per se* on the extraction process. Polkowska in 2000 produced a microwave assisted procedure which claimed to be more effective than classical open digestion procedure when determining concentrations of cobalt in plant materials.

Table 6.1 shows that copper concentration in all soil samples was higher when open extraction was used, however copper concentration in some vegetation samples was actually determined when open digestion extracts failed to yield quantifiable values. When higher copper concentrations were encountered in vegetation, open digestion produced a higher yield. Table 6.1 indicates that there were variations in efficiency of zinc extraction from soil, four samples with higher estimated organic content yielding

higher concentrations when mineralised using open digestion. Three soil samples yielded higher zinc concentrations when digested using pressurised microwave vessels. In all cases, pressurised microwave extraction was more efficient when determining zinc concentrations in all vegetation samples. Table 6.1 also illustrates that when lead concentrations in soil are low, then pressurised microwave extraction was more efficient; in this situation, however, the soils concerned have an estimated higher proportion of organic components. Conversely, open digestion technique successfully extracted lead from one of the vegetation samples, collected in the vicinity of an old lead mine, whereas pressurised microwave mineralization failed to extract detectable amounts of lead. Examination of Figures 6.2 – 6.7 illustrates generally that standard deviations are lower when open digestion is undertaken, an indication that the spread of replicate values is lower and hence subject to less variance (Miller and Miller, 2000). This is reflected in Table 6.2 where RSD values for open digestion are lower for copper and zinc determinations, hence this technique exhibits more precision than pressurised microwave extraction. The indications of precision, produced by replicate lead determinations, vary within each extraction method.

The use of pressurised microwave extraction as recommended by the USEPA does not appear to be as efficient or precise the process adapted for this research when digesting soil (SOP 4.2.1) however it produces overall similar results when vegetation is extracted when compared with SOP 4.2.2. Nitric acid is used as the extractant when pressurised microwave equipment is used and further evaluation using aqua regia should be undertaken.

Chapter 7

Evaluation of analytical procedures for the determination of concentrations of Cd, Cu, Pb and Zn in various samples associated with mining operations

Although FAAS instrumentation was available regularly for this research it was decided to evaluate other methods used as a matter of course by other researchers in the field of environmental analysis for heavy metals when the opportunity arose.

Introduction.

The determination of trace metal concentrations in soil, vegetation and organisms is an essential process used in environmental monitoring, surveying and research. A review of seventy five recent research papers and reports indicated that a variety of analytical procedures have been used.

Analysis of dried material directly would be an ideal method both for speed and the elimination of much experimental error due to further processing. Shotyk (1996) used X-ray fluorescence spectrometry to directly analyse dried peat samples and Brito *et al.* (2000) used the same method to determine lead concentrations in human bone. Clark, *et al.* (2001) and Pyatt *et al.* (1998) used Energy Dispersive Analytical X-ray to determine a range of elements in soil, ore and vegetation. Novel technology such as laser induced breakdown spectrometry combined with laser induced fluorescence (Hilbk-Kortenbruck *et al.* 2001.) has been used for soil analysis, claiming detection limits of 6.0 $\mu g\ g^{-1}$ for Cd and 17 $\mu g\ g^{-1}$ for Pb.

Wet or dry ashing of prepared samples of soil, sediment or vegetation is used to prepare homogenous aqueous extract for subsequent quantitative analysis. Samples taken from rivers, lakes, ponds etc can be similarly analysed following filtration and stabilisation (Gunderson *et al.* 2001).

Research papers surveyed used the following methods to analyse samples from a combination of soil, ore, sediment, vegetation and animal tissue.

Flame Atomic Absorption Spectrometry (FAAS)	36%
Graphite Furnace Atomic Absorption Spectrometry (GF-AAS)	12%
Inductively coupled plasma atomic emission spectrometry (ICP-AES)	18%
Inductively coupled plasma mass spectrometry (ICP-MS)	14%
X-ray Microanalysis	8%

The advantage of using ICP techniques is that multi-elemental analysis takes place simultaneously whereas with FAAS only single element detection is possible at any one time. These techniques require the preparation of an aqueous extract in order that suitable analyte can be introduced into the instrument.

It was considered necessary to compare the efficiency, accuracy and precision of FAAS, ICP-AES, ICP-MS for the analysis of soils and vegetation digested in aqua regia. Ion chromatography had been used by one team (Bruno et al, 2000) claiming detection limits of 10-15 ppb for Cu and Zn, 30 ppb for Cd and 60 ppb for Pb. Since the current author had used ion chromatography successfully in the past and the technique allows simultaneous determination of Cd, Cu, Pb and Zn, it was decided to include this analytical method in the experiment.

The use of Energy dispersive X-ray microanalysis would also be carried out directly on the samples after drying and grinding.

Materials and Methods

The spoil samples used for the comparison were taken from an old lead mine at Snail Beach, Shropshire, U.K.(see Figure 4.2) and a copper mine in British Columbia,

Canada (see Figure 9.1). Vegetation samples were obtained from trees at different altitudes surrounding the copper mine in British Columbia. Certified reference materials, CRM-101, Spruce needles and CRM-142R Light sandy soil obtained from Community Bureau of Reference, Brussels, Belgium, were included in the programme as a guide accuracy of the methods under examination..

Spoil samples were collected and prepared in accordance with SOP 3.3.1 and spruce (*Picea sitchensis*) needles were collected and prepared in accordance with SOP3.3.2.

Approximately 4.0g of spoil (2.0g of vegetation) sample was weighed into a 100 mL conical flask and 28.0mL of aqua regia (made by adding 150mL of 32% HCl to 50mL of 70% nitric acid) added and the flask left overnight. The flasks were then simmered on a hotplate (100-105^{0}C) for 90 minutes. After cooling a further 10.0mL of aqua regia was added and the flasks simmered for a further 30 minutes before cooling and and filtering through Whatman 541 paper into a 200mL graduated flask. All equipment was washed through and made up to the mark with 0.1M nitric acid. Four aliquots for each sample were prepared in this way and mixed together in a previously cleaned 1L HDPE bottle. 10 x 25mL aliquots of each solution were placed in clean 30mL HDPE bottles and these were used as replicates for each analytical determination. Extraction procedures were in line with SOP 4.2.1 and 4.2.2 and scaled up to produce sufficient analyte for comparison purposes.

Material for EDAX examination was taken from bulk spoil and spruce needle samples after drying and grinding etc at the same time as samples were taken for extraction and prepared for analysis as in SOP 3.6.3.

Analytical Equipment

1. FAAS. Analyses were carried out using SOP 3.6.1.

2. ICP-AES. Thermo Tracescan operating sequentially and measuring emissions in radial configuration (WSP Ltd., Blenheim Industrial Estate, Bulwell, Nottingham)

3. ICP-MS . Fissons Model PQ2 Turbo (Institute of Geography and Land Studies, University of Wales, Aberystwyth.)

4. EDAX. SOP 3.6.3 was used to carry out the analysis collecting data from ten different locations in the field of vision.

5. Ion chromatography. Procedure carried out as in SOP 3.6.4

Results.

Table 7.1 contains the metal concentrations and RSD values where possible for each procedure together with certified and indicative values, where appropriate, for both reference materials. EDAX analysis using this instrument detected quantities of target metals in only two samples and in these cases did not indicate target metal presence in all locations in the field of vision. Ion chromatography produced extremely variable results with high RSD values (up to 55.8%). Further examination and statistical analysis of results from these two techniques was not undertaken.

Figure 7.1 illustrates the high result obtained for zinc concentration in CM1 spoil by ICP-MS analysis and largest SD values for copper and zinc. Figure 7.2 shows the variable quality of comparative results between all of the analysis techniques examined. Figure 7.3 again demonstrates a high value for zinc concentration in CRM 142R soil reference material obtained from ICP-MS analysis again with higher SD values.

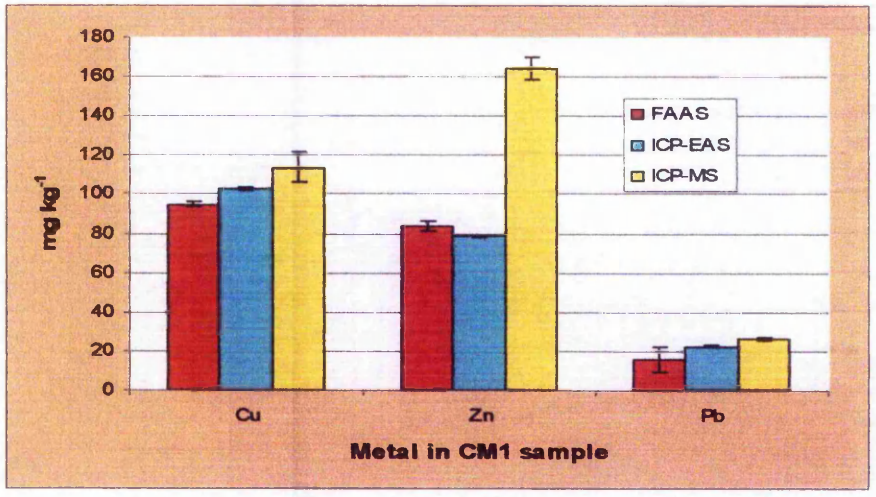


Figure 7.1 Metal concentration in CM1 spoil determine using different analytical techniques (error bars represent standard deviation)

Sample	Metal data	FAAS	ICP-AES	ICP-MS	Ion Chrom.	EDAX %	Certified values
CM1 spoil	Cu Mean mg kg^{-1}	94.89	102.8	113.9	126.8	ND	
	Cu RSD %	1.39	0.73	7.90	26.1		
	Zn Mean mg kg^{-1}	83.96	78.7	163.9	40.11	ND	
	Zn RSD %	3.02	0.58	5.87	35.0		
	Pb mean mg kg^{-1}	16.13	22.81	26.43	130.0	ND	
	Pb RSD %	41.21	1.68	3.51	55.8		
	Cd mean mg kg^{-1}	<LOD	<LOD	0.180	ND	ND	
	Cd RSD %			9.78			
SB2 spoil	Cu Mean mg kg^{-1}	130.2	132.8	183.5	103.0	ND	
	Cu RSD %	5.89	0.757	4.59	27.0		
	Zn Mean mg kg^{-1}	9347.0	10500.0	9041.0	885.0	0.36	
	Zn RSD %	0.98	0.63	5.46	14.1		
	Pb mean mg kg^{-1}	33285	32613	4337.0	194.3	0.21	
	Pb RSD %	2.10	1.38	31.53	9.89		
	Cd mean mg kg^{-1}	64.75	68.14	119.9	ND	ND	
	Cd RSD %	5.01	1.91	7.66			
LR3 CRM 142R	Cu Mean mg kg^{-1}	61.87	76.39	90.63	43.0	0.023	69.8*
	Cu RSD %	2.65	1.68	4.23	56.0		1.43
	Zn Mean mg kg^{-1}	90.66	84.4	189.41	94.8	ND	93.33
	Zn RSD %	1.35	1.23	3.38	23.7		3.64
	Pb mean mg kg^{-1}	21.51	29.79	31.96	156.3	ND	25.72
	Pb RSD %	15.39	1.23	3.87	26.6		6.45
	Cd mean mg kg^{-1}	<LOD	0.439	0.523	ND	ND	0.25
	Cd RSD %		4.34	7.78	ND		2.40
CV4 shore spruce	Cu Mean mg kg^{-1}		3.17	4.50	19.99	ND	
	Cu RSD %		8.23	4.95	14.5		
	Zn Mean mg kg^{-1}	12.67	12.84	28.61	27.12	ND	
	Zn RSD %	9.34	1.08	14.39	11.0		
	Pb mean mg kg^{-1}	<LOD	2.06	5.09	41.21	ND	
	Pb RSD %		23.3	9.74	11.4		
	Cd mean mg kg^{-1}	<LOD	0.110	0.208	ND	ND	
	Cd RSD %		19.73	8.79			
C225 spoil spruce	Cu Mean mg kg^{-1}	<LOQ	8.56	12.30		ND	
	Cu RSD %		1.56	3.45			
	Zn Mean mg kg^{-1}	12.69	13.19	27.97	5.54	ND	
	Zn RSD %	4.49	0.74	2.43	26.0		
	Pb mean mg kg^{-1}	<LOD	2.95	4.35	1.21	ND	
	Pb RSD %		11.2	8.38	17.8		
	Cd mean mg kg^{-1}	<LOD	0.055	0.110	ND	ND	
	Cd RSD %		58.94	12.67			
NM6 CRM 101	Cu Mean mg kg^{-1}	<LOQ	3.97	5.83	14.01	ND	4.9
	Cu RSD %		2.68	5.68	51.3		4.08
	Zn Mean mg kg^{-1}	31.25	30.58	59.94	28.42	ND	35.53*
	Zn RSD %	2.56	0.8	5.99	9.74		5.09
	Pb mean mg kg^{-1}	<LOQ	3.65	7.72	43.18	ND	2.6*
	Pb RSD %		8.48	20.06	34.12		7.69
	Cd mean mg kg^{-1}	<LOD	0.330	0.596	ND	ND	0.34*
	Cd RSD %		5.84	7.99			2.94

Table 7.1 Metal concentrations of each sample obtained with each analysis method and certified or indicative concentrations of reference samples together with respective relative standard deviations (ND = not detected)

*Indicative values included in certification reports.

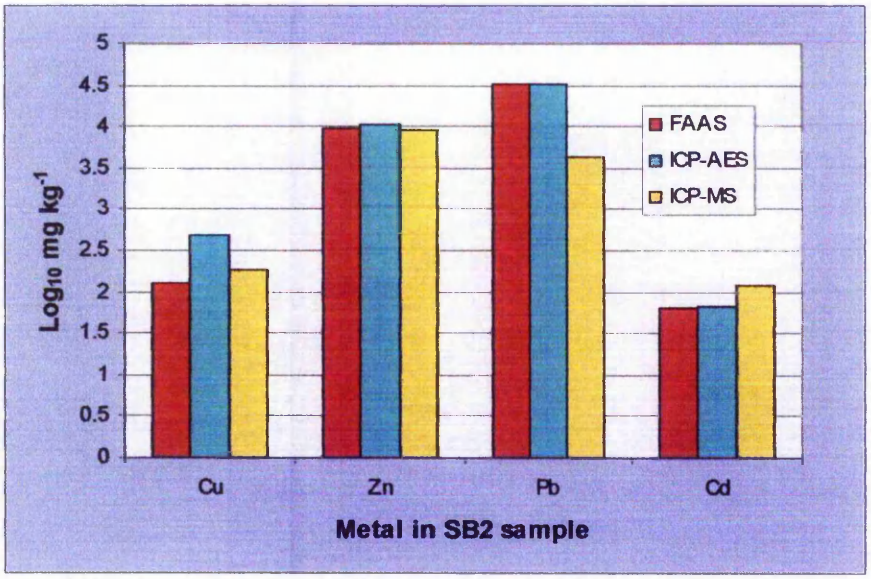


Figure 7.2 Log_{10} of metal concentration in SB2 spoil determine using different analytical techniques.

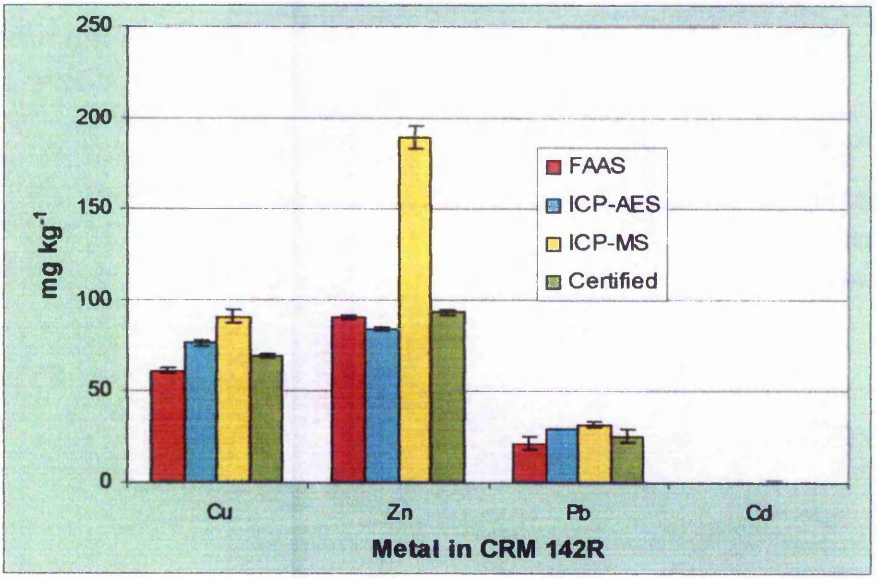


Figure 7.3 Metal concentration in CRM 142R Light sandy soil determine using different analytical techniques together with certified or indicative values from BCR report (error bars represent standard deviation)

Figure 7.4 indicates Cu and lead concentrations were below the LOQ for FAAS instrumentation used and ICP-MS recorded a relatively high zinc concentration (28.6 mg kg^{-1}) in CV4 spruce needles.

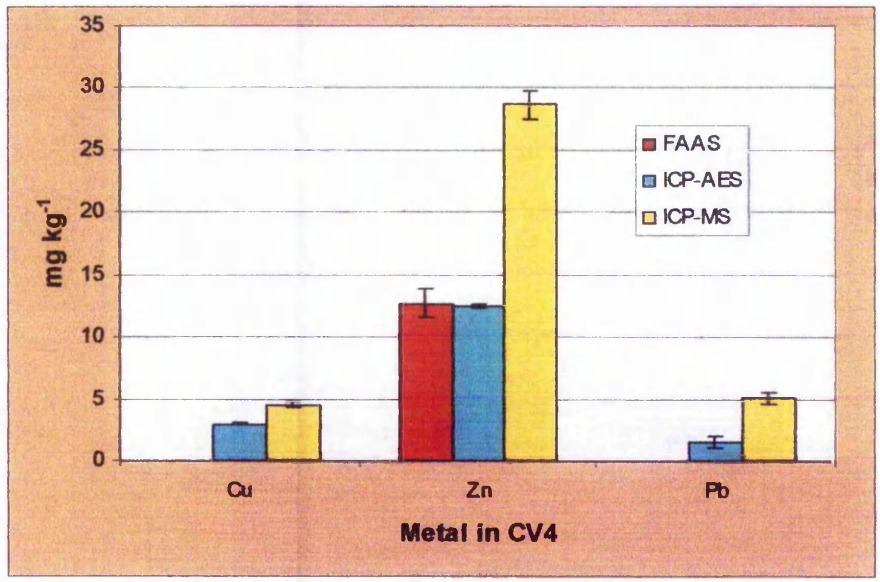


Figure 7.4 Metal concentration in CV4 spruce needles determine using different analytical techniques (error bars represent standard deviation)

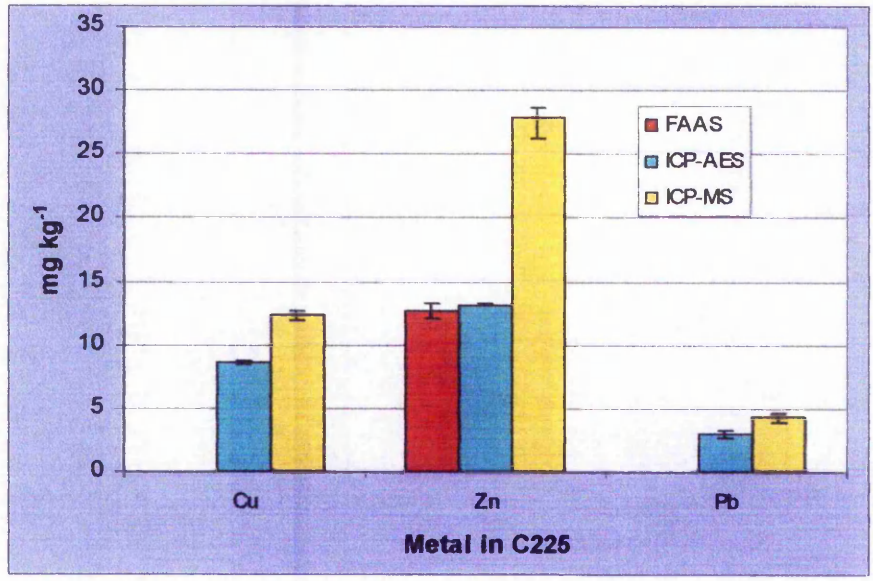


Figure7.5 Metal concentration in C225 spruce needles determine using different analytical techniques (error bars represent standard deviation)

Figure 7.5 indicates that FAAS did not quantify copper and lead concentrations in C225 spruce needles.

Figure 7.6 shows that ICP-AES achieved lower results than certified values but exhibited lower SD values. ICP-MS zinc concentration in CRM 101 spruce needles is again high (59.9 mg kg^{-1}).

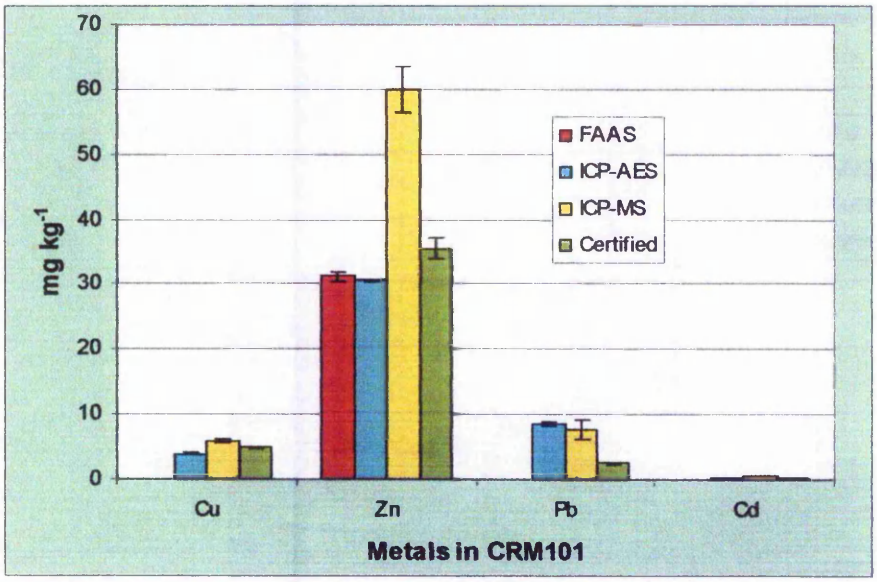


Figure 7.6 Metal concentration in CRM 101 Spruce needles determine using different analytical techniques together with certified or indicative values from BCR report (error bars represent standard deviation)

Two way ANOVA

Since the analytical data obtained using ion chromatography and EDAX was of poor quality, these methods were not subject to further examination. When FAAS, ICP-AES and ICP-MS were subjected to ANOVA as in SOP 3.7.5, it was apparent that there was no statistically significant difference between FAAS and ICP-AES at 5% probability level however ICP-MS was found to be significantly different.

Figure 7.7 is typical of the interaction plots obtained for each individual analyte and in statistical terms there was no significant difference between FAAS, ICP-AES and certified analysis of CRM 142R Light sandy soil; ICP-MS however did differ statistically. Similar conditions apply when CRM 101 Spruce needles are analysed (Figure 7.8)

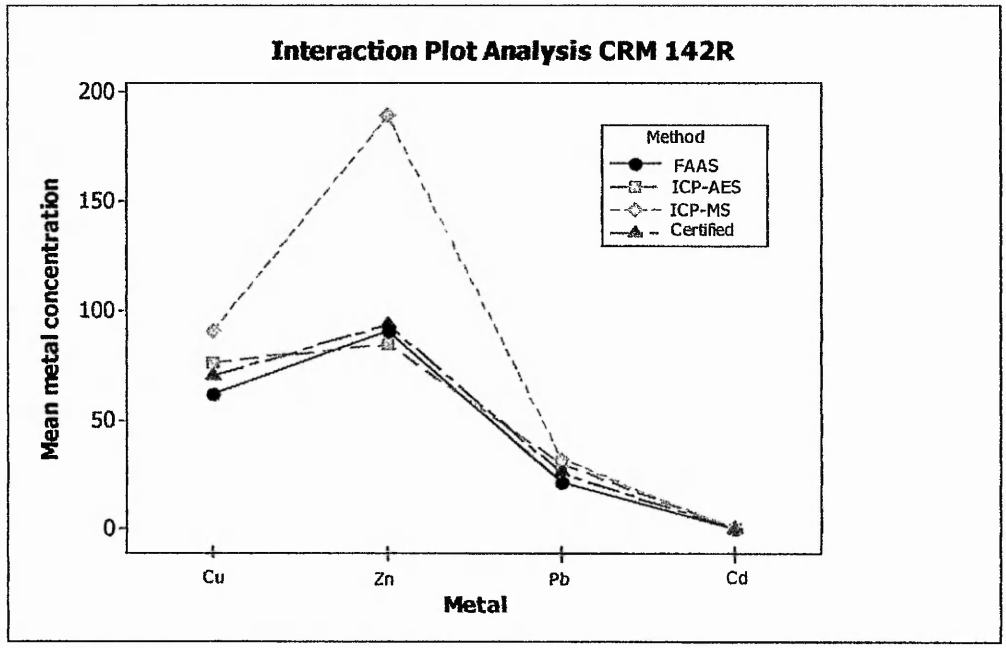


Figure 7.7 ANOVA interaction plot for analysis of CRM 142R

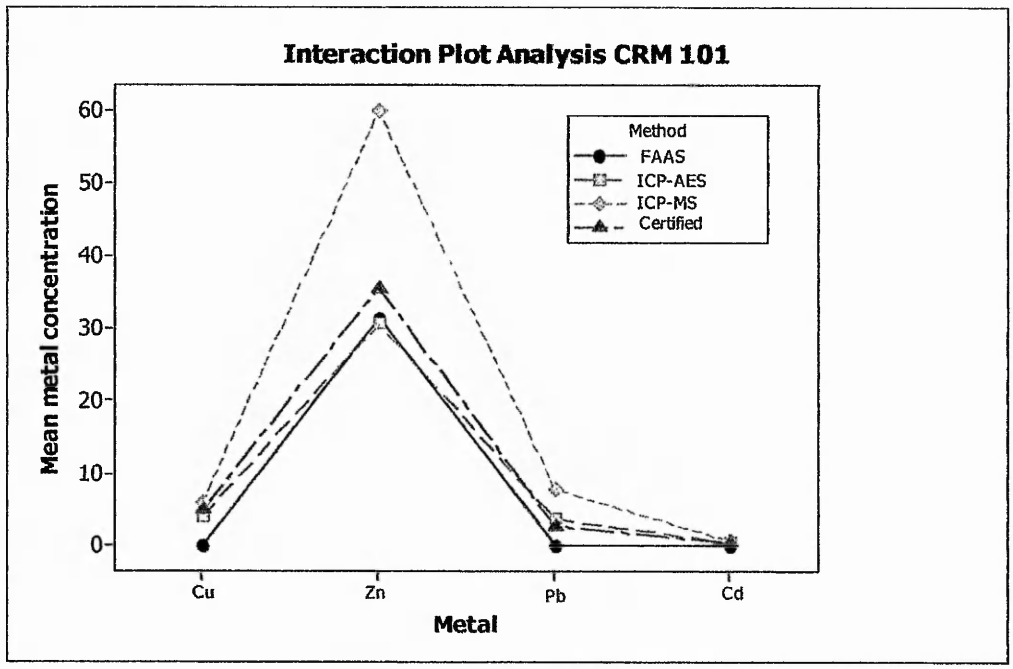


Figure 7.8 ANOVA interaction plot for CRM 101 analysis

In both cases the plot obtained from data generated by the ICP-MS instrument indicates that, in particular, the concentration obtained for zinc is considerably different from the certified value and values obtained by other instrumentation.

Accuracy

Figure 7.9 illustrates the differences in accuracy determine as in SOP 3.7.3 for three evaluated methods analysing Community Bureau of Reference materials. In both cases maximum accuracy for zinc concentration was achieved by FAAS and by ICP-AES for all other target metals.

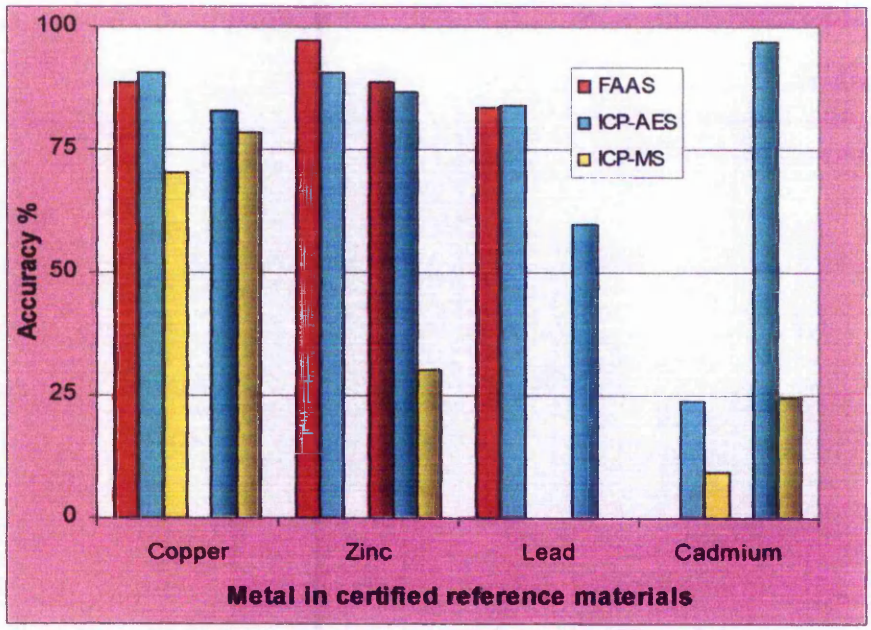


Figure 7.9 Accuracy comparison between analytical methods relating to BCR reference materials. Plain columns represent CRM 142R and shaded columns represent CRM 101.

Accuracy values could not be calculated for ICP-MS when used to determine zinc concentrations in CRM 142R and lead concentrations in both CRM 142R and CRM 101 as the results produced would have been indecipherable if included in Figure 7.9.

Discussion

Table 7.1 indicates the lack of results obtained using EDAX analysis of environmental samples as presented on this occasion. The instrumentation used for this research detects all elements above carbon in the periodic table and is considered to have a reliable quantifiable detection limit in the order of 0.1% or 1000ppm of the elements detected (*pers. com.* Steve Bursland). Zinc and lead were not detected in some of the scans taken at different locations on SB2 spoil, therefore the analysis is

not uniformly representative of the whole sampling volume. The analyst has to be content with knowing that the analysis for a bulk specimen is likely to come from a volume of about 1μm x 1μm x 1μm. (Goodhew and Humphreys, 1992) although during the current procedure an area of approximately 500 mm^2 was scanned to obtain the data. These methods are suitable in the context for which they were used i.e. comparative or qualitative purposes at concentrations in excess of 1000 ppm. Absolute concentrations however are necessary for most monitoring, surveying and research purposes. Pyatt, *et al.*, in 1998 detected a range of elements with a minimum concentration of 0.1%. West *et al.* (1997) point out that the technique has several limitations and at best can only be used for comparative purposes although heterogeneity still presents a major problem.

Ion chromatography results in Table 7.1 also exhibit major problems associated with RSD values hence precision seems to be a major problem with this technique when used for environmental analysis. Zinc concentrations are consistently lower and in most cases lead concentrations are greater than other methods. Discussions with the manufacturers indicate that complex matrices should be treated in order to chelate transition metals in order that they can be separated from alkaline earth elements which interfere with the elution and detection of metal complexes in the post column reaction phase. An attempt was made to extract the target metals using solid phase extraction (SPE) techniques but with little success. The author considered such additional processing would be unacceptable since further systematic errors would be introduced. Bruno *et al.* in 2000 claimed detection limits 30-60 ppb for the target metals using a bifunctional exchange column using an oxalic acid/sodium nitrate and the addition of sodium chloride solution to optimise separation and further work would be necessary to evaluate this procedure. Figure 7.10 illustrates interference encountered when soil extract is analysed when compared with a 4.0ppm standard prepared in analytical blank. Elution times have changed and the final seven minutes of elution time were subjected to increased interference causing displacement of and elimination of peaks. The report data shown in Figure 7.11 clearly indicates shift in elution times and elimination of detectable peaks due to interference.. Analysis by FAAS, ICP-AES and ICP-MS detected concentrations of lead in the order of five times less than the value indicated (and subsequently calculated) in this

chromatograph. Even larger variations were found when relatively small concentrations of lead were present; CV4 spruce leaves extract produced a factor of x20 for lead when determined using ion chromatography compared with ICP-AES (see Table 7.1).

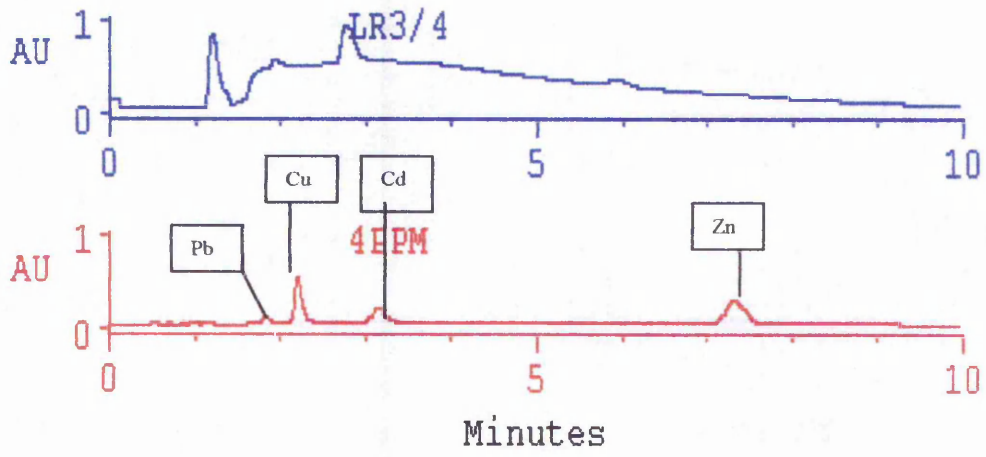


Figure 7.10 Chromatograms obtained when LR3 and 4.0 ppm calibration standard were analysed using Dionex 600X ion chromatography instrument.

|Data File : C:\PEAKNET\DATA\INVEQ010.DXD Report Date: 6/1/105 9:26:00 AM |
|Sample Name: LR3/4 Collected : 2/11/104 11:01:03 AM|

Pk. Num	Ret Time	Component Name	Height	Area	Bl
3	1.93	LEAD	63346	399423	
0	0.00	COPPER	0	0	
4	2.78	CADMIUM	410694	4911282	
0	0.00	ZINC	0	0	

|Data File : C:\PEAKNET\DATA\INVEQ005.DXD Report Date: 6/1/105 9:17:38 AM |
|Sample Name: 4PPM Collected : 2/11/104 9:57:22 AM |

Pk. Num	Ret Time	Component Name	Height	Area	Bl.
3	1.83	LEAD	66482	353842	
4	2.20	COPPER	459989	3370675	
5	3.15	CADMIUM	172321	2060582	
6	7.32	ZINC	256298	4355617	

Figure 7.11 data report from Dionex 600X illustrating elution time shift between LR3 and 4ppm calibration standard

Figures 7.1 – 7.6 illustrated much higher concentration of zinc detection in all substrates except SB2 Snail Beach spoil(Figure7.2) and interactive ANOVA plots shown in Figures 7.7 and 7.8 also illustrated this effect. Since copper, lead and where appropriate, cadmium concentrations determined using ICP-MS are of a similar magnitude to those determined using FAAS and ICP-EAS, then the author believes that zinc standards used to calibrate the ICP-MS instrumentation must have been inaccurately prepared. When cadmium was detected in various analytes ICP-MS produced higher values than ICP-AES although when compared to certified concentrations in reference materials, ICP-AES produce more correlated results. Additional problems associated with relatively high RSD values and hence precision, were encountered when ICP-MS instrumentation was used. Accuracy values as shown Figure 7.9 are consistently lower for this method of analysis, on this occasion, when compared with ICP-AES and FAAS. It should be born in mind however that the analysis calculations made for all of the methods, when possible, is not merely a reflection of the accuracy of the analytical method but a reflection of analytical method plus extraction procedure. However since the same extract solution was used for each analytical determination, then the accuracy values produced indicate the relative value of the analytical method.

Figure 7.1 – 7.9 illustrate the close relationship between FAAS determinations and those obtained using ICP-AES. When low concentrations of target metals were encountered, FAAS instrumentation did not detect the element because concentrations were below the LOD for that particular instrument or signals relating to absorption values were below the calculated LOQ for the instrument. Figure 7.9 shows that FAAS achieved higher accuracy for zinc in both reference materials but failed to quantify lead and cadmium in some cases. RSD values obtained when using ICP-AES are consistently lower than those exhibited by FAAS.

The predominance of FAAS techniques in research papers surveyed indicate that this method is suitable for environmental analysis however, from this evaluation, the use of correctly calibrated ICP-AES instrumentation would certainly produce more precise results and would detect and quantify lower concentrations of target metals.

Overall it was determined that ICP-AES was most suitable for this type of analysis, although FAAS produced comparable results in many instances. Statistically it was demonstrated that there was no significant difference between ICP-AES and FAAS, however LOD and LOQ values for ICP-AES were lower than those for FAAS in all cases. It was recommended that ICP-AES be purchased when possible.

Chapter 8.

Heavy metal dispersion, dilution, bioaccumulation and health implications in an area surrounding an ancient copper/zinc mine situated in Anglesey, North Wales, UK

Copper has been extracted from this site since the bronze age and is situated in a temperate costal location. The area is surrounded by igneous rock producing a generally acid environment. The site is an open pit copper mine, as are all of the mines investigated in this research.

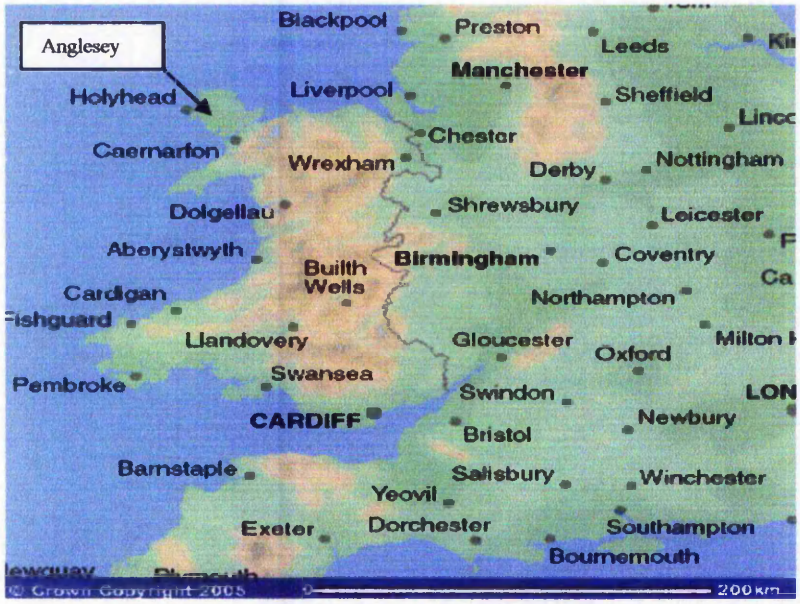


Figure 8.1 Location of Anglesey, UK.

Introduction

Parys Mountain is situated on the island of Anglesey in North Wales UK 4 km south west of the coast (Figure 1.) and 3 km from the town of Amlwch. Copper has been extracted from the site for over 4000 years initially by prehistoric populations; cakes of copper found on the north side of the workings have been identified as Roman (Rowlands, 1966). By the mid eighteenth century the area was extensively developed and copper from the mines dominated the world's markets in the 1780's. Zalasiewicz in 2000 stated that Parys Mountain was once the largest copper mine in the world but by the 1880's competition from cheaper ores from abroad led to the decline of the mines. Initially, ore extraction was from the surface followed by open pit mining. The inlet and subsequent port at Amlwch has been used for the shipping of ore and copper

metal to other parts of the UK. The ore was carried by horse and cart from Parys Mountain down to the harbour.

Figure 8.2 Parys Mountain, Anglesey, UK, from the south west.

Figure 8.2 The main pit at Parys Mountain, Anglesey, UK.

As the copper concentration of the surface ore decreased, underground mining became prevalent; however the extracted ore was broken up into smaller pieces for subsequent smelting on the surface (Amlwch Industrial Heritage Trust, 2000).
Mining ceased in 1904 and was briefly reopened in 1984 when 2000 tonnes of ore were extracted. Anglesey Mining plc currently hold the mineral rights to part of the site and have predicted that 6.5 million tonnes of ore with a combined average grade of over 10% of zinc, copper and lead are available. Small amounts of gold and silver are also contained in the ore (Kearney, 2003). Low metal prices apparently currently prevent extraction of the ore.

Smelting has been carried out both on Parys Mountain and at Amlwch and reference was made to the damage to vegetation in the latter part of the eighteenth century and a special allowance called 'smoke trespass' was paid to the vicars of Amlwch for the inconvenience caused by fumes from open kilns on the mountain, however it was suggested that the problem was sulphur release from the ore and the payment was withdrawn with the introduction of a new process which condensed the sulphur and other noxious substances (Rowlands. 1966).

Parys Mountain attains an altitude of 147m above sea level and the prevailing winds come from between south and west for 56% of the time according to Llansadwern Weather Station, Anglesey (www[8]). Thus the land to the north east of Parys Mountain, including the town of Amlwch suffered from atmospheric dispersion of gases and particulates (Figure 8.1) and evidence of heavy metal pollution has been documented (Curtis, 2004).

This research endeavours to determine the extent of the dispersion of heavy metals downwind of the workings at Parys Mountain and at the port of Amlwch. Evidence of bioaccumulation in vegetation and possible movement through the food chain were also investigated. The sites were approximately in line with the prevailing winds and it was expected that metal concentrations would decrease with distance from the workings at Parys Mountain. The site at Amlwch port was included to determine the degree of contamination due to the smelting of copper, extraction of sulphur and the loading of metal and ore onto ships for onward transport (Amlwch Industrial Heritage Trust, 2002).

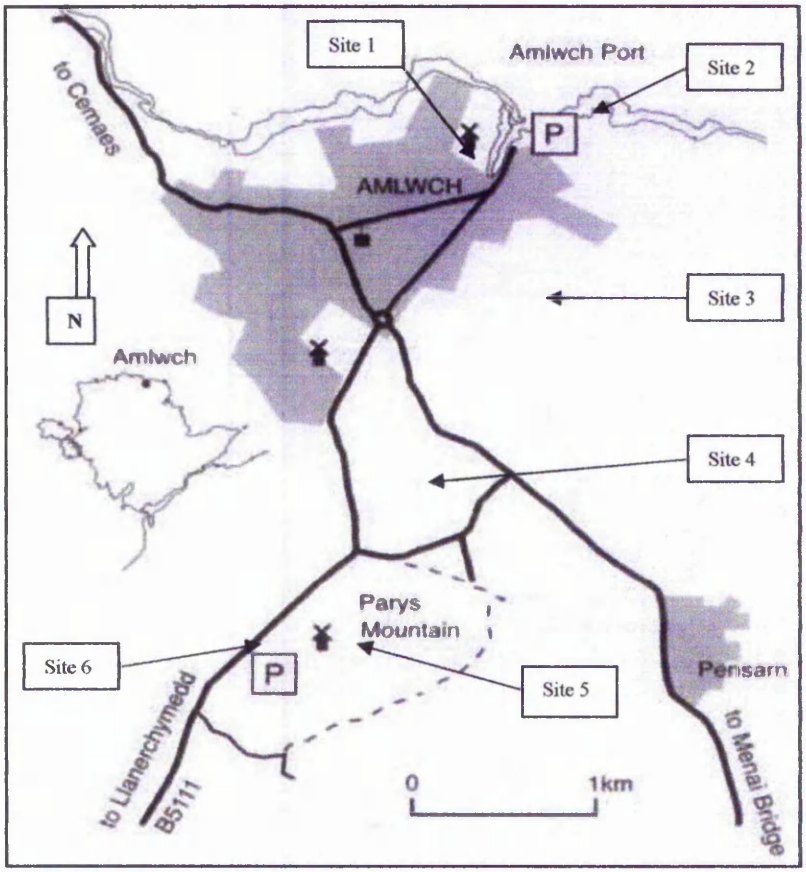


Figure 8.1 Location of Parys Mountain, Amlwch port and sample sites, Anglesey, North Wales, U.K.

The control site on the west slope of Yr Arwydd, 178m above sea level was 6 km south of the workings at a similar altitude to site 5 on Parys Mountain.

Materials and Methods

Samples were collected from the sites shown in Figure 8.2 on 16 September 2003.The exact location on the Ordnance Survey 1:25000 scale map of the area and the type of samples collected is shown in Table 8.1.

Soil samples were collected and prepared for analysis in accordance with SOP 3.3.1. Flowers from ling (*Calluna vulgaris*) plants were collected and prepared as in SOP 3.3.4.

Sample No.	Site No.	Location	km and direction from workings	U.K. National Grid Ref.	Type
9PM1	1	50m N of W end Amlych harbour	3.38 N	SH449935	Soil
9PM2	1	"		"	*Calluna vulgaris*
9PM4	2	Near coastal path	3.38 NE	SH462937	Soil
9PM5	2	"		"	*C. vulgaris*
9PM6	2	"		"	*Cladonis* sp.
9PM7	2	"		"	Cow faeces
9PM8	2	Stream in ravine near above site		SH461937	Water
9PM9	3	Heathland outcrop	2.13 NE	SH455924	Soil
9PM11	3	"		"	Cow faeces
9PM12	3	Steam adjacent to above site		SH456926	Water
9PM13	3	Mole hill adjacent to stream		"	Soil
9PM14	3	On path from road to site 5		SH457925	*C. vulgaris*
9PM15	4	Side of dry pond	0.75 NE	SH448913	Soil
9PM16	4	"		"	*C. vulgaris*
9PM17	4	"		"	*Cladonia* sp.
9PM18	4	"		"	Silt
9PM19	5	NE of workings on Parys M.	0.05 NE	SH445907	Soil
9PM20	5	"		"	*C. vulgaris*
9PM21	5	"		"	*C. vulgaris* wood
9PM22	5	"		"	*Cladonia* sp.
9PM23	6	Stream N of Parys Mountain		SH437906	Water
9PM24	7	Yr Arwydd (control)	6.0 S	SH470854	Soil
9PM25	7	"		"	*C. vulgaris*
9PM26	7	"			Cow faeces

Table 8.1 Samples collected in the vicinity of Parys Mountain, Anglesey, 16 September 2003

Samples of cattle faeces were collected and prepared as shown in SOP 3.3.8 and water samples were collected as in SOP 3.3.10.

Conductivity and pH determinations on soil samples were carried out in accordance with SOP 3.4.1 and 3.5.1 and these determinations for water were carried out as in 3.4.2 and 3.5.2

Soil and vegetation samples were extracted as in SOP 4.2.1 and 4.2.2 and analysis of digested soil and vegetation as well as stabilised water was carried out using FAAS in accordance with 3.6.1

Results

Conductivity and pH

Sample	Type	pH at 21^{0}C	Conductivity μScm^{-1} at 21^{0}C
9PM1	Soil	4.63	335
9PM4	Soil	3.52	282
9PM8	Water	6.22	-
9PM9	Soil	3.41	415
9PM12	Water	5.92	-
9PM13	Soil	4.39	505
9PM15	Soil	3.13	586
9PM18	Soil	2.83	421
9PM19	Soil	3.01	308
9PM23	Water	2.41	-
9PM24	Soil	3.42	361

Table 8.2. pH and conductivity of soil and pH of water samples collected in the vicinity of Parys Mountain, Anglesey, U.K. September 2003.

Table 8.2 illustrates the pH of water and soil samples measured on return to the laboratory, 24 hours after collection. All water samples were acidic but those from near the workings were the most acidic. Conductivity was highest (586 $\mu S\ cm^{-1}$) in the soil sample collected from site 4, by a dried out pond. Conductivity was lowest (282 $\mu S\ cm^{-1}$) at the coastal site (site 2).

Metal concentrations.

Table 8.3 illustrates the concentration of target metals in the dried samples together with the relative standard deviation (RSD) obtained from the analytical results of 10 replicates of each sample.

The soil sample collected from near the harbour at Amlwch contained major concentrations of copper zinc and lead with traces of cadmium whereas ling collected at this site contained only concentrations of zinc.

Sample	Type	Mean Cu mg kg^{-1}	RSD Cu %	Mean Zn mg kg^{-1}	RSD Zn %	Mean Pb mg kg^{-1}	RSD Pb %	Mean Cd mg kg^{-1}	RSD Cd %
9PM1	Soil	1569.9	7.52	2374.6	1.64	1528.3	11.91	3.36*	10.85
9PM2	C. vulgaris	<LOD		40.01	0.64	<LOD		<LOD	
9PM4	Soil	49.9	9.77	9.06	6.62	51.52	13.56	<LOD	
9PM5	C. vulgaris	<LOD		12.4	4.98	<LOD		<LOD	
9PM6	Cladonis sp.	<LOD		7.12	0.77	<LOD		<LOD	
9PM7	Faeces	28.1	10.26	87.3	1.86	<LOD		<LOD	
9PM8	Water	<LOD		<LOD		<LOD		<LOD	
9PM9	Soil	215.5	5.49	114.6	3.57	193.2	4.02	<LOD	
9PM11	Faeces	20.4*	17.12	153.5	2.26	<LOD		<LOD	
9PM12	Water	<LOD		<LOD		<LOD		<LOD	
9PM13	Soil	73.0	1.17	105.5	3.23	<LOD		<LOD	
9PM14	C. vulgaris	<LOD		18.5	2.05	<LOD		<LOD	
9PM15	Soil	858.9	2.11	160.8	2.82	475.3	8.82	<LOD	
9PM16	C. vulgaris	10.74*	28.6	13.3	4.56	<LOD		<LOD	
9PM17	Cladonia sp.	<LOD		11.6**	7.54	<LOD		<LOD	
9PM18	Silt	1220.0	1.95	221.4	3.17	205.1	3.10	<LOD	
9PM19	Soil	3344.0	3.10	1063.0	2.19	4067.0	3.40	1.37*	
9PM20	C. vulgaris	21.52	18.57	27.32	6.13	26.54	27.42	<LOD	
9PM21	C. vul. wood	49.81	11.58	44.06	4.19	163.89	5.27	2.11*	
9PM22	Cladonia sp.	<LOD		11.6**	5.26	<LOD		<LOD	
9PM23	Water	507.6	3.09	230.7	1.44	449.8	6.31	<LOD	
9PM24	Soil	13.11*	11.09	29.8	1.35	42.4**	9.33	<LOD	
9PM25	C. vulgaris	<LOD		15.74	4.42	<LOD		<LOD	
9PM26	Faeces	15.42*		51.88		<LOD		<LOD	

*Some signals below limit of detection. All signal values below limit of quantitation

**Some signal values below quantitation.

Table 8.3 Mean metal concentrations and relative standard deviation of environmental samples collected in the vicinity of Parys Mountain, U.K. Control samples shaded.

Soil from near the new workings on Parys Mountain contained the highest concentrations of copper and lead with a noteworthy concentration of zinc; cadmium was not detected. Ling flowers growing in this area contained quantities of copper, zinc and lead. The woody stems of the ling plants from which the flowers were collected contained higher concentrations of the same metals.

Water samples collected near the coastal path and site 3 produced FAAS absorption signals for all target metal concentrations below their respective LOD, however water sampled close to the workings contained significant concentrations of copper (508 mg L^{-1}) zinc (231 mg L^{-1}) and lead (450 mg L^{-1}). Water samples collected down wind of the workings were acidic with pH values of 6.22 and 5.92 respectively; however the stream water from near the workings had a pH value of 2.41

Some RSD values shown in Table 8.3 are relatively high (28.6% for copper) especially where relatively low concentrations (10.7 mg kg^{-1}) of target metals are determined. This could be a result of random errors particularly influenced by natural heterogeneity in soil and plant samples. The Community Bureau of Reference, 1994, make much of this point in the preparation and supply of there certified samples of soil and in another report, 1990, referring to their certified samples of spruce (*Picea abies*) needles. Values of RSD <4.0% are considered exceptionally good for this type of analysis (*pers. com.* Dr A. Braithwaite). Generally the results for RSD in Table 8.3 show values < 4 % for higher concentrations of all target metals in soil. Zinc determinations in all cases exhibit a maximum RSD of 6.62% whereas lead varies between 3.1% and 27.2%. Analysis carried out by various workers has shown large differences in metal concentrations and RSD in soil and vegetation samples (Pederson *et al.*, 2000, Pueyo *et al.*, 2001, Veinott *et al.*, 2003).

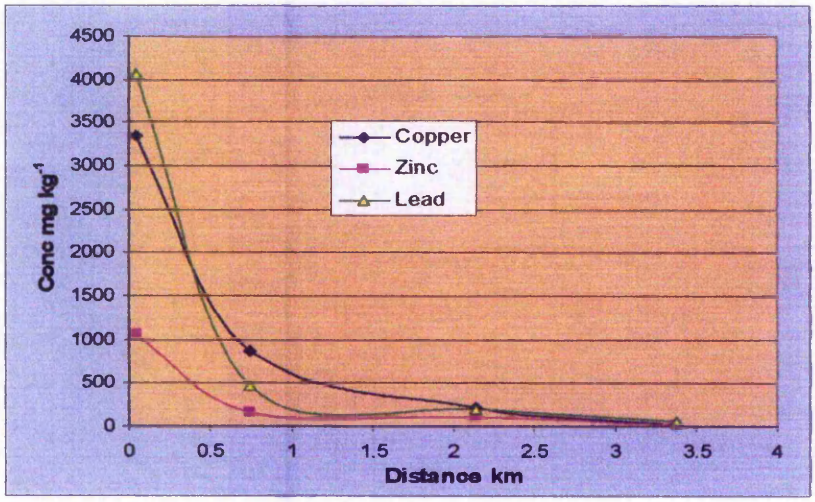


Figure 8.2. Variation in copper, zinc and lead concentrations in soil relative to distance downwind from mine workings at Parys Mountain, Anglesey, U.K.

Figure 8.2 shows the relationship between copper, zinc and lead concentrations in soil samples and distances downwind from the workings. Copper and zinc decline at a similar rate over the first 0.75km, however lead concentrations decline much more rapidly over the same distance due density effects related to the ore types.

Discussion

All soil samples collected down wind of the old workings exhibited pH values ranging from 3.5 at the coastal site to a value of 2.8 for silt collected from the dried pond at site 5. The soil collected from the control site had a pH value of 3.4. Soil collected from the site near the harbour had the highest pH value of 4.6. The sampling sites are all acidic and this would contribute to the release of metal ions from the soil matrix (Hur *et al.*, 2004. Yukselen and Alpaslan, 2001.), leading to subsequent uptake by plants or leaching into ground waters. One factor however was not determined, that of Pε, a measure of the redox potential of the soil, which would also affect the release of metals from soil into the surrounding environment.

The concentration of target metals in soil samples taken down wind of the workings on Parys Mountain declines with distance over a 3.4 km north easterly transect. Topography in the direct line to the sea does not interfere with air flow; therefore one would not expect to find areas of anything other than incremental reduction in metal concentrations from Parys Mountain to the coast. The control samples collected 6 km south of the workings from the slopes of Yr Arwydd produced low concentrations of copper, zinc and lead, however some of absorbance values recorded for copper and lead were below the LOQ and LOD values. The zinc concentration at the control site was higher than that downwind at the coastal site although copper and lead concentrations were higher at the coastal site. It is suggested that intermittent sea water spray could be responsible for leaching zinc from the soil at the coastal site similar in effect to the extraction of exchangeable metal ions using 1 molar magnesium chloride in the Tessier *et al.*, (1979) sequential extraction process. Typical sea water has a sodium and chloride concentration of 1.04M (de Mora, 1999) and this would preferentially extract zinc from the soil matrix.. Relatively high conductivity of soil at site 4 (586 $\mu S\ cm^{-1}$) is probably due to the fact that this site is flooded during the winter and residual soluble salts have influenced the value.

Figure 8.2 illustrates the dispersion and dilution of metal concentrations for target metals in soil at various distances downwind of the workings towards the coast. Copper, zinc and lead concentrations reduce almost exponentially with distance from

the workings. The decline in metal concentration over distance is indicative of atmospheric dispersion of the particulates resulting from mining activity. Bioaccumulation of target metals will have taken place from the soil by plants and subsequent movement through trophic levels to animals and humans. Ingestion and inhalation of airborne particulates would also have contributed to accumulation of heavy metals directly by animals including humans not directly associated with the mine workings.

Lichen (*Cladonia* sp.) collected at various distances from the workings exhibit only relatively small concentrations of zinc, other target metals were not detected. All lichen samples collected by the author from mine workings and relevant control sites in the U.K., British Columbia and Arizona contained zinc concentrations <50 mg kg^{-1} of dried lichen and it is considered that these values relate to the concentration of essential zinc in plants. This suggests that noteworthy airborne dispersion of particulates from the mine workings has not occurred recently. Therefore, it is conceivable that any accumulation of copper, lead and excessive zinc in other vegetation in the vicinity is a result of metal concentrations in the soil and not as a result of stomatal accumulation of particulates. The possible effect of soil pH on availability of metal ions for bioaccumulation will be investagated later in this research.

Concentrations of zinc were recorded in *C. vulgaris* florets collected at all sites but only samples collected at Amlwch harbour and near the workings exhibited noticeably higher concentrations than the control sample. Some additional accumulation of zinc has taken place at both of these sites, where high concentrations of zinc (>1000 mg kg^{-1}) are present in the soil. Florets collected on this occasion around the harbour however do not indicate accumulation of copper and lead which are present in major concentrations in the soil at this location. It is possible that the relatively high pH (4.63) of the soil may contribute to this lack of accumulation as well as the proximity of the site to the sea. This phenomenon was also recorded in the vicinity of Britannia Mine, Canada (Wilson *et al.*, 2005B).

C. vulgaris florets collected near the workings contained concentrations of copper, lead and zinc. Evidence of partitioning in plants has been reported by Kruckerberg and Wu, 1992, Ernst, 1993 and Pyatt, 2001; therefore a sample of the woody section was collected from the same *C. vulgaris* plants near to the workings. Table 8.4 shows the concentrations of metals recorded in the soil, *C.vulgaris* florets and woody section collected near to the mine workings.

	Soil	Floret	Wood	A.Ratio *
Cu mg kg^{-1}	3344.0	21.52	49.81	2.31
Zn mg kg^{-1}	1063.0	27.32	44.06	1.61
Pb mg kg^{-1}	4067.0	26.54	163.9	6.17
Cd mg kg^{-1}	1.37	<LOD	2.11	-

*Accumulation ratio between floret and wood.

Table 8.4 Metal concentrations in soil, *C. vulgaris* florets and wood collected adjacent to mine workings at Parys Mountain, Anglesey, U.K

Annual ring determinations on the freshly cut woody section of the plant indicated that the wood was at least five years old. The expected accumulation in florets would be lower than that of the woody section since the production of florets by the plant is an annual event. Accumulation of zinc and cadmium in both sections of the plant are high relative to the soil concentrations; however these metals are considerably more mobile than lead and copper in soil matrixes (Gale *et al.*, 2004)

Table 8.4 also shows an accumulation factor of target metals relating floret and woody sections of *C. vulgaris*. Lead concentrations are 6.2 times higher in the woody section of the plant than in the florets and copper concentrations are 2.3 times higher. Zinc however is only 1.6 times as much which may suggest that being an essential element in plant physiology, there is a mechanism which limits zinc uptake.

Concentrations of target metals were not detected in water samples collected down wind of the workings and pH values recorded were higher (>5.9) than the soil samples collected in the vicinity. All soil samples were collected in heathland habitats

however, in order to maintain consistency. Much of the surrounding area below Parys Mountain has been cleared for agricultural purposes, mainly grazing and the pH of the soil will have increased over time due to the input of organic matter and general decomposition.

Water collected near to the mine workings exhibited an intense yellow/brown colouration and the stream banks showed no signs of biotic activity. A pH value of 2.41 was recorded and major concentrations of copper, zinc and lead were detected. Jenkins *et al.*, (2000), carried out analysis of mine drainage at various sites in the workings around Parys Mountain recording similar pH and copper concentration values in some places. Zinc and lead concentrations were much higher in the sample examined on this occasion, which was collected after a prolonged period of drought. The stream in question appeared to sink into the hillside downhill from the workings.

Cow faeces samples collected at sites 2, 3 and 7 contain small concentrations of copper (15 – 28 mg kg^{-1}) and more significant concentrations of zinc (52 -153 mg kg^{-1}). The samples were collected in heathland areas along with soil, *C. vulgaris* etc. however it was not necessarily the case that the animals had been grazing in that area extensively or exclusively. Copper concentrations at all three sites were similar but only the coastal path sample produced consistent signal values above the LOQ for that element. Soil from this site exhibited the lowest copper concentration and therefore it was proposed that a.) cattle could have been grazing in an area where copper concentrations in the soil and vegetation were much higher or b.) levels of copper ingested were as a result of accumulation from the large amounts of vegetation consumed, in which copper is an essential element. Similar arguments could be applied to the accumulated zinc concentrations in the cow faeces, however the concentration of zinc appears to increase with increased soil concentration at sites 2 and 3 and the control collected at Yr Arwydd contained the lowest concentration of zinc. Zinc was not detected in *C. vulgaris* collected at any of these sites, however it is probable that soil has been ingested by the animals in question.

Dispersion and dilution of metalliferous pollution was demonstrated in the direction of the prevailing winds but this phenomenon appears to have ceased since lichens collected in the area do not exhibit evidence of target metal accumulation. The soil in the areas was found to be acid and the vegetation collected demonstrated partitioning within the plant tissues, annual tissue in the species accumulating less of the target metals than perennial tissue. Animal faeces collected in the vicinity produced inconclusive evidence of bioaccumulation by the animal concerned as result of pollution from the mine.

Chapter 9

The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada.

Britannia mine was selected due to its position in a temperate coastal climate condition and its location within an area of igneous rock. In contrast to Parys Mountain, Anglesey, UK. this site operated for a shorter (76 years) but more intensive period essentially in the 20th century. Mining ceased on a similar d ate

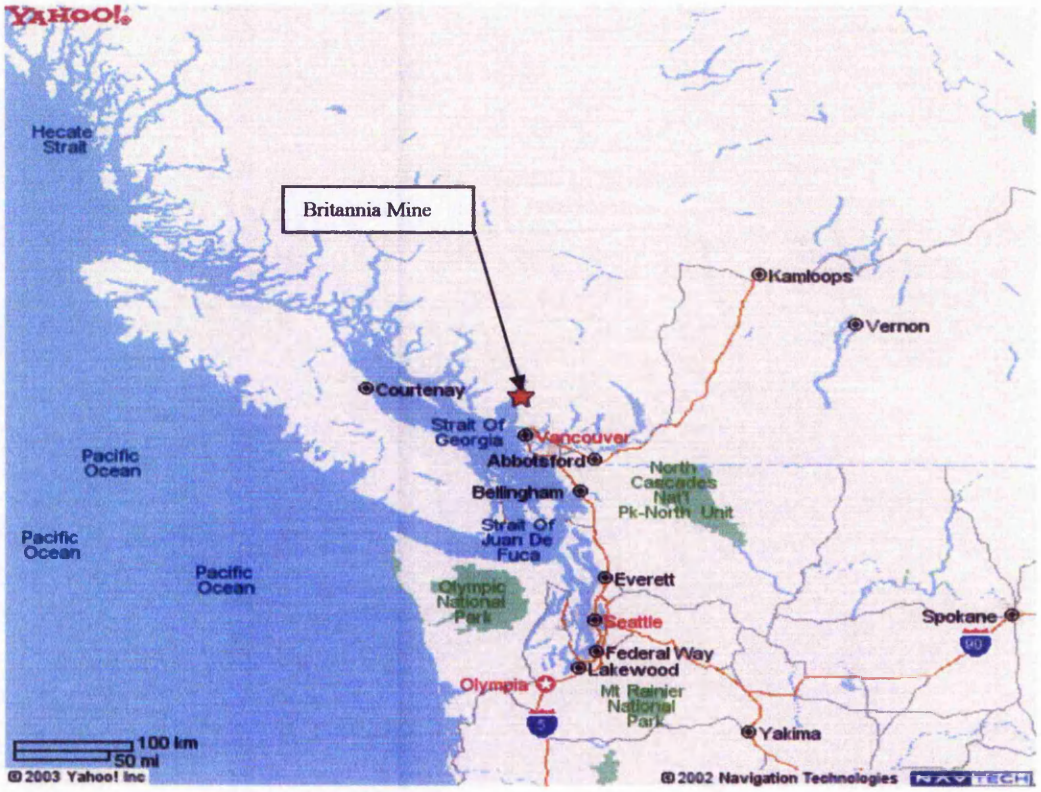


Figure 9.1 Location of Britannia Mine

Introduction

Britannia Mine is situated above the settlement of Britannia Beach some 50 km north of Vancouver (Figure 9.1) on Highway 99, also known as the Sea to Sky Highway. The nearest large town is Squamish, 11 km north of the site.

Figure9.2 Approach to Britannia Mine from the shore.

The mine at Britannia Beach operated from 1898 until 1974 and in the 1920's was said to be the largest copper mine in the then British Commonwealth (URS, 2002). Between 1905 and 1974 the mine produced 47.8 million tonnes of ore (Mills, 2003). Secondary products were Zn, Ag, Au, Cd and Pb (URS, 2002)

The site (Figure 8.3) is currently considered to be one of the worst sources of water pollution in North America (Warkentin, 2000). The mine covers an area of some 28 km^2 and consists of a series of tunnels and some open-cast mining. The mine originally commenced on a hill at an elevation of 1400m above sea level (Figure 9.2) and now extends to below sea level. Five open pits were also created near the summit of the hill. A series of portals exit onto the hill side at different altitudes and aspects, but all have waste rock dumps (spoil tips) nearby eventually washing down into a series of creeks. When the mine was abandoned, acid mine drainage (AMD) became a major problem, issuing from several portals and a submerged drain extending into Howe Sound. Britannia Creek which collected AMD from Jane Creek and Mineral Creek was also a source of discharge into Howe Sound. A concrete plug is reported to have closed off AMD from the 2200 level portal (Britannia Mine Remediation Project, 2002) and AMD from the mine is being discharged via the submerged drain.

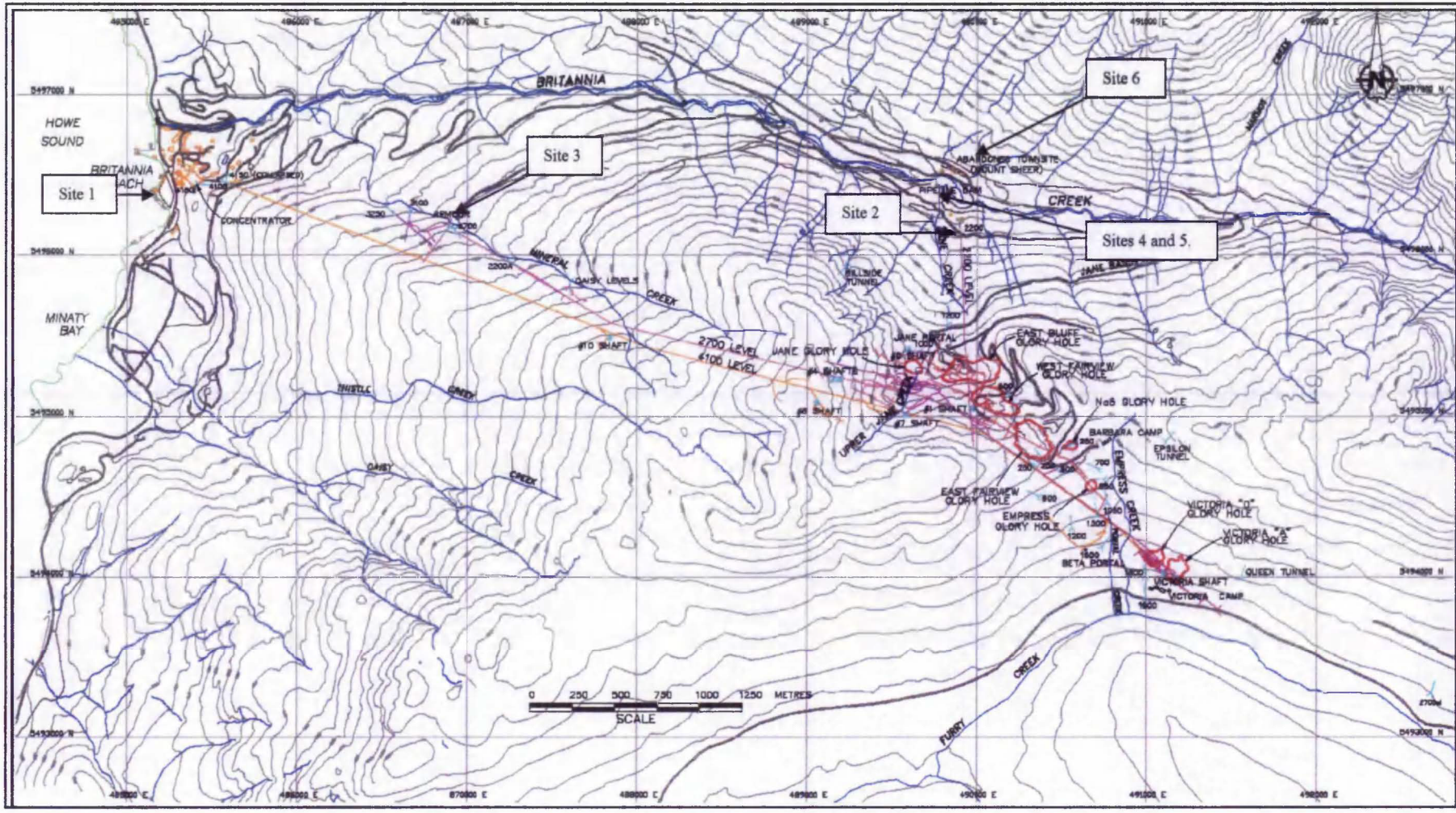


Figure 1. Position , layout and sample sites of Britannia Mine, British Columbia, Canada

Prof. John Meech of UBC Department of Mining estimates that at least one tonne of copper is discharged into the Sound each day (*pers. comm.*, 2002).

The provincial government have obtained reparations from various previous owners of the mine to carry out remedial work and currently employs several consultants who are determining the extent of the remediation required to make the site 'safe'. They have identified many 'areas of environmental concern' (AEC) the majority of which centre around the Fan Area where the old concentrators were sited.

This area is inhabited by approximately 300 individuals who live in either old mine housing or trailers. Copper Beach Estates, who owned the site in January 2003, were extremely helpful by providing vehicular access to the site and at the time were considered to be 'potentially responsible persons' (PRP) in terms of remediation but they were still in negotiation with the provincial government agencies. In April 2003, however, Copper Beach Estates declared themselves bankrupt and ownership of the site has now been assumed by the provincial government.

A second visit to area was undertaken in November 2003 approximately 10 months after the initial visit. Vehicular access to the site was not possible on this occasion since the Government of British Columbia, Department of Mines did not respond to several requests for access. There was however public access on foot to the site and on this occasion further soil and vegetation samples were collected from the site of the old township. This visit was undertaken to assess the impact of the workings on the old township in the past and at the present time. The township was fully occupied from 1915 until 1958 (www[3]) by mine workers and their families. During this period the initial underground workings were supplemented by the introduction of open pit mining in the so called 'glory holes'. (Figure 9.3). The substantial township (Figure 9.4.) was gradually abandoned and finally demolished in 1969 (*pers.comm.* curator, BC Museum of Mining)

An assessment of the impact of mining operations during the occupancy of Mount Sheer was undertaken by the analysis of soil and vegetation samples collected in the area within the old site.

The old town site (Mt. Sheer) has been levelled and the area has now been invaded by *Alnus* spp. with some early growth of *Picea sitchensis*, the usual successional sequence in western Canada after 'clear cutting' lumber (Figure 9.5). The prevailing winds are either northerly or southerly up and down Howe Sound and have little effect in the valleys on either side of the mine. The area is classified as a coastal wet climatic zone with high levels of precipitation (1117mm annually) most of the year (minimum 36.1mm in July) but with very warm summers. Precipitation in winter regularly falls as snow above about 300m.

Figure 9. 4 Mount Sheer township circa 1950 (courtesy BC Museum of Mining).

Much work has been carried out examining the effect of AMD into the Sound and the effects of some 40,000 tonnes of 'tailings' (from the concentrators) which were discharged from 1902 to 1974 into Howe Sound (Hagen, 2001). Such examinations range from underwater surveys from diving vessels (Levings and McDaniel 1973), to the effects of the local sea water on transplanted Rainbow trout (Barry *et al.* 2000), and estimations of benthic organism reduction around the Sound (Ellis and Hoover, 1990).

Figure 9.5 Collecting samples in November 2003 on the site of Mount Sheer township site, Britannia Mine.

There has been no assessment on the dispersion, accumulation and partitioning of copper and associated metals into the surrounding biosphere. Local residents have expressed concern over health issues for many years although such concerns have been refuted by the various consultants employed by the provincial government. Hagen, in 2001, noted that groundwater and upland influences will need to be studied. It should be noted that a pilot water treatment plant has been installed in the local primary school to allay concerns regarding the health of children in the area (Golder Associates, 2002A).

Materials and Methods

Samples of spoil were collected in January 2003 near the entrances of the 2200 level and 2700 level tunnels and from the fan area adjacent to the sea shore. Foliage from small spruce trees (*Picea sitchensis*) was collected from the spoil area at the 2200 level and the sea shore. Samples were collected from the same aspect and height above the ground for each tree. Trees in a similar condition of health were selected. Water samples were collected from Britannia Creek above and below the confluences

with Jane Creek and from Jane Creek just above the confluence with Britannia Creek. In November 2003, at the old township site, a recently fallen *Alnus rubra* estimated to be 30 years old, determined by dendrochronology, was used as a possible monitor for bioaccumulation. Samples of various tissues from the same tree were collected in order to assess the degree of partitioning within the species (Kruckerberg and Wu, 1992, Ernst, 1993 and Pyatt, 2001). Two soil samples were collected at Mount Sheer site one at the base of the fallen alder and the second approximately 100m north of the fallen alder.

Lichen species were abundant in the vicinity of the old township and the control site and they were collected in an endeavour to determine the extent if any of current atmospheric dispersion of heavy metals from the old workings (Pyatt *et al*, 1999. Bari *et al*, 2001)

The sample collection sites are shown in Figure 9.3

Site No. 1. Shore, just above high tide mark.

Site No. 2. 2200 level, adjacent to the portal entrance.

Site No. 3. 2700 level, adjacent to the portal entrance.

Site No. 4. Jane Creek adjacent to the gauging station.

Site No. 5. Britannia Creek below the confluence with Jane Creek.

Site No. 6 Old township (Mt. Sheer) site

A control site approximately 50km north of Britannia mine, at a similar altitude, near the town of Pemberton was used to collect samples of soil and lichens.

All samples were collected and prepared as outlined in Standard Operating Procedures (SOP) method numbers as follows:

Soil	SOP 3.3.1
Leaves	SOP 3.3.2
Bark	SOP 3.3.3
Catkins, Strobiles	SOP 3.3.6
Lichens	SOP 3.3.7

pH and conductivity of relevant samples was carried out using the following SOP methods:-

Soil SOP 3.4.1 and 3.5.1

Acid digestion of prepared samples was carried using the following SOP methods:

Soil SOP 4.2.1

Leaves SOP 4.2.2

Bark SOP 4.2.2

Catkins, Strobiles SOP 4.2.2

Lichens SOP 4.2.2

Analysis of all extracted samples and water samples was carried out using SOP 3.6.1 (FAAS).

Results

Sample	Type	Site No.	Area	Mean Cu mg kg^{-1}	Rel SD Cu %	Mean Zn mg kg^{-1}	Rel SD Zn %	Mean Pb mg kg^{-1}	Rel SD Pb %
CM1	Soil	1	Shore	94.9	1.39	84.0	3.02	16.1	41.2
BB22S	Spoil	2	2200 portal	1571	6.87	1064	2.25	451.6	14.55
BB27S	Spoil	3	2700 portal	407	6.13	220	5.31	651	7.04
CV4	Spruce	1	Shore	<LOD		12.7	9.34	<LOD	
C225	Spruce	2	2200 portal	<LOQ		12.7		<LOD	
BJ3	Water	4	Jane Creek	0.27	10.4	0.58	2.14	<LOD	
BC2L	Water	5	Britannia creek	<LOQ		0.07	13.4	<LOD	
1103C1	*Usnea* sp	6	Mt Sheer	<LOD	<LOD	23.6	6.64	<LOD	
1103C2	*Parmelia* sp	6	Mt Sheer	7.11 <LOQ	13.9	23.2	5.6	<LOD	
1103C3	Bark	6	Mt Sheer	8.52	7.0	78.5	2.3	<LOD	
1103C4	Leaves	6	Mt Sheer	15.4	5.9	101.0	4.6	<LOD	
1103C5	Soil	6	Mt Sheer	1120	2.7	5.57	5.6	47.4	11.2
1103C6	Strobiles	6	Mt Sheer	21.0	2.8	32.8	3.9	<LOD	
1103C6A	Staminate catkins	6	Mt Sheer	26.0	4.1	41.0	6.2	<LOD	
1103C7	Soil	6	Mt Sheer	1100	1.8	157.0	3.7	25.1	14.5
1103C8	Water	5 Nov 2003	Britannia Creek	0.058 <LOQ	15.1	0.049 <LOQ	5.5	<LOD	
1103C10	*Usnea* sp		Control	<LOD		29.4	2.6	<LOD	
1103C11	Soil		Control	17.3	4.6	30.7	2.4	<LOD	
1103C12	*Parmelia* sp		Control	6.65 <LOQ	14.4	26.8	1.6	<LOD	

Table 9.1 Target metal concentrations in samples collected in the vicinity of Britannia Mine in January and November 2003. Control samples shaded.

Table 9.1 illustrates the concentrations of Cu, Pb and Zn determined in each sample analyzed together with the mean and other statistical information. In all cases where Pb was detected, 'noisy' signals were encountered especially where lower concentrations were present. Generally however the results show good reproducibility when related to similar results obtained in this area of environmental analysis.

The values in Table 9.1 show that Britannia Beach shore spoil exhibits noteworthy concentrations of Cu and Zn with lower concentrations of Pb. Britannia Mine 2200 level spoil contains large concentrations of Cu, Zn and Pb. Only spoil from the 2200 level exhibited a significant concentration of Cd. (3.5 mg kg^{-1}). All other spoil samples produced signals below the LOQ for the instrument in use.
This table also shows that spoil at the 2700 level portal contains lower concentrations of Cu, Zn and than the spoil at 2200 level but a higher concentration of Pb. Cd was not detected in this spoil sample.

The spruce needles collected at the shore site contained significant concentrations of Zn and traces of Cd. The spruce needles collected at the 2200 level portal, exhibit similar concentrations of Zn to those collected by the shore, however traces of Cu were detected but Cd was not detected. Target metals were not detected in water from Britannia Creek above the confluence with Jane Creek. Table 9.1 shows that Jane Creek however contains small concentrations of Cu and Zn. Samples taken from Britannia Creek below the confluence with Jane Creek (BC2L series,) contain only traces of Cu and low concentrations of Zn.

Figure 9.6 illustrates the variation in metal concentrations in the spoil samples. 2200 level spoil has the highest concentration of Cu, Zn and Cd whereas the sample of 2700 level spoil exhibits the highest Pb concentration.

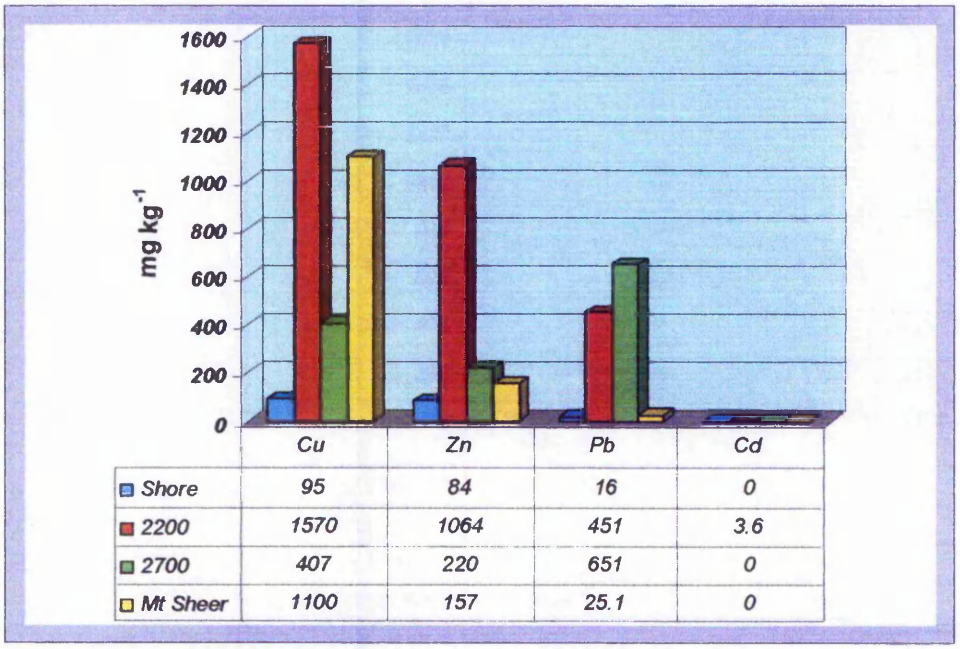


Figure 9.6 Concentration of target metals in spoil and soil samples collected at Britannia Mine, British Columbia, Canada.

Table 9.2 shows that pH values obtained from the spoil at 2700 and 2200 level portals is higher than values obtained by the samples collected at the township site. Conductivity values obtained at both portals were again higher than those obtained at the township sites.

Sample	Date	Type	Location	pH	Cond μScm^{-1}
BB22S	01/03	Spoil	2200 portal	3.0	860
BB27S	01/03	Spoil	2700 portal	2.9	850
1103C5	11/03	Soil	Mt Sheer	5.4	190
1103C7	11/03	Soil	Mt Sheer	5.9	141
1103C11	11/03	Soil	Control	5.1	594

Table 9.2 Conductivity and pH of soil samples collected in January and November 2003, Britannia Mine, BC, Canada.

Discussion

All spoil samples collected in January 2003, contained noteable concentrations of Cu, Zn and Pb (see Figure 9.6) The spoil around the 2200 level portal contains relatively high proportions of all target metals and the 2700 level spoil contains the highest concentration of Pb (651 mg kg^{-1}). Cadmium however was not detected in the spoil from the 2700 level. Surprisingly, the lowest concentration of target metals detected was in the sample collected on the sea shore at the edge of the fan area. The fan area was the site of the concentrators in the past and one would have expected a higher concentration of Cu in particular in this area. Much of the analysis by visiting

consultants has been carried out in this area (Golder Associates, 2002b. URS,2002). The sample from this site was taken from an area close to the high tide mark and may be subject to 'splashing' with subsequent accelerated leaching of metals. Core sampling methods in this area have been used in the past (Golder Associates, 2002B) and higher values of Cu, Zn and Pb were recorded. It would appear that metal concentrations on this area vary since some areas have been overlaid with gravel, earth etc. The values recorded from core samples taken in February 2001(Golder Associates, 2002B) in the same area are however much higher suggesting that additional leaching due to sea water splashing had taken place.

The dispersion of metals from these spoil heaps must be considerable due to heavy rainfall causing general erosion and eventual dissolution. All of the roadways and railways were constructed using spoil consequently further dispersion in the region must have taken place. Thompson and McComas, (1974), recorded Cu concentrations of 1300 $\mu g\ g^{-1}$ in the sediment in Howe Sound, collected in the vicinity of Britannia just after the mine closed. This gave rise to concern and according to Hagen, 2001, this has now reduced to below 300 $\mu g\ g^{-1}$. The concentration in 2200 level spoil collected at the surface of the spoil heap was determined as 1570 $mg\ kg^{-1}$ in January 2002.

Copper concentrations in the soils collected in the vicinity of the old township are between the concentrations determined in the soil around the 2200 and 2700 level mine entrances. Zinc and lead concentrations are generally lower than the mine entrance samples. Since Mount Sheer site is in the Britannia Creek valley at a lower altitude than either the 2200 or 2700 level entrances it is suggested that the metal content of the soil is to some extent, related to the use of spoil from the mine workings as foundations for the township or the metal contamination is as a result of atmospheric transport from the open cast mines further up the valley. Atmospheric transportation of metalliferous pollution from mine workings has been reported by many researchers including Shparyk and Parpan (2004) in the Ukraine, Gregori *et al.*, (2003) in Chile and West *et al.* (1997) in England. Copper and lead concentrations are higher at both township sites when compared with the control and zinc concentrations are higher in the vicinity of the fallen *A. rubra*. Since background concentrations of the copper zinc and lead at the control site are much lower than the

concentrations in the vicinity of the township, then the metal content of the soil here is the result of importing mine spoil for foundation work and atmospheric transfer from the 'glory hole' workings.

The samples of spoil collected near the 2200 level portal contain copper, lead and zinc concentrations in excess of limits outlined by CCME (2002) for all classifications of subsequent use (Table 9.3). Similarly spoil from near the 2700 level portal contains copper and lead concentrations in excess of all permitted values, however zinc concentrations exceed only agricultural and residential/parkland permitted values. Samples collected at the township site contain only copper concentrations in excess of all permitted values, other metal concentrations fall within the limits for types of end use.

Metal	Agricultural	Residential/Parkland	Commercial	Industrial
Cadmium	1.4	10.0	22.0	23.0
Copper	63.0	63.0	91.0	91.0
Lead	70.0	140	260	260
Zinc	200	200	360	360

Table 9.3 Environmental quality criteria in Canada for contaminated sites (CCME, 2002). Concentrations given in mg kg^{-1}

The analysis of spruce needles collected from the spoil tips adjacent to the 2200 and 2700 portals showed significant concentrations of Zn only (12.7 mg kg^{-1}). This may well be a normal concentration for this species since it is an essential trace element. This could be verified by comparison with samples collected away from the immediate area of the workings, probably in the next valley north and at a similar altitude. Markert in 1993 concluded that temporary seasonally conditioned differences in concentration exercise a greater influence for individual elements. Comparisons of target metal concentrations in samples collected in summer during the growing season would also confirm or otherwise this hypothesis. Traces of Cu were recorded in the spruce needles collected at the 2200 level. The trees sampled on this occasion were apparently very young specimens (shore site 2m high, 2200 site 3m high) therefore any bioaccumulation would be in initial stages. A further consideration is that the outer bark of a trunk is more enriched by heavy metals than

the inner bark. The least accumulation is in trunk wood. The advantage of analyzing bark and twigs as opposed to leaves is their permanence throughout the year (Ernst, 1993).

pH values for the township soil samples and the control site were all acidic ranging from pH 4.8 to pH 5.4. These values are not unexpected in this general area due to geological (predominantly granite) and ecological (boreal forest) structure. Spoil samples from the 2200 and 2700 level, collected in January 2003 yielded lower pH values ie. more acid than soil collected around the township.. Conductivity values of the 2200/2700 level spoil samples are also higher than the township samples. The acidity of the spoil samples could be as a result of contact with AMD which until 2000 passed through the portals and eventually into Britannia Creek (*pers. comm.* Brennan Lang). The higher conductivity (860μs cm^{-1}) could be a result of contact with AMD releasing more soluble ions from the matrix. Interestingly the control soil sample has a higher conductivity then the town ship samples but a similar pH value. In theory metals should be leaching from the spoil near the portals at a much faster rate than from the soil in the township due to the lower pH values and the fact spoil areas are on a steep slope. Long term monitoring would be necessary to confirm this proposal.

The lichen, *Usnea* sp., collected from the township site and the control site exhibited similar concentrations of zinc which with other heavy metals are necessary for many enzymatic and physiological processes occurring within plants (Mathys, 1980). Similarly *Parmelia* sp. collected from the control site produced comparable zinc concentrations to the sample collected from the root area of the fallen *A. rubra* despite differences in the zinc concentration of the adjacent soil samples. *Parmelia* sp. indicated a small accumulation of copper whereas *Usnea* sp. collected from the branches of trees at the township and control sites had not accumulated major concentrations of copper. This indicates that atmospheric dispersion of target metals have ceased in the vicinity of Mount Sheer. It is however suggested that the low concentrations of copper found in *Parmelia* sp. at both sites is probably due to rain water splashing particles of copper from the surrounding soil onto the lichen.

Alnus rubra is an angiosperm which produces strobiles and staminate flowers or catkins (Brockman, 1979). These tissues together with bark and leaves were analysed and the metal concentrations detected are shown in Table 9.1. The accumulation factor (Arias *et al*, 1998) for the detectable metals in each type of tissue relative to the soil in the vicinity of the roots is shown in Table 9.4.

Alnus rubra	Accumulation Factor %	
Tissue	Copper	Zinc
Bark	0.77	14.0
Leaves	1.4	18.0
Strobiles	1.9	5.9
Staminate catkins	2.4	7.4

Table 9.4 Accumulation factor of copper and zinc in different tissues of *Alnus rubra*.

Bark from *A. rubra* has accumulated less copper than other tissue samples and staminate catkins have accumulated the highest concentration of copper. Gabriel and Patten, 1994, did research in Arizona on leaves from honey mesquite trees and indicated that these tissues from this species were good accumulators of heavy metals. This research shows that the flowers and fruits of *A. rubra* accumulate more copper than the other tissues analysed. Zinc concentration appears to be higher in leaf tissue followed by bark, strobiles collected from this tree exhibit the lowest zinc accumulation factor, though this may be a function of species physiology.

Spruce trees growing in the 2200 portal area, where metal concentrations are generally higher than those in the township site, have not accumulated copper whereas *A. rubra* has accumulated relatively high concentrations of copper (15.4 mg kg^{-1}). Zinc accumulation in spruce needles is similar in samples collected from the shore area and in the vicinity of 2200 portal, although zinc concentrations in relative soils are very different. Zinc accumulation in *A. rubra* is higher than that accumulated by spruce needles despite the fact that soil surrounding *A. rubra* contains significantly less zinc than that surrounding the spruce trees under investigation. These data reflect different accumulation rates between species.

Target metal concentrations in the creek waters were low with only the water in Jane Creek registering Cu and Zn. The plugging of the 2200 portal with a structure underground would seem to have prevented major outflow of mine drainage into Jane Creek and subsequently into Britannia Creek. It should be noted however that the water samples were collected in January; with snow around the 2200 level consequently the water temperature would be in the order of 3 - 4^0C. Water samples collected in November 2003 at the same collection point exhibited similar target metal concentrations when water temperatures was recorded as 5^0C. Work carried out in the UK (Wilson 2002) demonstrated that the concentration of ions in river and stream water reduced rapidly with lower temperatures and it could be that summertime concentrations of heavy metals in Britannia Creek would increase.

Metal pollution in the vicinity of Britannia Mine is extensive and persistent. All target metal concentrations on the hillsides adjacent to the portals exceed the maximum values outlined by Environment Canada for residential/parkland. Copper and Zinc concentrations in the soil within the old township also exceed the commercial/parkland values. Lack of bioaccumulation within lichen species collected in the vicinity of the old township indicates that atmospheric pollution has ceased. Partitioning in *A. rubra* had occurred but in this case the annual tissue (leaves) accumulated more than perennial tissue.

Chapter 10

Heavy metal concentrations in an arid environment around Lavender Pit, Bisbee, Arizona, USA.

Lavender Pit was chosen for investigation because of its location in an arid climatic zone and sedimentary rock geology resulting in alkaline soil conditions. The mine was operated for 25 years but the scale of the operation is approximately12 times more extensive than Parys Mountain. Additionally the town of Bisbee is located on the very edge of the workings.

Figure 10.1 Location of Bisbee, Arizona, USA.

Figure 10.2 Central Bisbee, Arizona USA

Introduction

The town of Bisbee (elevation 1632m) is situated 325 km south east of Phoenix and about 11.0 km from the Mexican border on AZ Highway 80, on the edge of the Chihuahuan desert (Figure 10.1). Lavender Pit lies 1.0 km south east of Old Bisbee adjacent to Highway 80 (Figure 10.3.).

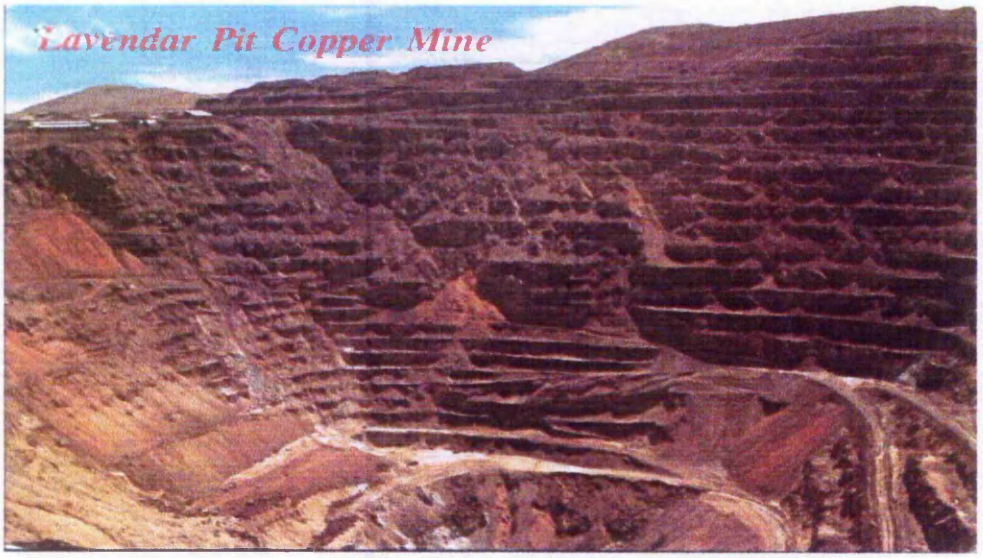


Figure 10.3 Lavender Pit, Bisbee, Arizona, USA, viewed from the highway.

The prevailing winds are from the south to southeast and are described as 'strong gusty'. Rainfall is limited averaging 467mm annually (Brown, 1994). The habitat

surrounding the town and mine workings is described as Madre and evergreen woodland rapidly fading into Plains and Great Basin grassland to the south and east with Chihuahuan desert scrub to the west (USEPA, 1997).

Copper mining activity has been carried out in the Bisbee area since 1880. The town of Bisbee, named after one of the first investors in mining, Judge DeWitt Bisbee, was founded in the Mule Mountains solely as a copper mining camp. The development of Lavender Pit began in 1950 and was worked continually until 1974. Three small communities disappeared as the pit was developed (Francaviglia, 1982). As well as mining, several smelters were operated in the vicinity over the years, the final unit at Douglas being closed in 1987 because of air pollution laws and regulations (Leaming, 1998).
The area, presently, is surrounded by old workings and spoil tips although the old town has undergone rejuvenation with the influx of various artisans. The area has experienced an increase in tourism in recent years which has helped increase prosperity, mainly as a result of mine tours.

Several underground mines were operated here before open cast work was carried out, initially in the Sacramento Pit, followed by the much larger Lavender Pit in 1954. Extraction took the form of daily blasting, followed by the removal of ore to the local concentrator and spoil to waste dumps. Smelting of ore was carried out in three smelters in the Bisbee area until World War 2 but ore from the Lavender Pit was transported 42 km east to Douglas, the last smelter operating in the area (Leaming, 1998).

Lavender Pit embraces an area of 121 hectares (300 acres) and was mined in 15 meter benches. The ores mined were principally chalcite, chalcopyrite and bornite with associated deposits of galena and sphalerite. It is estimated that 386 million tonnes of material were removed from the pit of which 96 million tonnes was copper ore, 113 million tonnes of leach material and 177 million tonnes was waste rock (Francaviglia, 1982).

Little research appears to have been done in the area with reference to the dispersion and accumulation of heavy metals. A thesis was written by Lewis at Arizona State

University in 1977 entitled 'Influence of an open pit copper mine on the ecology of an upper Sonoran intermittent stream.' and a paper by Gabriel and Patten (1994) proposed a tree species (honey mesquite) as a useful biomonitor for emissions from copper smelters.

Towne, (2000) published a report on ground water quality in the vicinity and collected samples from the area surrounding mine tailings in Mule Gulch near Bisbee. The analysis carried out determined fluoride, sulphate, nitrate and total dissolved solids. Cation concentrations were not reported.

Due to the intensive mining and smelting operations carried out in the Bisbee area during the last century, large quantities of ore particulates and smelter emissions would have been carried on the prevailing winds into the old town of Bisbee. Brewery Gulch, a narrow valley projecting generally northwards from the town, would have been particularly affected due to topographical effects similar to those produced by prevailing winds on spoil tips in the vicinity of smelters in Canada (Goodarzi *et al.*, 2002).

Past mining and smelting of sulphide ore (pyrite – chalcopyrite – sphalerite) sites can represent important sources of metal pollutants for watercourses and soil (Yukselen and Alpaslan, 2001, Arias *et al*, 1998). Accumulation of heavy metals by plants has been shown to be related to their concentration in the soil and leaves have been reported as the most consistent accumulator of Cu, Zn and Pb (Pyatt, 1999. Murphy *et al.*, 2000. Luyssaert *et al.*, 2003.). Some workers have reported that wood in *Betula* spp. was a better bioindicator than the leaves (Scharenberg and Ebeling, 1996). Tree bark has also been reported as a good indicator of atmospheric deposition from stationary sources (Bellis *et al.*, 2001) however this is related to the duration of exposure to direct deposition onto the bark surface (Bellis *et al*, 2003).

The aim of this current research was to study the extent of the dispersion of copper and associated heavy metals from Lavender Pit into the old town and in particular along Brewery Gulch, by analysing samples of soil and vegetation at intervals down wind from the pit.

Materials and Methods

Initial samples were collected in June 2003. Soil and leaves were collected from three sites in Brewery Gulch and a site near the observation point on the edge of Lavender Pit (Figure 10.4). Samples of soil were collected from an area in San Jose, 4 km. south of the pit and unaffected by any visible workings or spoil heaps (sample AZ12). This site was selected as a control since it was up wind from Lavender Pit, however long and detailed discussion with local inhabitants highlighted the fact that the area from which the sample was collected had been levelled about twenty years ago by the addition of waste rock originally from Lavender Pit. It was then decided to use an alternative site for control purposes. Soil and leaf samples were collected from an area 10 meters south of mile post 3 on McNeal Road, approximately 16 km north of Lavender pit on the other side of the Mule Mountains.

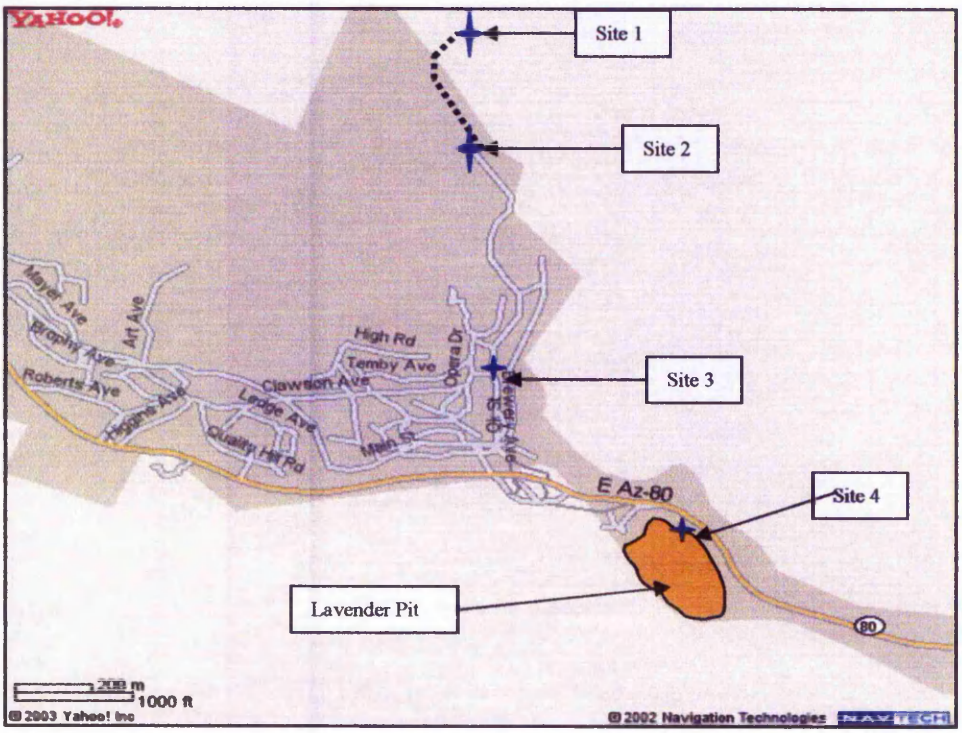


Figure 10.4 Sample site locations, Bisbee, Arizona, USA.

The tree species used were identical at sites 1, 2, 3 and 4 (*Salix amygdaloides*), however this species was absent in the area surrounding the control site and an alternative species (*Rhus glabra*) of similar height was employed. The use of

mesquite species as suggested by Gabriel and Patten (1994) was not possible since this species was not available at the majority of sites. Additionally bark samples were collected from the same branch as the leaves from the tree at site 1 to determine whether such tissue would accumulate more of the target metals and would therefore be a better bioindicator for future investigations.

Site 1 was a level platform adjacent to the footpath north east of the ruins beyond the road end in Brewery Gulch about 2.0 km directly down wind of Lavender Pit. Site 2 was on the west side of the paved road approximately 1.5 km from the pit and site 3 was between the paved road and the town park, about 1.0 km from the pit. Site 4 was immediately adjacent to the northern rim of the pit.

All samples were collected and prepared as outlined in Standard Operating Procedures (SOP) method numbers as follows:

Soil	SOP 3.3.1
Leaves	SOP 3.3.2
Bark	SOP 3.3.3

pH and conductivity of relevant samples was carried out using the following SOP methods:-

Soil	SOP 3.4.1 and 3.5.1

Acid digestion of prepared samples was carried using the following SOP methods:

Soil	SOP 4.2.1
Leaves	SOP 4.2.2
Bark	SOP 4.2.2

Analysis of all extracted samples and water samples was carried out using SOP 3.6.1 (FAAS).

Ten replicates of each sample were digested/filtered and stored in a refrigerator for subsequent analysis. Only 6 replicates of the bark sample were processed.

Results

Table 10.1 shows Cu, Zn and Pb concentrations and RSD obtained from samples of vegetation and soil samples collected at sites around Lavender Pit. Cadmium was detected at a concentration of 3.5 mg kg^{-1} in soil collected from site 4 on the rim of the pit.

Dried leaves of *S. amygdaloides* from all sites have similar concentrations of Cu and Zn however only leaves from sites 2 and 3 had noteable concentrations of Pb. Dried leaves of *R. glabra* from control site exhibited the lowest concentrations of Cu and Zn. Bark from site 1 exhibited higher Cu and Pb concentrations than the leaves from the same branch but a lower Zn concentration.

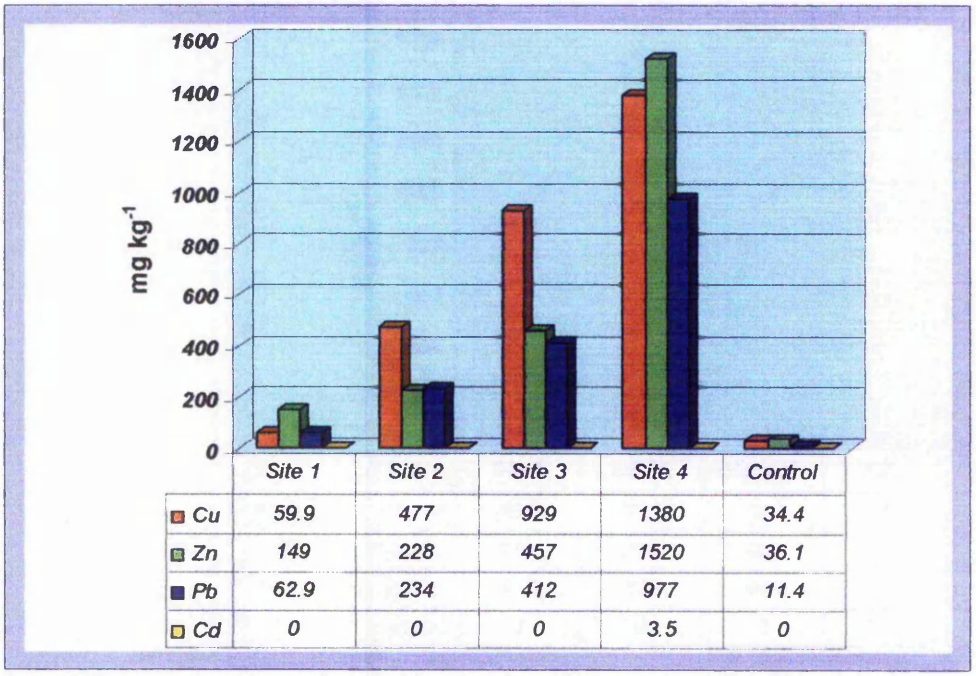
Sample	Site	Type	Mean Cu mg kg^{-1}	RSD Cu %	Mean Zn mg kg^{-1}	RSD Zn %	Mean Pb mg kg^{-1}	RSD Pb %
AZ1	1	Bark	18.7	34.8	16.4	12.8	77.36	20.4
AZ3	1	Leaves	14.7	10.38	26.6	5.4	<LOD	
AZ4	1	Soil	59.9	2.84	149	1.93	62.9	11.2
AZ6	2	Leaves	16.0	23.9	22.0	11.0	38.6	19.3
AZ7	2	Soil	477	15.7	228	9.64	234	11.2
AZ8	3	Leaves	16.9	3.54	22.5	3.42	34.4	40.0
AZ9	3	Soil	929	6.44	457	3.91	412	11.14
AZ10	4	Leaves	19.0	11.4	28.0	5.98	<LOD	
AZ11	4	Soil	1380	1.61	1520	2.46	977	5.33
AZ12	5	San Jose	215	6.34	186	2.52	225	3.49
AZ13	Control	Leaves	2.84 <LOQ		21.6	5.8	<LOD	
AZ14	Control	Soil	34.4	5.5	36.1	3.0	11.4	7.9

Table 10.1 Metal concentration and RSD values obtained from soil and vegetation samples collected in the vicinity of Lavender Pit, Bisbee, AZ, USA. Control samples shaded.

RSD values calculated for various samples vary considerably although when metal concentrations are relatively high, (>950 mg kg^{-1}) RSD values are lower. Vegetation samples generally produce higher variation in RSD values. Such variation is normal in environmental sample analysis due heterogeneous nature of the material under

examination and in particular the natural heterogeneity in the contamination of the samples (Kozlof *et al.*, 2000).

Figure 10.5 illustrates that dried soil from site 1, furthest down wind from Lavender Pit exhibits the lowest concentrations of Cu, Zn and Pb and soil from site 4 exhibits the greatest concentration of all target metals.



	Site 1	Site 2	Site 3	Site 4	Control
Cu	59.9	477	929	1380	34.4
Zn	149	228	457	1520	36.1
Pb	62.9	234	412	977	11.4
Cd	0	0	0	3.5	0

Figure 10.5 Metal concentration in soil samples collected from sites in the vicinity of Lavender Pit, Bisbee, AZ, USA.

Table 10.2 illustrates pH and conductivity values obtained from soil samples collected from the various sites.

Sample	Site	pH	Conductivity µs cm^{-1}
AZ4	1	7.33	138
AZ7	2	8.49	104
AZ9	3	8.04	247
AZ11	4	8.48	158
AZ14	control	8.83	126

Table 10.2 Conductivity and pH of dried soil samples collected in the vicinity of Lavender Pit, Bisbee, AZ, USA.

The lowest pH (7.33) was recorded at site 1 and the highest pH (8.83) was recorded at the control site. Highest conductivity value (247 $\mu S\ cm^{-1}$) was obtained at site 3.

Discussion

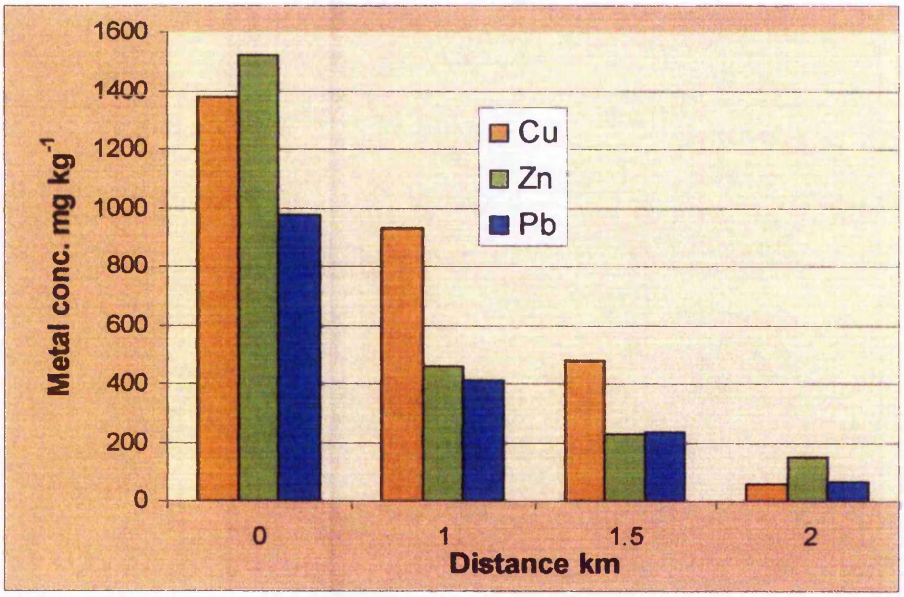


Figure 10.6. Metal concentrations in soil samples related to distance from Lavender Pit.

Figure 10.6 illustrates the decreasing concentration of the target metals with increasing distance from Lavender Pit. Background concentrations of lead and zinc as illustrated by soil samples collected at the control site (Table 10.1) are approximately five time less than the concentrations of these metals collected the end of Brewery Gulch (site1) although background copper concentrations at the control site are approximately half of those detected at the head of Brewery Gulch.. The ratio of the metals in the soil however varied with zinc exhibiting approximately 3 times the concentration of Cu and Pb at Site 1. At sites 2 and 3 the concentration of Cu was approximately twice that of Zn and Pb and at site 1 the concentration of Cu and Zn was similar but approximately 1.5 times higher than Pb. Natural heterogeneity in the soil background concentrations could be a factor here but more likely is the fact that some heavy metal concentrations decrease through the source-transport-deposit process (Charlesworth and Lees, 1999). This could be due to selective onward transport of finer materials, relative specific gravity of the minerals concerned or other anthropogenic processes such as the contribution of Pb from gasoline in the

past. This latter factor may have a bearing on the high Pb concentration (77.3 mg kg^{-1}) in bark samples collected at site 1 when compared with the leaf Pb concentration. This site is in the narrowing section of Brewery Gulch and motor vehicles have and still do use the approach to this area extensively. The effects of wash out and rain out in the past cannot be ignored. More than half of the total annual rainfall occurs in July, August and September (Brown, 1994) and this would influence the deposition rate of airborne minerals (Clarke, 1997) and indeed the deposition of lead from gasoline in the past. The author considers that wash out and /or rain out would have more effect on Pb emissions due to gasoline and consequently this would have the greatest effect on site 1. This is highlighted by the concentration of Pb in the bark samples from this site.

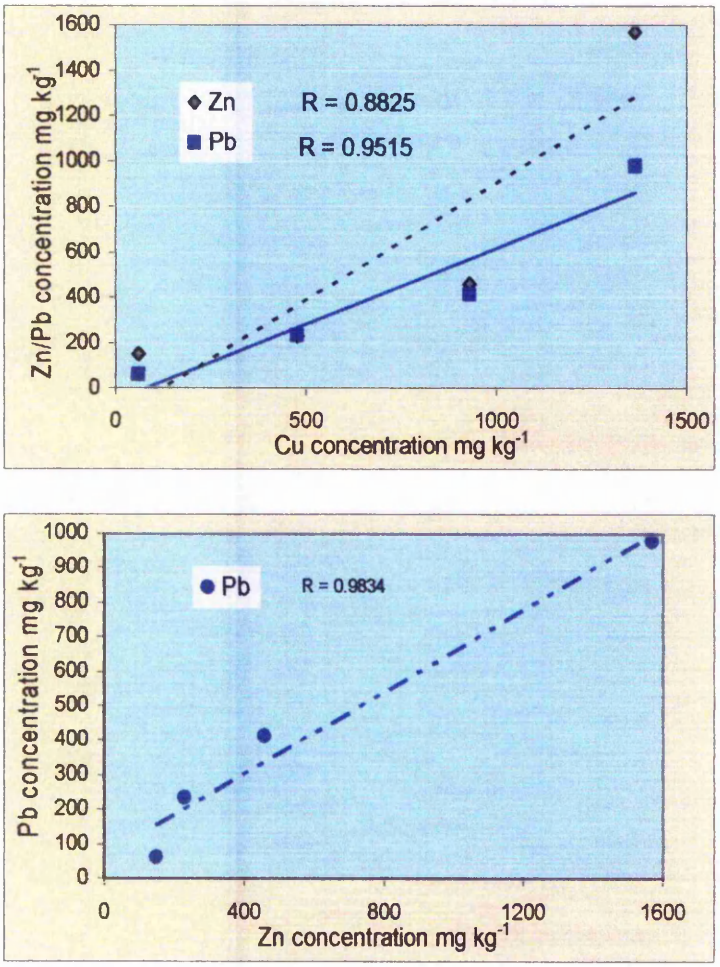


Figure 10.7 Correlation coefficients obtained for the concentrations of Cu and Zn, Cu and Pb and Zn and Pb in dried soil samples collected in the vicinity of Lavender Pit, Bisbee, Arizona.

Correlation coefficients obtained for metal to metal concentrations as illustrated in Figure 10.7 show good linearity (Miller and Miller, 2000) confirming the consistency

of the dispersion processes along Brewery Gulch almost certainly due to the canyon effect rather than open area dispersion. The coefficient for value and graph for zinc demonstrates that this metal is more variable throughout the dispersion pattern than copper or lead due to its amphoteric nature.

The metal concentrations at site 5, south of San Jose, approximately 4 km from Lavender Pit were unexpected because the area is not subject to particulate pollution from any of the mine workings due to the site being south of the workings and the prevailing winds deposit particulates to the north. The nearest possible source of contamination would have been the old tailings pond 1.5 km due east of the site (Francaviglia, 1982).

The prevailing winds however would not deposit mineral dusts from the pond in this area. Additionally the site is situated in the mainly gravel deposit geological area isolated from the granite porphyry intrusion which served as the primary host for the deposits of copper minerals (Leaming, 1998). The site was by necessity located at the side of the road leading south to Mexico. It is suggested that the hardcore used in the road construction came from the spoil heaps surrounding Bisbee, confirming the ideas put forward by the local population. Metal concentrations in the soil collected at the control site on McNeil Road were lower than those detected at the furthest site from Lavender Pit in Brewery Gulch and probably represent the general background concentrations of soils in south eastern Arizona.

Metal concentrations in leaves taken from *S. amygdaloides* at all sites exhibited similar concentrations of Cu but where Pb has been accumulated i.e. at sites 2 and 3, zinc concentrations were lower. This suggests that Pb may replace Zn in the accumulation process. Cu concentration in leaves of *R. glabra* collected at the control site was lower than other sites although Zn concentration was similar to leaves collected at site1. Although Luyssaert *et al* in 2003 indicated that metal accumulation was specific for chemical elements, tree species and deposition regimes, the low metal concentrations detected are probably the result of low metal concentrations in the surrounding soil.

Significant concentrations (>34 mg kg^{-1}) of Pb in the leaves of *S. amygdaloides* collected at sites 2 and 3 were not unexpected because the soil collected at these sites exhibit relatively large concentrations of Pb. However, the concentrations in the leaves were of a similar order of magnitude and did not appear to be related to the relative concentration of Pb in the respective soil sample. Additionally Pb was not detected in leaf samples from the same species collected at sites 1 and 4 despite a relatively high concentration of Pb in the soil at site 4 (977 mg kg^{-1}). Soil from site 1 does have the lowest Pb concentration and the Pb concentration in the leaf samples could be below the detectable limit of the instrument in use. Some relationship to historical output of Pb from motor vehicles could be responsible for the Pb content of the leaves under examination since sites 2 and 3 are in an urban environment. Site 4 is on the windward side of the highway which has been moved progressively northwards as the size of Lavender Pit was increased. Analysis of bark from the trees in question might confirm this possibility. (Bellis *et al*, 2001). An alternative factor may be the effect of rain water collection in the vicinity of the trees from which samples were taken, since sites 2 and 3 lie in a definite drainage pool zone whereas sites 1 and 4 are in a raised position and would not be subject to drainage or drainage retention.

Accumulation of heavy metals is still taking place and is likely to continue for some considerable time. Similar dry, desert scrub areas in the Middle East still exhibit high concentrations of heavy metals (>11,000 ppm) several millennia after metalliferous workings have ceased (Pyatt *et al*, 2000). Evdokimova and Mozgona (2003) and Wilson *et al.* (2005B) demonstrated that heavily contaminated soil remained toxic for a forecasted period of at least 100 years in a wet temperate environment in Russia and Canada respectively.

The control site on McNeil road is however subject to flash flooding and excessive drainage which may contribute to the relatively high pH value of soil (8.83) recorded in this vicinity. pH values of all soil samples collected in this area are alkaline due to the presence of limestone, shale and sandstone throughout the region. Site 1 at the head of Brewery Gulch is the most vegetated of the sites examined and subsequent decomposition of vegetative detritus would result in lower pH values (Table 10.2); other sites in Bisbee have similar pH values. Conductivity values are of a similar

magnitude at all sites except site 3 which has a higher value, reflecting higher concentrations of soluble material in this area.

Element	Soil Limit Value mg kg^{-1} dry matter.
Cadmium	1 - 3
Copper	50 - 140
Lead	50 - 300
Zinc	150 - 300

Table 10.3 Environmental quality criteria in the EU. Soil quality criteria , maximum admissible concentration.(after Radojevic and Bashkin, 1999).

Table 10.3 illustrates the European Union maximum admissible concentration of the metals under examination. The control site and site 1 comply with the soil limit values for all four metals. Site 2 and site 3 exceeded the Cu limit values while sites 3 and 4 exceeded Cu, Zn and Pb limit values and site 4 also exceeded the Cd limit value. The landscape in the vicinity of greater Bisbee has a barren, neglected appearance with only patchy 'colonies' of vegetation. On abandoned mine workings in California where much lower concentrations of copper and associated metals were recorded, re-vegetation is extremely slow (Kruckeberg and Wu, 1992) and the mines under investigation were abandoned in 1947. Clearly the concentrations of heavy metals in the soil and vegetation in the vicinity of Bisbee will last for a considerable time in the future.

The accumulation of heavy metals through trophic levels is important and generally well understood; grazing animals in the area could have accumulated significantly higher levels of heavy metals than the surrounding soil. (Smith and Rongstad, 1982). Ingestion of such animals together with ingestion of airborne particles and drinking of contaminated water would have had an adverse effect on the health of the human population living in the area (Pyatt and Grattan, 2001). The trees under examination were approximately 3 m high and were less than 30 years old. It is probable that the accumulation of heavy metals in the bark is mainly related to wind deposition of dust up to the present day although mining operations have ceased. Dust from tailings dumps in South Africa and the Peruvian Andes were reported to be affecting the amenity and health of residents in near by townships (Balkau, 1999, Bech *et al.*, 1997) and the same could be said of Bisbee.

Dispersion and dilution has polluted the brewery Gulch area of Bisbee and the pollution is persistant. Copper, zinc and lead concentrations at all sites within the town exceed EU soil limit values and the Dutch Housing Ministry 'T' values. Bioaccumulation factor for copper within the leaves of *S. amygdaloides* (1.4%) is similar to the factor obtained at Britannia Mine.

Chapter 11

Heavy metal concentrations in the vicinity of a relatively new mine (Mission Mine), near Tucson, Arizona, USA.

Mission mine is a relatively new mine and has been operating since 1961. It is situated within a Native American reserve and any dispersion from the site could have major effects on health.

Figure 11.1 Location of Mission Mine, near Tucson, Arizona, USA

Figure 11.2 San Xavier Mission, near Tucson, Arizona, USA.

Figure 11.3 Aerial view of Mission Mine, AZ, USA (San Xavier Mission marked in the upper right distance)

Introduction

Mission Mine complex (elevation 850 m.) is situated 28km south of Tucson (Figure 11.1) and about 6 km south of San Xavier Mission (Figure 11.2) between Interstate 19 and South Mission Road, The prevailing winds are from the south to southeast and are described as strong gusty. Rainfall is limited averaging 290mm annually (www[4]). The habitat type surrounding the mine workings is desert scrub with the Sierrita Mountains to the south west of the workings.

The mine is in the area where small underground mines existed before the First World War. These mines produced mainly lead and zinc with only small amounts of copper (Heinrichs, 2000). Asarco Inc. commenced production from Mission Mine pit

in 1961 partly on land leased from the O'odham tribe (Anonymous, 1977). The complex consists of a series of pits and the current pit is 4km long, 2.4km wide and 366m deep with 12m extraction benches. The complex produced 28,100 tonnes of copper and 15.34 tonnes of silver in 2003 and currently operates one mill with crushing, grinding and flotation facilities and had 188 employees (www[5]). The peak of production was in 1973 when Mission Mine produced 90,000 tonnes of copper and had just under 900 employees (Heinrichs, 2000). The extracted rock contained a maximum of 25% chalcopyrite of which 0.67% was copper metal (www[5]). In January 2005, the mill had 65 employees operating only to keep the mine and mill open, however the price of copper was rising and there were plans to increase production by April 2005 (*pers.comm.* Mark J Karmi) It is calculated that on average the excavated material before separation etc. contains 1900 mg kg^{-1} of copper. Rock extraction was carried out by daily blasting using ANFO explosive followed by collection by large electrically powered shovels. Ore separation and concentration was carried by crushing and subsequent froth flotation. Copper concentrate was shipped out of Mission Mine complex for smelting elsewhere (www[6]).

The mining operations at the Mission complex have been environmentally monitored by the mining company since the very early days of operation and a wild life reserve has been constructed and managed by the company on former tailings storage sites. Vegetation on the reserve is said not to accumulated copper or other heavy metals (*pers. comm.* Al Cooper). All operations in the pit have been constantly damped with water to prevent dust emissions.

The Mission complex adjoins the San Xavier Indian Reservation, the majority of which is down wind from the workings. Past mining and smelting of sulphide ore (pyrite – chalcopyrite – sphalerite) sites can represent important sources of metal pollutants for watercourses and soil (Yukselen and Alpaslan, 2001, Arias *et al*, 1998). Additionally, Mission Mine complex was reported to have the largest amount of toxic releases to air land and water of all reporting mines in Arizona. Toxic releases totalled approximately 1,410 tonnes in 2002, of which 2050kg was emitted to the air (USEPA, 2004)

The aim of the current research was to determine the extent, if any, of the dispersion of copper and associated heavy metals from the Mission Mine into the St Xavier Tribal lands and the future potential health implications for flora and fauna by analysing samples of soil taken from near the milling operations and at intervals east of the road down wind from the mine site, visible in Figure 10.3 emerging from the centre of the hills behind the mine and progressing left of San Xaviour Mission.

The mine was visited in January 2005 in order to view operational procedures and to collect further samples relating to possible trophic level accumulation within the boundaries if the reserve. In preparation for the January 2005 visit, contact was made with Tohono O'odham Nation office, Sells, Arizona, without response. Attempts to contact the office whilst in Arizona proved negative; therefore data from June 2004 samples was used in this assessment.

Sample collection

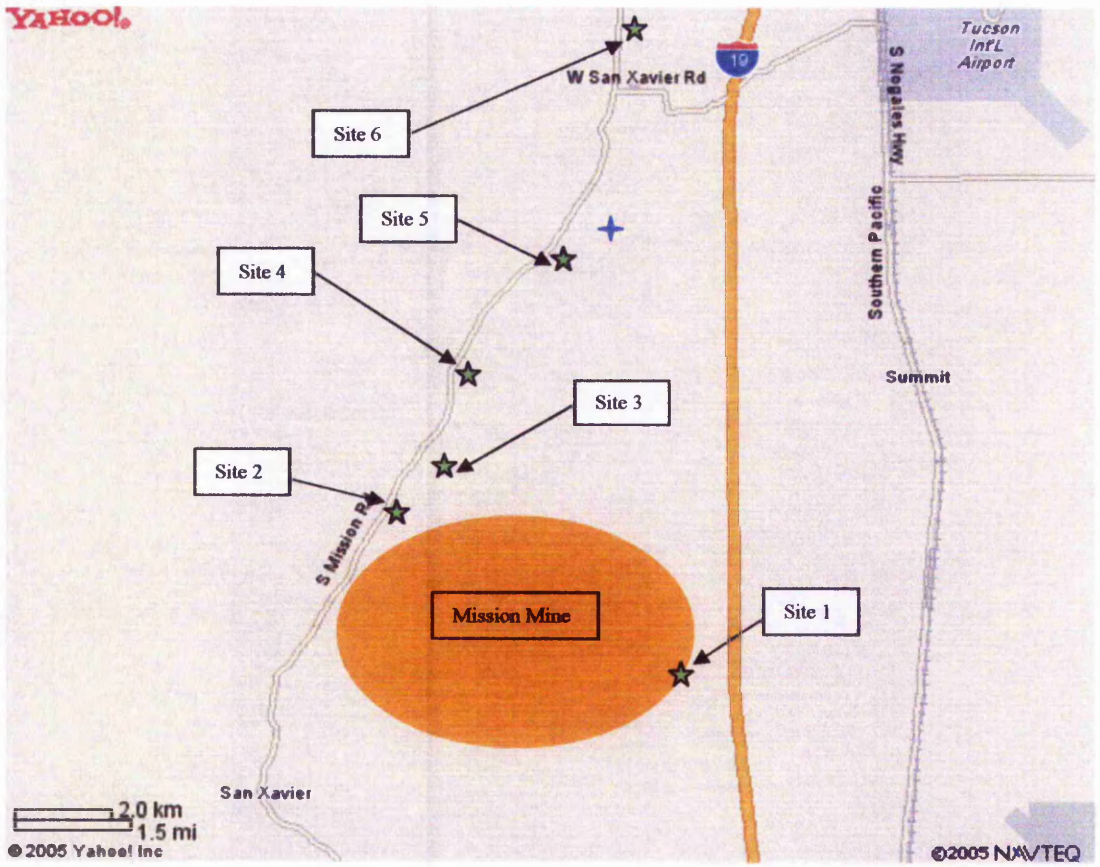


Figure 11.4 Location of sample sites in the vicinity of Mission Mine, AZ, USA

Materials and Methods

Samples were in collected June 2004 and prepared in accordance with SOP 3.3.1 for soil, 3.3.5 *Opuntia violacea* (prickly pear cactuus) and 3.3.8 for faeces. Sampling sites 1 – 6 are shown in Figure 11.4. Site 7 is 4 miles north of site 6 and the control site was situated 16 km northwest of site 2. Table 11.1 illustrates the exact location and types of samples collected.

Site	Sample	Type	Location
1	64A01	Soil	Pima Mine Rd, on edge of tailings dump
1	64A02	*O. violacea* pads	Pima Mine Rd, on edge of tailings dump
2	64A03	Soil	South Mission Road, mile post 13, approx. 200m north mine perimeter.
2	64A04	Cattle faeces	"
3	64A05	Soil	South Mission Road, mp 13.5
4	64A06	Soil	South Mission Road, mp 14
5	64A07	Soil	South Mission Road, mp 15
6	64A08	Soil	South Mission Road, mp 17
7	64A09	Soil	South Mission Road, mp 21
8	64A10	Soil. Control	Control. 22 km NW of Mission Mine

Table 11.1 Sampling sites, location and sample types collected in the vicinity of Mission Mine complex, near Tucson, Arizona, June 2004. Control sample shaded

Ten replicates of each sample were extracted using the appropriate SOP and analysed using SOP 6.6.1 (FAAS)

Vegetation in the vicinity of the sample site was either non-existent or too variable to be useful as biomonitors for each site. *O.violacea*, however, was abundant on the southern edge of the mine, adjacent to a tailings dump, therefore a representative sample was collected for examination. This plant, a species of Prickly Pear cactus,

presented several problems of handling, transportation and preparation and eventually was processed in the same manner as woody sections of other plants.

Results

Each sample extract was analysed for cadmium but signals in most cases obtained were below the LOD for that element for the instrument in use. Cadmium was detected in soil from site 1 however signals obtained were below the LOQ for the instrument

Sample	Site	Type	Mean Cu mg kg^{-1}	RSD Cu %	Mean Zn mg kg^{-1}	RSD Zn %	Mean Pb mg kg^{-1}	RSD Pb %	pH 20°C	Conductivity µs cm^{-1} 20°C
64A1	1	Soil	2377	2.0	435	1.0	83.2	2.0	8.52	110
64A2	1	*O. violacea*	32.2	5.7	89.3	6.1	<LOD			
64A3	2	Soil	42.3	2.8	40.9	1.3	<LOD		8.42	45
64A4	2	Faeces	247	3.0	94.2	2.7	<LOD			
64A5	3	Soil	26.6	6.2	34.1	3.5	<LOD		7.72	28
64A6	4	Soil	38.3	4.2	48.6	4.2	<LOD		7.43	33
64A7	5	Soil	20.5	3.3	39.6	2.0	<LOD		7.19	47
64A8	6	Soil	33.9	3.8	55.9	2.7	9.8 <LOQ	11.2	7.39	29
64A9	7	Soil	30.6	3.5	56.4	3.0	12.1 ≤LOQ	7.8	8.68	76
64A10	8	Soil	18.4 <LOQ	8.0	51.3	2.5	14.7	14.2	8.34	42

Table 11.2 Mean metal concentration,relative standard deviation, pH and conductivity obtained after analysing replicates samples of soil and other substrates in the vicinity of Mission Mine,Arizona. Control sample shaded.

The highest copper concentration in soil was obtained from site 1. Zinc concentration of soil samples showed relatively small variation between sites 2 to 8. Cd was detected only in soil from site 1 near the tailings dump.

Discussion

The highest concentrations of copper, zinc and lead were found in soil sample collected adjacent to Pima Mine Road (Site 1) together with a low concentration of cadmium. This site is approximately 20m from a tailings heap apparently used as a retaining barrier for the site as a whole. The copper content of the soil here is higher than the calculated average concentration for the extracted ore. It is suggested that this phenomenon is as result of either leaching from adjacent tailings, wind dispersion of ore from the extraction operations and the mill or spillage from transport vehicles removing the concentrated ore from the site for smelting. Lead and Zinc concentrations of the soil sample from this site are also significant (435 and 83.2 mg kg^{-1} respectively), however their source would be confirmed if the total composition of the ore being extracted was calculated and possible correlation of composition confirmed (Shparyk and Parpan, 2004)

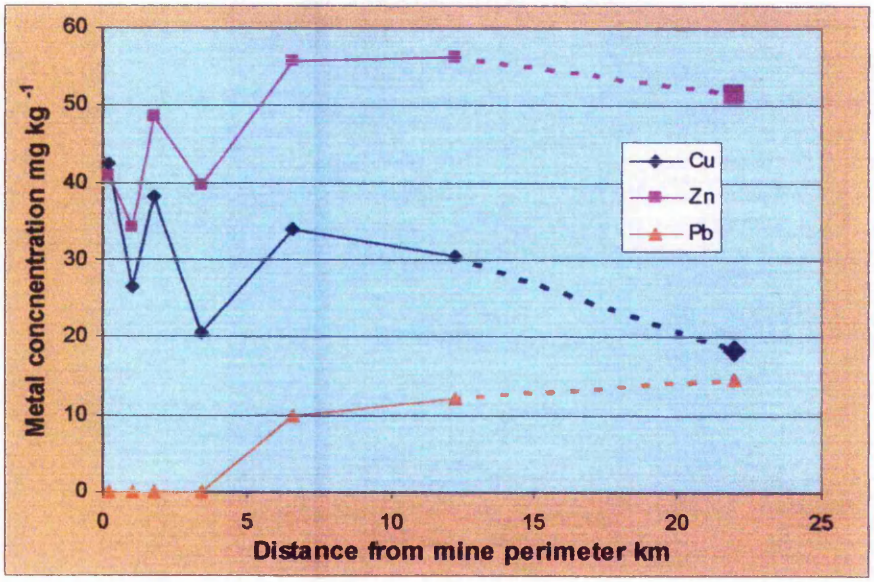


Figure 11.5 Graph illustrating distribution of heavy metals north of Mission Mine, AZ (final data point at 22km is the control site 8)

The sample of *O. violacea* was found to contain an average of 32.2 mg kg^{-1} of copper, which is higher than the level of 21 ppm in plant tissue considered to be an excessive or toxic accumulation of the element by Wallingford and Simkins in 1977. Cox and Hutchinson (1980) however demonstrated that some plants can develop tolerance to

much higher concentrations of copper. Zinc concentration in the pad was not excessive, according to Groet (1980), who recorded concentrations in excess of 100 ppm. in species of trees and grasses collected in areas remote from possible zinc pollution.

Copper concentrations of the soil collected at increasing intervals along a 12km transect north of the workings (Figure 11.5) are generally of the same magnitude with a decreasing concentration trend. The copper concentration in the transect samples range from 2.3 to 1.1 times that of the control site (site 8) 22 km to the north west of the mine and represented in Figure 11.5 as the final data point on each graph at 22 km from the perimeter of Mission Mine. Zinc and lead concentrations generally increase with distance from the mine. It is likely that increased zinc concentrations are the result of decomposition of a greater abundance of vegetation present in the vicinity of sites 4,6 and 7. Increased lead concentrations at site 6, 7 and 8 are possibly due to the more urban situation of these sites subject to much higher volumes of road traffic and subsequent deposition of lead from gasoline in the past.

Replicates of dried cattle faeces sample collected nearest to the workings contained a higher concentration of copper (247 mg kg^{-1}) than the surrounding soil, suggesting that the beast had been ingesting and excreting large quantities of copper compounds. As in the case of Parys Mountain, cattle faeces are not necessarily indicative of bioaccumulation in the area where the faeces were collected since the cattle concerned could have been moved from another area. Cattle faeces are however an accumulator of metals in the vegetation as cattle behave as a vegetation 'concentrator'. This is an interesting aspect of the work and requires further study to establish its usefulness in environmental research. Luckey and Venugopal stated in 1977 that mammals tend to accumulate quantities of copper as well as being able to excrete a proportion of the ingested material; however sheep generally are exceptional in that they accumulate copper relative to supply available to the point of death (Martin, 1998). Similarly zinc concentrations in the faeces are higher than the surrounding soil but this is probably the result of homeostatic processes controlling the absorption of zinc in mammals (Miller and Neathery, 1980).

The conductivity values of all soil samples was relatively low suggesting low soluble salt content (Radofevic and Bashkin, 1999) and the pH values were all above 7.0 suggesting that metal mobility from the soil would be restricted due to sorbtion into the soil structure of much of the bioavailable metal (Alloway, 1999) being present as complex hydroxides rather than simple cations. Torrential rain episodes when naturally acid rain would increase mobility and subsequent possible accumulation by vegetation. Since such episodes of torrential rain are limited in this area analysis of leaves and woody sections of trees or shrubs would indicate episodes of accumulation of metals and analysis of bark samples would determine if atmospheric dispersion had been occurring (Bellis *et al*, 2001). Suitable lichen thalli were not observed in this part of Arizona, preventing possible determination of recent dispersion of metalliferous particulates from the workings.

The dispersion of metalliferous pollutants from this operation has been severely curtailed when compared with the other site in this research due to the introduction of new mining practices. Metal concentrations in the direction of the prevailing winds are less than the soil limit values for all target metals proposed by the EU.

Chapter 12

Heavy metal bioaccumulatuion by *Olea europaea* and *Citrus sinensis* in the vicinity of ancient mine workings at the Limni mine, near Polis, Cyprus.

This site was examined because of the presence of pollution since the Byzantine period and the cultivation of plants producing edible fruit. An investigation in partitioning between annual and perennial tissues could also be carried out.

Figure 12.1 Location of Polis, Cyprus.

Figure 12.2 location of sample and control sites in the vicinity of Limni spoil tips, Cyprus.

Introduction

Polis is located in northwest Cyprus (Figure 12.1) and the Limni workings are approximately 8 km to the east of the town. The control site was situated on the Akamas peninsular approximately 25 km west of Limni. (Figure 12.2). The prevailing winds are from the south west and should not effect the deposition of mine dust on the control site.

Copper exploitation occurred on the island of Cyprus during the Chalcolithic Age (5900 – 4500 BP) and increased during the Bronze Age (4500 – 3050 BP) due to the strategic position of the island (Pyatt, 1999). Forty nine metallurgical and other related sites were recorded in the area east of Polis by Raber (1984) and these sites represent in excess of 2000 years of metal extraction activities from the late Iron Age to the late Medieval period (i.e. from around 2800 BP to 600 BP). Altogether, Cyprus has more than 40 spoil heaps containing more than 4 million tonnes of historic spoil, showing the massive scale of the industry over time (www[7]). Raber, (1984) stated that copper mining and smelting was conducted in the Polis area through the Byzantine and Late Medieval periods and that copper production remained relatively small scale throughout the 2000 years of its history. Pyatt (2001), however, argued that the extent of the site around Limni would cast doubt on this point.

Koucky and Steinberg (1982) recognised five main peaks of mining activity in Cyprus ranging from the late Bronze age to the last period of intensive mining between 1950 and 1970. The dominant copper mineral on Cyprus is chalcopyrite ($CuFeS_2$), which would have been the probable source of copper from the late Bronze Age (Rapp, 1982).

The spoil tips adjacent to the sample collection site at Limni are large covering an area of approximately 2 x 2 km and are often steep sided and prone to atmospheric, sheet and gulley erosion (Pyatt and Birch, 1994) together with leaching after rainfall.

Research on bioaccumulation has been carried out by many workers at metalliferous workings sites around the world (see previous chapters) and Pyatt (2001) investigated

the plant accumulation of copper and lead by *Acacia retinoides* and *Eucalyptus torquata*, two tree species which had grown on the contaminated landscape for a long period.

The aims of the current research were to examine edible plant species currently growing in the vicinity to determine copper, zinc, lead and cadmium concentration in *C. sinensis* fruits (oranges) and also to determine the degree of partitioning, if any in *O. europaea* fruits (olives). Samples of soil, olives and oranges were collected from the control site on the Akamas peninsular on the same day from randomly selected trees of a similar height and from the same aspect.

Methods and Materials.

Soil samples were collected and prepared in accordance with SOP 3.3.1

Olives were collected in accordance with SOP 3.3.6, after drying the olives were separated from the stones using a clean stainless steel scalpel. Fleshy parts of olives were processed as in SOP 3.3.6 together with any oil which had exuded onto the storage dishes. Five stones from olives collected from Limni and the control site were placed in a clean 50 mL polypropylene centrifuge tube with 20 mL of Certified grade acetone (Fisher Chemicals Ltd) and shaken for 30 min in a IKA model KS130 orbital shaker at 360 oscillations per min. This washing procedure was repeated, decanting away the acetone each time, stones were wiped with clean tissues and allowed to dry at room temperature overnight. The stones were prepared for analysis in the same many as the flesh i.e. using SOP 3.3.6.

Olive leaves were collected and prepared using SOP 3.3.2 and bark as in SOP 3.3.3. Additionally oranges, were collected and sliced at the sites using a clean stainless steel scalpel, before placing in polythene bags and returning them to the laboratory. Material from the preparation process was digested using either SOP 4.2.1 (soil) or 4.2.2 (vegetation). Ten replicates of each sample were extracted and analysed except olive stones from each site (4 replicates) and Limni olive stems (4 replicates).

Analysis was carried out using ICP-AES techniques in accordance with SOP 3.6.2.

Results

Sample Ref	Location Type	Mean Cu mg kg^{-1}	RSD Cu %	Mean Zn mg kg^{-1}	RSD Zn %	Mean Pb mg kg^{-1}	RSD Pb %
25FBP01	Limni Spoil	674.0	2.78	<LOD		<LOD	
25FBP02	Limni Soil	193.0	5.8	<LOD		<LOD	
25FBP03	Limni olives Flesh	8.93	3.53	5.02	14.9	0.50	44.44
25FBP03A	Limni olives stone	<LOD		6.40		<LOD	
25FBP04A	Limni olives leaves	5.82	3.46	9.83	6.5	0.4	44.44
25FBP04B	Limni olives stem	4.43		15.3		0.21	
25FBP04C	Limni olives bark	4.38	8.16	7.29	4.34	0.36	<LOQ
25FBP05	Akamas olives flesh	7.11	16.74	5.72	13.47	0.5	66.18
25FBP05A	Akamas olives stone	<LOD		7.15		<LOD	
25FBP06A	Akamas olives leaves	4.77	3.76	13.4	4.22	0.5	61.0
25FBP06B	Akamas olives stem	2.73	14.9	4.54	38.91	0.22	<LOQ
25FBP07	Akamas soil	53.4	2.80	8.40	19.4	345.0	9.14
25 FBP 08	Limni orange	10.4	17.3	9.23	8.5	<LOD	
25 FBP 09	Akamas orange	8.0	13.25	7.28	0.9	<LOD	

Table 12.1 Metal concentrations in soil and vegetation samples collected in the vicinity of Limni spoil tips and the control site. Control samples shaded.

Cadmium was not detected in any of the samples digested and analysed.

The spoil sample collected from the Limni workings contained the highest concentration of copper (674 mg kg^{-1}) but lead and zinc were not detected in 10 replicates of this sample. Similarly a relatively high concentration of copper (193 mg kg^{-1}) was detected in the soil sample collected adjacent to the spoil tips but zinc and lead were not detected in 10 replicates of this sample.

Soil from the control site however had a relatively high concentration of lead and lower concentrations of copper and zinc.

Discussion.

The sample of spoil collected from the Limni workings had a lower concentration of copper than the samples examined by Raber (1982) and Pyatt (2001). Table 12.2 illustrates the metal concentrations recorded.

Metal	Source: Raber	Source: Pyatt	Limni current work
Cu	2000	3600	674
Zn	67	Not recorded	Not recorded
Pb	Not recorded	1200	Not recorded

Table 12.2 Metal concentrations in Cypriot spoils presented as mg kg^{-1}

Metal concentrations in the spoil varies according to collection site, however it should be noted that both Raber and Pyatt expressed their copper and lead concentrations in terms of percentage and the current author has adjusted these values to mg kg^{-1} in order to facilitate comparisons. Table 12.2 demonstrates the variability in composition of samples collected in the same area and on different occasions. The results obtained for the current samples reflect the re-contouring of the spoil tip since 2001 and the recent addition of soil overburden.

The soil sample at Limni was collected from within 1 m of the base of the olive trees and close to the orange trees and was found to contain a copper concentration of 193 mg kg^{-1}. This sample site was situated approximately 35 m west of the spoil tip and would have accumulated there as a result of atmospheric transfer and leaching during rainstorms. The soil collected on the Akamas peninsular contained approximately one quarter concentration of copper when compared to Limni soil. The Akamas soil did however contain a high concentration of lead (345 mg kg^{-1}), although this value does not exceed the soil guide line values for residential and allotment use. (DEFRA, 2002).

Many workers have demonstrated phyto-accumulation of heavy metals (Kruckerberg and Wu, 1991. Bech *et al.*, 1997. Boon *et al.*, 1998. Malawska and Wilkomirski, 2001. Yukselen and Alpaslan, 2001. Walker *et al.*, 2004. Wilson *et al.*, 2005a). Lead

concentrations in all the plant tissues examined were very similar and copper concentrations in the plant tissues were higher at the Limni site than those collected at the control site. Orange slices collected at Limni contained 30% more copper than the orange sample from Akamas.

Investigations into the extent of partitioning within plants themselves have been much less apparent. Pyatt (2001) and Wilson *et al.*, (2005b) demonstrated the varying concentrations of specified metals in different tissues of two plant species and the metal concentrations values shown in Table 12.1 corroborates these findings, with copper and zinc concentrations in various plant tissues being elevated where metal concentrations in soil are higher. Partitioning of metal concentrations within the olive plants examined can be expressed as follows:-

Cu mg kg^{-1} Limni Flesh>leaves> stem>bark>stone

Cu mg kg^{-1} Akamas Flesh>leaves>stem>stone

Zn mg kg^{-1} Limni Stem>leaves>bark>stone>flesh

Zn mg kg^{-1} Akamas Leaves>stone>flesh>stem

Pb mg kg^{-1} Limni Flesh>leaves>bark>stem>stone

Pb mg kg^{-1} Akamas Flesh >leaves>stem>stone

There appears to be little or no accumulation of copper or lead in the stone or seed of the olive and the flesh of these fruits contain the highest concentration of these two metals.

Maisto *et al.* (2004) used an accumulation factor, which they based upon the ratio between metal accumulated in *Quercus ilex* L. leaves and the concentration of metal in the soil surrounding the plant, as a method of comparing the accumulation of different metals. This author has investigated the ratio between metal concentrations in soil and various plant tissues, but expressed this ratio as a percentage i.e.

$$A = \frac{Cpt}{Cs} x100$$

where A = accumulation factor %

Cpt = metal concentration in plant tissue

Cs = metal concentration in soil.

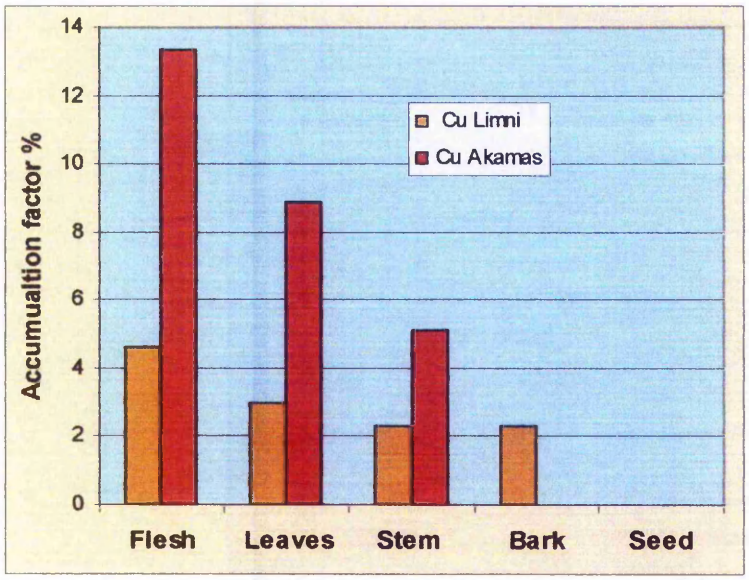


Figure 12.1 Partitioning of copper in olive plants at Limni and Akamas based upon accumulation factors.

Figure 12.1 illustrates the accumulation factors (A) obtained when comparing the copper concentrations in various plant tissues collected at Limni and Akamas. The flesh of ripe olive collected at both sites had the highest relative accumulation factor for copper thus a greater proportion of soil copper was accumulated by this tissue than other components examined and would therefore be the most useful as an indicator of copper contamination in soil. Unfortunately zinc was not detected in Limni soil, therefore a similar comparison cannot be carried out. Lead concentrations were similar in tissue samples from both sites, therefore on the basis of this data, *O. europaea* does not accumulate significant concentrations of lead (< 0.5 mg kg^{-1}) in any of the tissues examined.

Figure 12.1 also illustrates that all olive plant tissues collected from the control site, with the exception of seed, have higher accumulation factors (>5.0%) than the same tissues collected near the spoil tips. It would appear that a higher proportion of the copper concentration in the soil on the Akamas peninsular is available for bioaccumulation. Unfortunately all of the available soil samples from both sites were consumed during the extraction process and further work relating to pH, organic content and conductivity should be carried out in the future.

The extent of partitioning within the annual and perennial tissues of the olive were demonstrated together with the development of a factor (accumulation factor) for the comparison of metal uptake by plants relative to metal concentrations in soil.

Chapter 13

An examination of the possible mobility of heavy metals in selected soil samples from areas surrounding metalliferous mine workings using sequential extraction and a single extraction procedure.

The possible effect of soil pH and climate on the bioavailability of metals concentrations in soil were investigated using two methods suggested as determinants for the availability of metals subject to possible speciation changes.

Introduction

Soil and spoil analysed during this research have varied in pH, conductivity values and metal concentrations as a result of ecological domains, ore body composition and climate due to the location of the mines under examination. Lavkulich stated in 2002 that soil composition is a function of parent material, climate, biota and time, therefore composition, pH and surface properties of resultant soils can affect the speciation of metals and their release under varying biotic and abiotic conditions.

Polluted metal mining sites, both abandoned and current are subject to varying rates of distribution and deposition of heavy metals as seen in previous chapters and their mobilisation due to weathering under exogenic conditions is favoured (Perez and Valiente, 2005). The mobility of heavy metals, their bioavailability and related ecotoxicity to plants depend strongly on their specific chemical forms or ways of binding (Pueyo *et al.*, 2001). Aqua regia digestion of soil samples and subsequent analysis for heavy metals produces a value considered to be the pseudo total concentration of the metals under examination (Ure, 1996; Gupta *et al.*,1996; Ščančar *et al.*, 2000) but this extraction method does not address speciation or its effects on lability, mobility and persistence of metals in soil nor does it provide sufficient criteria for estimating their ecological and biological effects (Cid *et al.*, 2001). Bioavailabilty of heavy metals depends greatly on the characteristics of the particle surface, the kind of strength of the bond and on the properties of the solution in contact with the solid phase (Filgueiras *et al.*, 2002) hence the solutions used to determine phyto-availability of varying species of heavy metals in soil.

In summary soils in and around old mine workings under examination are composed of a matrix of bedrock and metalliferous materials combined with varying proportions of organic matter in which exists a degree of solid partitioning into 2 or more phases. Van der Waals and ionic forces bind particulate matter containing metals to other particles within the soil as well as the formations of ligands and other organic metal complexes due to the presence of humic and fulvic acids in the soil. Temporal, climatic and pH, influenced by both biotic and abiotic factors, will effect the strength of these various bonding processes as will the degree polarity of the soil components. The ease by which these bonds can be broken will greatly affect the bioavailability of the target metals contained in the soil under investigation.

The development of procedures to address the question of speciation of heavy metals in soils and sediments has been addressed by many workers over the years, however Tessier in 1979 produced a sequential extraction scheme (SES) to determine exchangeable, bound to carbonates or specifically absorbed, bound to Fe-Mn oxides and bound to organic matter and sulphides fractions. Maiz *et al.*(2000) proposed a shorter procedure to determine labile, mobile and mobilisable fractions. A single stage extraction has been used to evaluate the bioavailable fraction of copper and antimony in agricultural soils and their transfer to plants (De Gregori *et al.*, 2004)

The Community Bureau of Reference (BCR) organised a workshop which was recognised a "turning point" in the use of SES in Europe (Pueyo *et al.* 2001). The main drawbacks of SES had been identified as re-adsorption and re-distribution of metals (Kheboian and Bauer, 1987; Xiao-Quan and Bin, 1993; Whalley and Grant, 1994). Certification of a BCR reference material containing 6 heavy metals (Table 13.1) was carried out in 2000 using an optimised three step extraction procedure (Pueyo *et al.*, 2001). Recent work carried out by Perez and Valiente (2005) used the same procedure to reveal the potential of SES application to a highly polluted mining site. The metal concentrations determined in each step of the BCR SES are classified as :-

1. Acid soluble
2. Reducible
3. Oxidizeable

Any remaining metals after SES were termed residual and were unlikely to be available for bioaccumulation for a considerable time(Sutherland and Tack, 2003).

Single stage extraction methods have also been suggested, especially when examining heavy metal pollution in agricultural soils (De Gegory *et al.*,) and since a BCR sequential procedure was being evaluated it was decided to use a single stage ethylene diamine tetra-acetic acid (EDTA) procedure developed by that organisation (Ure *et al.*, 1993)

Soil samples collected for this reearch from mining areas in Anglesey, UK, British Columbia, Canada and Arizona, USA exhibited a wide range of pH values and total metal concentrations, therefore it was decided to examine selected soil samples using the BCR optimised SES together with the EDTA procedure mentioned above. The latter was included using a selection of soil samples from those subjected SES procedures with a view to comparing the results obtained.

Materials and methods.

The soil samples used for this assessment were selected from samples already collected from Parys Mountain, Britannia Mine, Lavender Pit and Mission Mine (see Chapters 8, 9, 10 and 11 respectively). The choice of which samples to use were dictated by availability, metal content and pH value in an effort to encompass as much variety as possible. All soil samples were collected and prepared for extraction in accordance with SOP 3.3.1.

Sequential Extraction

The following solutions were made up in preparation for sequential extraction using analytical grade reagents supplied by Fisher Scientific UK Ltd.

Solution A. 0.11M acetic acid was prepared by making 6.25mL of glacial acetic acid up to 1 L in a graduated flask with deionised distilled water. The resultant solution was stored in a clean dry HDPE bottle and used within 1 month of preparation.

Solution B. 0.5M hydroxylammonium chloride was prepared by placing 34.75 g of hydroxylammonium chloride in a beaker and adding 400 mL of deionised distilled water and dissolving the salt completely. The solution was transferred to a 1 L graduated flask and 25 mL of 2M nitric acid was added by means of a volumetric pipette. The volume was made up to 1L using deionised distilled water and thoroughly agitated. This solution was prepared on the same day as the extraction was carried out and excess discarded.

Solution C. Certified hydrogen peroxide 300 mg g^{-1} as supplied.

Solution D. 1.0M ammonium acetate was prepared by dissolving 77.08 g of ammonium acetate in 800 mL of deionised distilled water in a beaker. The pH was adjusted to 2.0 ± 0.1 with concentrated nitric acid and the volume made up to 1 L with deionised distilled water. The resultant solution was stored in a clean HDPE bottle and used within 1 month of preparation.

Extraction procedure

1.0 ± 0.05 g of soil was weighed into a 50 mL centrifuge tube at the commencement of the procedure. A minimum of 6 replicates of each soil sample was extracted. Certified reference material BCR 701 Lake sediment was used as a quality assurance sample in order to check the accuracy of the processes involved. The certified metal concentrations in the reference material are shown in Table 13.1.

Element	Mass Fraction Certified Value mg kg^{-1}		
	Stage 1	Stage 2	Stage 3
Cd	7.34	3.77	0.27
Cr	2.26	45.7	143
Cu	49.3	124	55.2
Ni	15.4	26.6	15.3
Pb	3.18	126	9.3
Zn	205	114	45.7

Table 13.1 Certified metal concentrations in BCR 701 (European Commission 2001).

Step 1.

20 mL of solution A was added to each tube which was closed and shaken on a IKA model KS130 orbital shaker for 16 h at a speed of 560 oscillations per minute and at a temperature of 22 ± 5^{0}C. There was no delay between the addition of the extractant solution and the beginning of shaking. The extract was separated from the solid residue by centrifuging at 4000 rpm for 20 min. The supernatant liquid was decanted into a clean HDPE bottle and stored at 5^{0}C until analysed. The residue was washed using 20 mL of deionised distilled water, shaking for 15 min as before and centrifuging for 20 min at 4000rpm. The supernatant was decanted and discarded taking care not to discard any of the solid residue.

Step 2.

20 mL of solution B was added to the residue from Step 1. in the centrifuge tube. The tubes were closed and residue was re-suspended by shaking manually and then placed in the orbital shaker for 16 h at the same speed and temperature as Step 1. No delay occurred between the addition of the extractant and the beginning of shaking. The extract and solid residue were separated by centrifuge and decanting as in Step 1. The extract was stores in an HDPE bottle at 5^{0}C for subsequent analysis. The remaining residue was washed with 20 mL of deionised distilled water, shaking for 15 min and centrifuging at 4000 rpm. Supernatant was discarded taking care not to discard any of the solid residue.

Step 3.

10 mL of solution C was added to the residue in each tube, in small aliquots to avoid possible violent reaction. The tube was loosely covered and left at room temperature for 1 h, shaking occasionally. The digestion was continued by placing tubes in a water bath at 85 ± 2^{0}C with occasional manual shaking for the first 30 min. The volume was reduced to less than 1 mL over a period of approximately 6 hours. 20 mL of solution D was added to each tube, the tube closed and shaken for 16 hours as in the other stages. There was no delay between the addition of the extractant and commencement of shaking. The extract was separated from the solid residue by

centrifuge and decantation as in the other steps and stored in a clean HDPE bottle at 5^0C until analysed.

Blanks were prepared for each stage and with each batch of extractions and stored in a clean HDPE bottle prior to analysis.

EDTA extraction (after De Gregori *et al.*, 2004)

EDTA solution

0.05M EDTA solution was prepared by dissolving 18.612 g of disodium ethylene diamine tetra-acetic acid salt dihydrate in 800 mL of deionised distilled water and the pH was adjusted to 7.0 ± 0.1 with ammonia solution (S.G. 0.88). The reultant solution was transferred to a 1 L graduated flask and the volume made up with deionised distilled water.

Extraction

2.0 ± 0.1 g of soil was weighed into a 50 mL centrifuge tube and 20 mL of EDTA solution was added using a volumetric pipette. The tubes were closed and shaken for 1 h on a IKA model KS130 orbital shaker at a speed of 560 oscillations per min and at a temperature of 22 ± 5^0C for 1 h. The tubes were then centrifuged for 20 min at 4000 rpm, the supernatant decanted into a clean HDPE bottle and stored at 5^0C for analysis. Eight soil samples already subjected to SES procedures were extracted using EDTA including BCR701 certified reference material.

Analysis

All extracts and blanks were analysed according to SOP 3.6.1 (FAAS).

RSD values were calculated as in SOP 3.7.2. Residual metal concentrations for all samples were calculated by subtracting the total of the SES stages from the total metal concentration value obtained in Chapters 8,9,10 and 11 when extracted using SOP 4.2.1 aqua regia extraction.

Results

Table 13.2, 13.3 and 13.4 indicate metal concentrations in various stages of SES and

Parys Mountain samples.	Metal data	SES Step 1	SES Step 2	SES Step 3	Total	Residual	EDTA Extract
SE9PM1	Cu Mean mg kg^{-1}	89.9	329	471	1570	680	87.2
pH = 4.63	Cu RSD %	9.6	1.7	5.0			2.4
Cond. = 235	Zn Mean mg kg^{-1}	144	195	147	2374	1889	6.6
	Zn RSD %	1.0	4.2	5.3			1.9
	Pb mean mg kg^{-1}	15.9	475	65.2	1528	972	146
	Pb RSD %	5.4	1.0	22.1			2.75
	Cd mean mg kg^{-1}				3.4		
	Cd RSD %						
SE9PM9	Cu Mean mg kg^{-1}	4.8	10.9	152	216	48.3	
pH = 3.4	Cu RSD %	4.3	0.7	2.5			
Cond = 415	Zn Mean mg kg^{-1}	6.3	4.0	11.2	115	93.5	
	Zn RSD %	5.7	10.1	5.0			
	Pb mean mg kg^{-1}	3.9	66.1	38.0	193	85.0	
	Pb RSD %	8.8	5.8	12.9			
	Cd mean mg kg^{-1}						
	Cd RSD %						
SE9PM19	Cu Mean mg kg^{-1}	36.2	56.7	235	3344	3016	38.5
pH = 3.01	Cu RSD %	1.5	10.8	4.0			1.1
Cond = 308	Zn Mean mg kg^{-1}	5.9	3.5	39.3	1063	1011	4.9
	Zn RSD %	3.6	12.6	4.9			1.7
	Pb mean mg kg^{-1}	70.6	619	106	4067	3271	277
	Pb RSD %	3.9	10.2	10.3			2.1
	Cd mean mg kg^{-1}						
	Cd RSD %						

EDTA obtained from soils evaluated together with pH and conductivity values.

Table 13.2 Metal concentrations obtained for different stages in SES and EDTA extraction of soil samples collected in the vicinity of Parys Mountain together with pH and conductivity data.

Britannia Mine	Metal data	SES Step 1	SES Step 2	SES Step 3	Total	Residual	EDTA Extract
SE1103C5	Cu Mean mg kg^{-1}	231	165	570	1120	154	442
pH = 5.4	Cu RSD %	1.3	3.7	2.8			0.6
Cond. = 190	Zn Mean mg kg^{-1}	27.9	10.7	10.1	557	508	20.8
	Zn RSD %	3.4	2.6	5.9			0.7
	Pb mean mg kg^{-1}		8.4	4.9	47.4	34.1	12.6
	Pb RSD %		22.4	10.3			4.6
	Cd mean mg kg^{-1}						0.7
	Cd RSD %						10.7
SE2700	Cu Mean mg kg^{-1}	38.5	7.6	7.9	407	353	
pH = 2.9	Cu RSD %	0.3	2.3	2.2			
Cond = 850	Zn Mean mg kg^{-1}	7.6	5.2	4.9	220	202	
	Zn RSD %	3.9	7.0	12.0			
	Pb mean mg kg^{-1}		4.4		651	647	
	Pb RSD %		8.2				
	Cd mean mg kg^{-1}						
	Cd RSD %						
SE1103C11	Cu Mean mg kg^{-1}			7.6	17.3	9.7	2.2
pH = 5.1	Cu RSD %			3.7			6.4
Cond. = 594	Zn Mean mg kg^{-1}	7.6	5.2	4.9	30.7	13	10.1
	Zn RSD %	3.9	7.0	12.0			0.7
	Pb mean mg kg^{-1}						
	Pb RSD %						
	Cd mean mg kg^{-1}						
	Cd RSD %						
Reference material							
SEBCR701	Cu Mean mg kg^{-1}	29.5 (49.3)*	74.6 (124)*	66.7 (55.2)*	275**		119
pH = 6.25	Cu RSD%	1.0	0.6	4.0			0.3
Cond. = 785	Zn Mean mg kg^{-1}	164 (205)*	126 (114)*	57.5 (45.7)*	454**		164
	Zn RSD%	0.6	1.9	2.0			0.7
	Pb Mean mg kg^{-1}	<LOD (3.18)*	102 (126)*	24.1 (9.3)*	143**		67.8
	Pb RSD%		1.6	2.8			1.1
	Cd Mean mg kg^{-1}	5.8 (7.34)*	4.6 (3.77)*	0.6 (0.27)*	11.7**		7.4
	Cd RSD%	1.2	1.5	8.5			0.9

* Certified values (Report EUR 19775EN)
** Indicative values (Report EUR 19775EN)

Table 13.3 Metal concentrations obtained for different stages in SES and EDTA extraction of soil samples collected in the vicinity of Britannia Mine and BCR 701 Lake sediment together with pH and conductivity data.

Lavender Pit	Metal data	SES Step 1	SES Step 2	SES Step 3	Total	Residual	EDTA Extract
SEAZ11	Cu Mean mg kg^{-1}	23.5	237	549	1366	557	
pH = 7.98	Cu RSD %	1.5	77.8	3.2			
Cond. = 292	Zn Mean mg kg^{-1}	357	622	135	1569	455	
	Zn RSD %	1.5	1.6	3.9			
	Pb mean mg kg^{-1}	23.8	164	340	977	449	
	Pb RSD %	3.8	6.4	3.4			
	Cd mean mg kg^{-1}	0.8	0.8	0.5	3.5	1.4	
	Cd RSD %	7.8	7.8	9.5			
SE64A18	Cu Mean mg kg^{-1}	1.1	4.2	1.4	34.4	27.7	10.8
pH = 8.83	Cu RSD %	30.9	5.6	17.3			1.1
Cond. = 126	Zn Mean mg kg^{-1}	0.7	3.0	0.4	36.1	32.0	1.0
	Zn RSD %	6.1	5.2	11.0			3.9
	Pb mean mg kg^{-1}		6.8	2.1	11.4	2.5	1.9
	Pb RSD %		8.8	18.9			16.5
	Cd mean mg kg^{-1}						
	Cd RSD %						
SEAZ9	Cu Mean mg kg^{-1}	82.8	370	171	929	305	324
pH = 7.78	Cu RSD %	6.2	6.6	2.1			3.3
Cond. = 384	Zn Mean mg kg^{-1}	102	151	50.7	457	153	103
	Zn RSD %	1.5	1.6	3.9			1.3
	Pb mean mg kg^{-1}	5.6	263	48.7	412	94.7	200
	Pb RSD %	15.4	3.7	13.6			2.7
	Cd mean mg kg^{-1}	0.8	0.7				1.2
	Cd RSD %	5.2	6.1				10.2
Mission Mine							
SE64A01	Cu Mean mg kg^{-1}	37.5	281	1770	2377	288	
pH = 8.52	Cu RSD %	0.9	2.1	1.3			
Cond = 110	Zn Mean mg kg^{-1}	77.7	123	52.1	435	182	
	Zn RSD %	1.2	2.8	1.0			
	Pb mean mg kg^{-1}	2.4	60.0	12.2	83.2	8.6	
	Pb RSD %	20.8	1.8	9.3			
	Cd mean mg kg^{-1}	0.6	0.3	0.3	1.8	0.6	
	Cd RSD %	7.2	20.3	0.8			
SE64A03	Cu Mean mg kg^{-1}		7.5	13.6	42.3	21.2	6.4
pH = 8.42	Cu RSD %		5.4	13.8			3.4
Cond. = 45	Zn Mean mg kg^{-1}	1.2	2.9	4.1	40.9	32.7	1.6
	Zn RSD %	10.8	2.4	4.6			6.7
	Pb mean mg kg^{-1}		6.2		13.1		3.1
	Pb RSD %		8.5				9.8
	Cd mean mg kg^{-1}						
	Cd RSD %						
SE64A06	Cu Mean mg kg^{-1}		8.6	9.6	38.3	20.1	
pH = 7.43	Cu RSD %		10.7	5.5			
Cond. = 33	Zn Mean mg kg^{-1}	2.7	5.2	5.8	48.6	34.9	
	Zn RSD %	9.5	3.2	10.3			
	Pb mean mg kg^{-1}		8.9		16.2		
	Pb RSD %		5.4				
	Cd mean mg kg^{-1}						
	Cd RSD %						

Table 13.4 Metal concentrations obtained for different stages in SES and EDTA extraction of soil samples collected in the vicinity of Lavender Pit and Mission Mine together with pH and conductivity data.

RSD values for EDTA extraction shown in Table 13.1, 13.2 and 13.3 are generally lower than those obtained in any of the sequential stages and copper concentrations of the two samples extracted using EDTA procedure equate to copper extracted during the first stage of SES

Data recorded for BCR 701 reference material showed some variations from the certified values. All metal concentrations determined from stage 1 extraction were lower than certified values, and all metal concentrations from stage 3 were higher than the certified values. Figure 13.1 illustrates the relationship between determined and certified metal concentrations in the certified material, stages 1, 2 and 3.

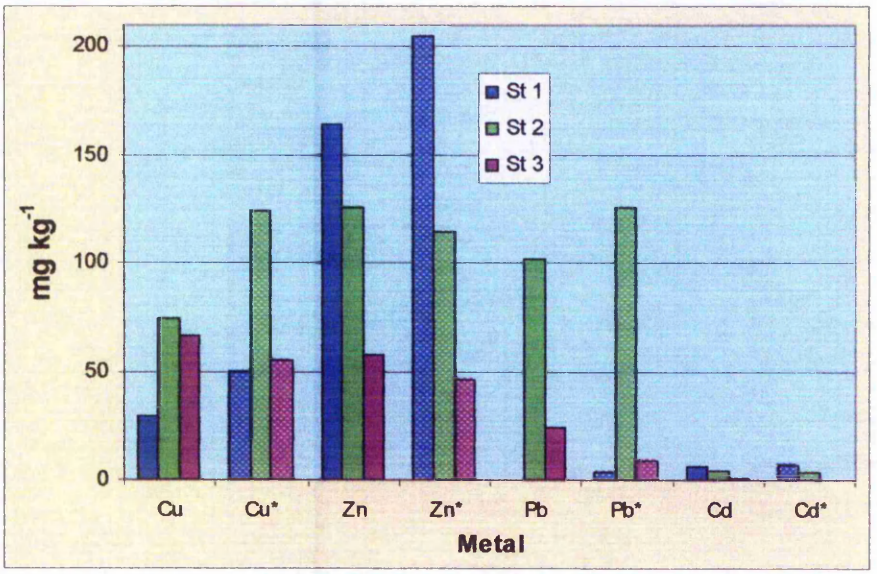


Figure 13.1 Determined and certified (*) concentrations of target metals in BCR 701 reference material using BCR SES procedure.

Figure 13.2 shows the percentage of total metals extracted by 3 stages of the BCR extraction process related to the total of the certified concentrations for the same three stages. 75% of total copper was extracted and other metals exceeded 90% extracted of the certified total.

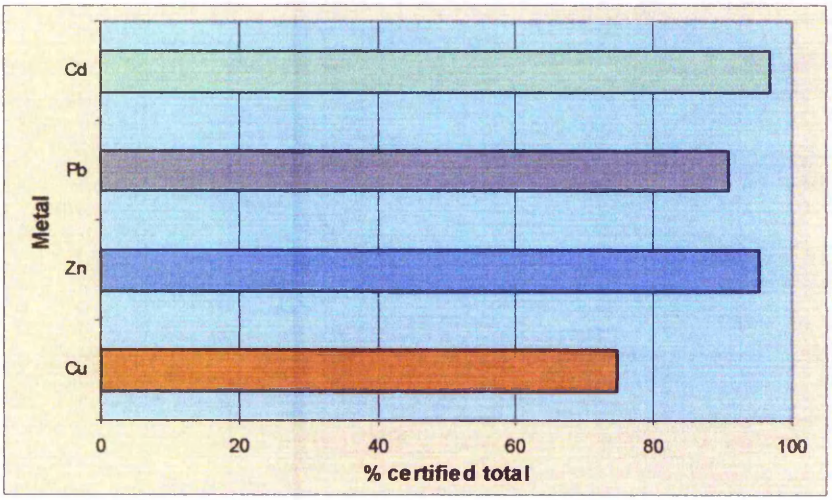


Figure 13.2 % of total metal extracted by 3 stages of BCR sequential extraction procedure related to certified total.

Discussion.

Cadmium was detected during sequential extraction in BCR701 reference material, whereas only one soil sample in this sequence contained noteable concentrations of cadmium and the concentrations sequentially extracted were below the LOD for the FAAS instrument in use, data relating to this element was no processed further.

Data obtained for BCR 701 reference material using the same SES procedure showed low results for copper concentrations extracted during stage 1 and stage 2 with a higher result for stage 3 extraction. The total copper extracted over three stages was only 75 % of the total certified extraction; other target metals produce values in excess of 90% for the total extracted over three stages (Figure 13.2). RSD values throughout this procedure were generally below 5% except where metal concentrations were relatively low, reflecting a satisfactory degree of precision since RSD values of <13% obtained for a similar study indicated samples obtained for that study were relatively homogeneous and confirmed the precision of the analytical work (Jong and Parry, 2004). The report relating to the certification process (European Commission 2001) indicated that 18 laboratories were involved during the certification process and the spread of means obtained for each metal and each extraction stage is detailed. Only zinc and cadmium stage 2 results fell within the range of means which indicated that the process used by the author was not identical

to that used by the certifying laboratories. Investigation showed that the shaker used in the BCR process was in fact a planar mixer producing regular end over end agitation, whereas the author used an orbital shaker which could reduce particular exposure to the extracting reagents. The data produced for the soil samples under investigation however was considered worth examining.

Tokalioğlu *et al.* (2003) proposed that an important consideration in the reliability of sequential extraction data was the percent recovery relative to a single digestion with aqua regia and they defined recovery as follows:-

$$\text{Recovery}_n = \frac{\Sigma_n \text{ Sequential extraction procedure}}{\text{Single digestion with aqua regia}} \times 100$$

Where n is the concentration of a given element and the single digestion with aqua regia was carried out in accordance with SOP 4.2.1.

Figure 13.3 illustrates the mean recovery of each target metal for each stage for all samples of soil evaluated including BCR 701 reference material. Lead was the least acid extractable (stage 1) whereas zinc was more available (Cottennie *et al.*, 1979) or labile (Maize *et al.*, 1997, 2000). This data may account for the consistently relative high concentration of zinc in the variety of plants and faeces examined at mine workings under examination. The average recovery of reducible lead (stage 2) is much higher than either zinc or copper and in reducing conditions will become readily available to plants. Copper has the highest average recovery at stage 3 (oxidisable).

The mobility, bioavailability, and related ecotoxicity depends strongly on their specific chemical form and localisation (Ipolyi *et al.*, 2002) and soil pH could have a profound effect on the subsequent release and accumulation by plants and animals.

All alkaline soils subjected to SES and analysis were collected in the vicinity of Lavender Pit, Bisbee, Arizona (3 samples) and Mission Mine, Tucson, Arizona (3 samples). Acid soils were collected at Parys Mountain, Anglesey, UK (3 samples), Britannia Mine, British Columbia, Canada (3 samples). The BCR reference material

was also acid and that was collected from lake sediment in Piemonte, Italy (European Commission, 2001).

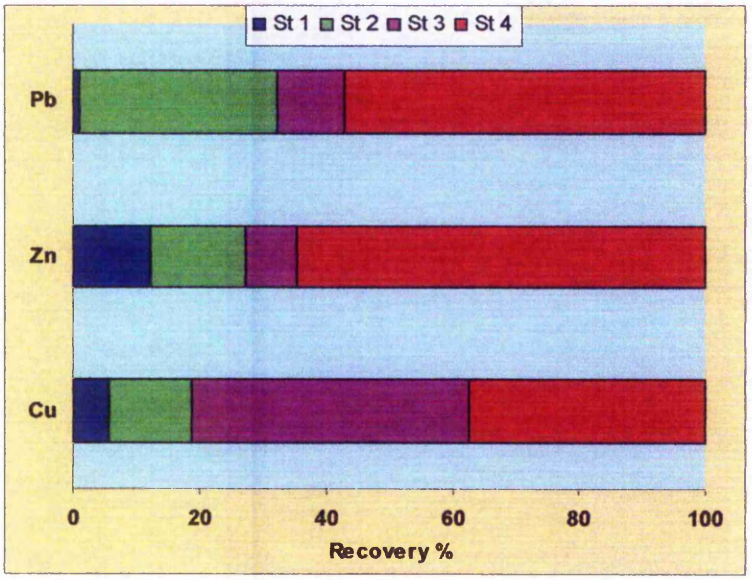


Figure 13.3 Mean recovery of target metals based on total concentration obtained using aqua regia extraction.

Figure 13.4 indicates that soils with acid pH values released lower overall percentages of all target metals when compared with soils with alkaline pH (Figure 13.5). This phenomenon is probably due to leaching of trace metals from the soil during periods of rain when aqueous acid conditions would prevail but could also be influenced by ionic bond strength. Additionally the alkaline soils were collected in Arizona where rain fall is relatively low when compared to rainfall experienced in Anglesey, UK and British Columbia, Canada. The largest concentrations of lead were recovered at stage 2, i.e. the reducible fraction at both acid and alkaline pH. Workers using a similar procedure for the extraction of lead from Scottish hillside soils (Bacon *et al.*, 2004) found that the highest concentration was released during stage 3 (oxidiseable). Figure 13.5 illustrates that alkaline soils release the highest concentrations of lead and zinc at stage 2 when compared with acid soils, however, a higher recovery of copper is obtained from acid soils at stage 2 (Figure 13.4).

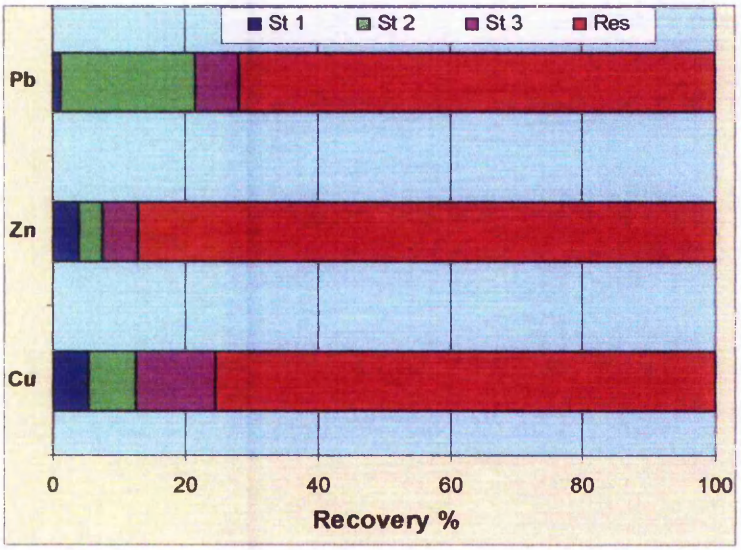


Figure 13.4 Mean SES recovery of target metals from soil samples with pH in the range 2.9-6.3

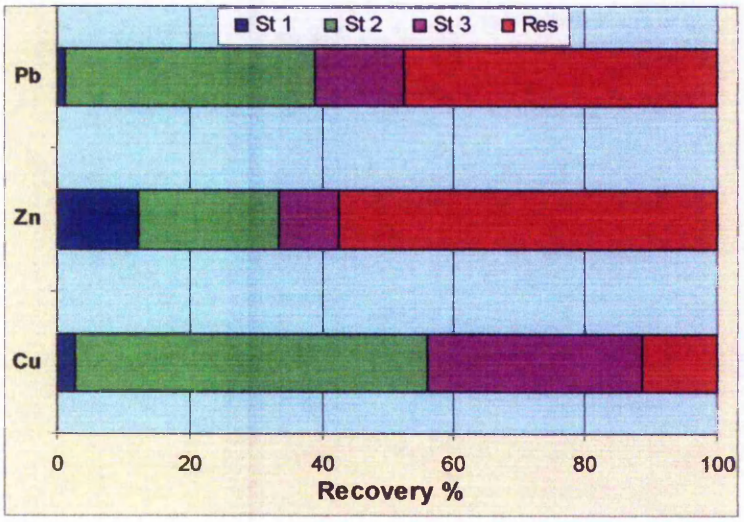


Figure 13.5 Mean SES recovery of target metals from soil samples with pH in range 7.4-8.8

Examination of the data produced during this study, collected from 7 samples of acid soil and 6 samples of alkaline soil, demonstrated that soil pH appeared to be affecting the release of target metals at various stages of SES and therefore affecting bioavailablity.

Many researchers (Tessier *et al.*, 1979. Murphy *et al.*, 2000. Perez and Valiente, 2005) have noted that heavy metals are bound to various forms of organic matter in soil such as biotic detritus, organic coatings on inorganic particles and living organisms. Additionally, humic acid content in soil, a component of decayed organic

material, has been shown to have an effect on the binding and chelating of trace metals and their subsequent release (Cottenie *et al.*, 1979.Maize *et al.*, 2000) additionally heavy metal complexes formed with humic and fulvic acid, constituents of the organic matter associated with soil, are easily oxidiseable (Filgueiras, 2002). The organic content of the various soil samples were not taken into account during the current research, however, it can be assumed that soil collected in the vicinity of Parys Mountain and Britannia Mine would have an elevated organic matter content when compared to soil from Arizona, due to the much higher abundance of vegetation with consequent high levels of humous from decomposition.

Research carried out by Qin *et al.* (2004) using low molecular weight organic acids as extractants for soil concluded that pH is not a dominant factor governing the release of metals from the soil. There a number of research papers with contradictory conclusions regarding the effects of pH on heavy metal release from soils. Qin *et al.* (2004) in the same paper stated that low soil pH and low organic matter content contributed to higher concentrations of metals released, Walker *et al.* (2004) said that increase in soil pH reduces plant availability whereas Hur *et al.* (2004) demonstrated that copper leaching from debris was pH dependant with more leaching occurring at low pH.

EDTA single stage extraction has been determined as a more reliable method of relating metal concentrations in agricultural soil to that bioaccumulated by plants growing in that soil (Quevauviller *et al.*, 2004).

Figure 13.6 illustrates the target metal recovery percentage extracted by EDTA compared with those extracted by SES, from acidic soils and Figure 13.7 demonstrates similar data for alkaline soils. Zinc concentrations extracted by EDTA are comparable to stage 1 extraction in both types of soil. The amount of lead recovered in both cases lies between stage 1 and 2, whereas copper recovery by EDTA exceeds stage 1 and stage 2 of the SES procedure. pH seems to have little effect of recovery values for lead and copper, however zinc recovery values are depressed in alkaline soils when compared with similar values in acid soils. This again suggests that zinc is more mobile in alkaline conditions.

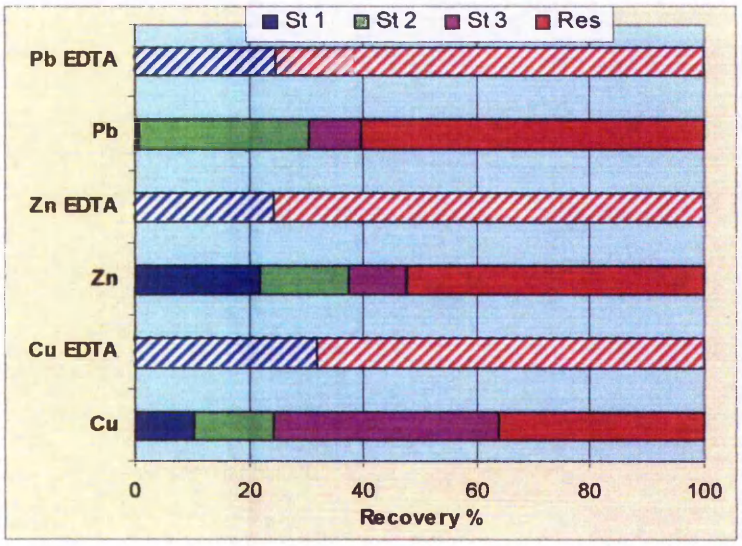


Figure 13.6 Comparison of SES and EDTA single stage extraction of acidic soils.

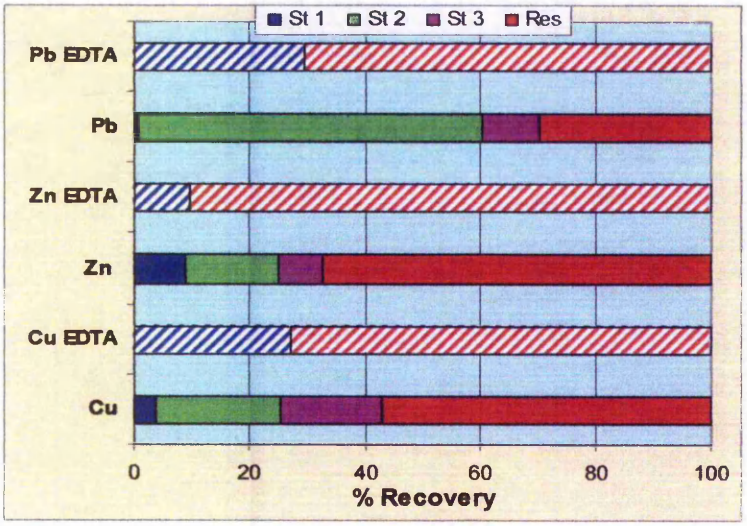


Figure 13.7 Comparison of SES and EDTA single stage extraction of alkaline soils.

Finally it must be born in mind that pH, organic content and ore type are not the only factors which control the release of metals from soil for bioaccumulation. Microbial biotransformations such as oxidation and reduction, methylation, chelation with organic ligands and bio-immobilisation are significant factors causing changes in metal speciation and mobility (Weber and Karczewska, 2004) as well as particle size and hardness of the rock matrix.

It was found that pH did effect the release of metal for bioavailability and that climate in the areas investigated had a limited effect on this property.

Chapter 14

Examination of data from metalliferous mine workings relating to bioaccumulation.

Bioaccumulation patterns within different plant species were examined for differences and evidence of mechanisms curtailing bioaccumulation from soil and distribution in plants.

Introduction

Concentrations of heavy metals in soils can be accumulated by plants in varying degrees. Some plants are intolerant to heavy metal accumulation whereas other species can tolerate high concentrations (> 1000 mg kg^{-1}) of certain heavy metals without exhibiting any apparent toxic effects such as discolouration, mutation or impeded growth. Different species of plant can tolerate different concentrations of varying heavy metals and this phenomenon dictates the presence or otherwise of plant species in the vicinity of metalliferous spoil tips and on land contaminated by heavy metal pollution. Mechanisms which influence the bioaccumulation of metals by plants include soil pH, organic matter content, moisture content and microbial activity and such cations can be distributed within plants from the roots via the xylem and from stomata in the leaves by the phloem.

It has been proposed that the presence of specific plant species on land surrounding old mine workings can indicate the metals present in the soil and can also indicate the presence of metals in the vicinity which have not yet been extracted (Salt *et al.*, 1998).

Plants which are tolerant of heavy metal accumulation have been be used as a means of remediation for land which has been contaminated by heavy metals (Schwitzguébel *et al.*, 2002), so called phyto-remediation. After the growing season during which the selected plants accumulate heavy metal from the soil, they are removed. SA similar process, termed phyto-mining has been used to accumulated precious metals in plants propagated on old precious metal mine spoil heaps. The

plants are then removed and burnt under controlled conditions to obtain the metal. Metal tolerant plants have also been used extensively as biomonitors for past and present heavy metal pollution from smelters (Gabriel and Patten, 1994, Bellis *et al.*, 2001) and mine workings (Balkau, 1999)

Various researchers have demonstrated that when heavy metals are bioaccumulated by plants partitioning occurs, that is, the metal concentrations in different plant tissues vary within the same plant. The degree of partitioning differs between plant species and between different tissues within plants. (Pyatt, 2001, Wilson *et al.*, 2005B)

Metal uptake by plants can play a key roll in the entry of metals to terrestrial food chains because vegetation creates habitats for animals and is foraged alive by herbivores and dead by detritivores (Vassilev *et al.*, 2004). When plants accumulate these metals are ingested by animals thus creating the potential of toxic effects at higher trophic levels (Crowder, 1991). Plants and animals which have accumulated heavy metals can be consumed by other mammals including humans and have a high toxicity potential for reproductive and developing tissues and can induce teratogenicity (Domingo, 1994)

Materials and Methods

Samples of varying vegetation types were collected from copper mine workings at Parys Mountain, Anglesey, UK., Britannia Mine, British Columbia, Canada, Lavender Pit, Bisbee, Arizona,Mission Mine, near Tucson, Arizona, USA and Limni workings near Polis, Cyprus. Other metals, notably zinc, lead and to a lesser extent cadmium have been detected in soil from all sites, indeed zinc and lead have been co-produced at all of the mines together with quantities of silver in Arizona.

Parys Mountain and Britannia Mine are situated in temperate coastal areas and represent mining activities which ceased in the mid twentieth century. In both areas the soil is acid and heavy metal contamination is persistent and distributed in the vicinity of the workings (see Chapters 8 and 9)

Lavender Pit ceased extraction operations in 1974 and Mission Mine started extraction operations in 1961 and they will continue into the foreseeable future. The soil surrounding these workings is alkaline and in the case of Lavender Pit, the soil in the area contaminated with copper, zinc and lead.

Limni workings are an ancient mine where copper has been extracted since tha late iron age (circa 2000 years BP). The soil is alkaline and heavy metal pollution is persistant although some attempt at land remediation was carried out three years ago by adding some soil overburden and re-contouring spoil heaps to curtail run off.

Samples of soil and vegetation were collected from each area and from control sites considered to be unaffected by the mine workings in each location. The samples were collected, prepared, digested and analysed as outlined in the relevant chapters.

Vegetation samples collected at the various sites are shown in Table 14.1

Site	Species	Common name
Parys Mountain, UK	*Calluna vulgaris*	Ling
Britannia Mine, Canada	*Alnus rubra*	Red Alder
Lavender Pit, USA	*Salix amigaloides*	Peachleaf Willow
Limni, Cyprus	*Olea europaea*	Olive

Table 14.1 Vegetation samples collected in the vicinity of metalliferous mine workings

Results

The author has adopted Accumulation Factor (%A) in order to facilitate comparison od accumulation in different plant tissues and species. It is an expression of the proportion of the soil metal content accumulated in the plant tissue and is calculated as follows:-

$$\%A = \frac{Cpt}{Cs} x100$$

where $\%A$ = accumulation factor

Cpt = metal concentration in plant tissue

Cs = metal concentration in soil.

Plant	Tissue	Location	Cu mg kg^{-1}	Cu %A	Zn mg kg^{-1}	Zn %A	Pb mg kg^{-1}	Pb %A
Ling	Floret	Parys M	21.52	0.64	27.32	2.6	26.54	0.65
Ling	Floret	Control	<LOD	-	15.74	53.0	<LOD	-
Ling	Stem	Parys M	49.8	1.5	44.06	4.1	163.9	4.0
Ling	Stem	Control	2.7	4.8	23.7	79.0	<LOD	-
Alder	Leaf	Britannia	15.4	1.4	101.0	+100*	<LOD	-
Alder	Strobile	Britannia	21.0	1.9	32.8	+100*	<LOD	-
Bark	Bark	Britannia	8.52	0.08	78.5	+100*	<LOD	
Willow	Leaf	Bisbee	19.0	1.4	28.0	1.8	<LOD	-
Willow	Leaf	Control	<LOQ	-	21.6	60.0	<LOD	-
Olive	Leaf	Limni	5.82	3.0	9.83	ND	0.4	ND
Olive	Flesh	Limni	8.93	4.6	5.02	ND	0.5	ND
Olive	Seed	Limni	<LOD	-	6.4	ND	<LOD	-
Olive	Stem	Limni	4.43	2.3	15.3	ND	0.21	ND
Olive	Leaf	Akamas	4.77	11.2	13.4	+100*	0.5	0.01
Olive	Flesh	Akamas	7.11	13.3	5.72	68.0	0.5	0.01
Olive	Seed	Akamas	<LOD	-	7.15	85.0	<LOD	-
Olive	Stem	Akamas	2.73	19.6	4.54	54.0	0.22	0.01

Table 14.2 Metal concentrations and accumulation factors for various plant tissues collected from Parys Mountain, UK, Britannia Mine, Canada, Lavender Pit, USA, Limni Mine and Akamas peninsular, Cyprus. Shaded values were obtained from designated control sites.

- +100 indicates that the metal concentration in the plat tissue is higher than that recorded in the soil surrounding the plant.

Discussion

Table 14.2 indicates that copper zinc and lead concentrations and subsequent accumulation factor values in annual plant tissues (ling florets) collected at Parys Mountain were lower than the same metal concentrations in the perennial tissue (woody stem) collected from the same individual plant. Ling stems accumulate approximately 2.3 time more copper, 1.6 times more zinc and 6.2 times more lead

than florets collected from the same plant.One could hypothesise that this degree of partitioning was not unexpected since the 'perennial' tissue has had more growing seasons in which to bioaccumulate the target metals than floret tissue which is an annual event. Copper and lead concentrations were not detected in ling florets at the control site but a small concentration of copper (2.7 mg kg^{-1}) was detected in the woody stem material. Zinc was also detected in both types of ling tissue at the control site.

Annual tissue collected from an alder tree at Britannia Mine, however, showed a reverse pattern of partitioning with annual tissue such as strobiles and leaves exhibiting a higher copper and zinc concentration than bark obtained from the same tree.

More extensive sample collection was undertaken at Limni mine on Cyprus in order to further assess partitioning particularly within tissues of a species which produces an edible fruit.

Table 14.2 indicates that copper and lead concentration in leaves and the flesh of olive plants ie. annual tissue exceeded the concentration of these metals in the woody stem, a perennial tissue by a factor of at least 1.3 for copper in the leaf and 2 for copper in the fleshy part of the fruit. Zinc concentrations were higher in perennial woody stem than either the leaf or the flesh. Concentrations of all three of the target metals in the annual tissues collected from this species exceeded the concentrations present in woody stem tissue.

Pyatt, in 2001, found that seeds of *Acacia retinoides* and *Eucalyptus torquat*a grown in an area adjacent the Limni olive site, to accumulated the least concentrations of copper when compared to other tissue such as leaves and woody stems. Lead accumulation in the seeds of both species was not detected, however annual tissue from both of these plants accumulated more lead and copper than perennial tissue, in this case woody stems.

Accumulation of zinc in some of the tissues examined, exceeded the concentration of zinc in the surrounding soil. This apparent anomaly could be due to the soil sample

collected at the base of the tree not being representative of the soil surrounding the roots of the tree or simply a case of this species being a hyper-accumulator of zinc. When calculated accumulation factors in Table 14.1 are examined the values for zinc are generally higher for zinc than for copper and lead. This is particularly the case when accumulation factors for control site tissues are examined. Values in excess of 50% are evident for zinc; copper accumulation factors are also higher at control sites (4.8 – 19.6%). Relevant data for lead collected during this research does not reflect such differences. Zinc is considered to be an essential macro element for plant development and it is suggested that zinc is tolerated in higher concentrations than copper and lead. Additionally concentrations of zinc in plant tissues located in polluted area are of a similar magnitude to those values obtained from control sites.

The use of accumulation factor proposed in this research enables a comparison to be made between the relative proportions of metals accumulated by the tissues under examination. The values calculated at control site are always higher than those for plant tissue collected from contaminated sites. Figure 12.1 using data from Cyprus illustrates this point and suggests to the author that some type of homeostatic mechanism was being carried out by the plants in question, restricting the amount of heavy metals being accumulated by the plant. In other words a form of protection against toxic concentrations being accumulated is in place. Similar concentrations of zinc (6.4 and 7.15 mg kg^{-1}) were determined in olive seeds collected at both sites in Cyprus, however copper and lead were not detected again suggesting that some form of protective mechanism is being undertaken.

There were indications that homeostasis was controlling the uptake of heavy metals from soil by plants and seeds in two species examined appeared to be protected from heavy metal accumulation.

Chapter 15

Outcomes and conclusion

15.1 Standard Operating Procedures

The sampling and preparation procedures were in the main satisfactory, however, same problems became evident with regard to soil sampling and preparation of vegetable tissues using the Waring commercial blender which need further examination.

Bioaccumulation of target metals in some vegetation samples occurred when that metal was not detected in the soil sample collected in the vicinity of the plant under examination, suggesting that the soil sample obtained was not representative of the soil in the immediate area or was not representative of the soil adjacent to the roots of the plant being investigated. Two anonymous reviewers of papers submitted for publication have commented on this fact and have suggested that the sampling procedure should be modified to reflect the natural heterogeneity of soil. Various methods have been considered however since such phenomena occurred infrequently and it was decided to continue using the current SOP in the interests of practicality.

Pulverisation of woody sections and cones of plants as in SOP 3.3.5 and 3.3.6 produced a variety of fragment and particle sizes with resultant higher RSD values than other vegetation tissues prepared by grinding in a pestle and mortar. One solution may be to add an equal weight of distilled demineralised water and blend/mix for a longer period and dry out the resultant slurry. The dried slurry could then be reworked to produce a more homogenous material for digestion before analysis.

15.2a Evaluation of digestion procedures.

The conclusions to this section of the research was fully documented at the end of chapter 4 in order to establish a standard operating procedure for the duration of the

current research, however the following comments became apparent as the project approached completion.

The established procedure utilising hot aqua regia produced adequate results with all of the materials extracted. RSD values reduced as the research progressed as a result of increasing the number of replicates used and increasing familiarity with the operating the procedure. The approved procedure proved to be robust when compared with possible replacement used by other researchers.

It is therefore suggested that the number of replicates for all samples to be extracted and analysed be reduced to seven, allowing calculation of satisfactory values of standard deviation and the possibility of maintaining that degree of precision if one of the samples is discarded for a variety of operational errors such as contamination or spillage.

15.2 Use of micronisers as a grinding method for soil samples.

The use of a micronising mill produces a material with a much reduced particle size and size distribution then the use of pestle and mortar grinding. The resultant product is suitable for acid digestion and subsequent determination of heavy metal concentrations. The precision obtained after analysis for some heavy metal concentrations is better than that obtained using the pestle and mortar method and the extraction efficiency for some metals is improved.

The potential for greater homogeneity in the ground sample is improved due to the lower particle size and size variation, however further processing will be required to improve homogeneity and hence precision of analysis

Ease of processing and time reduction factors commend the use of miconising mills as a valuable addition to the procedure for analysing heavy metals in dried soil, spoil and sediment samples, however an extended investigation using a variety of soil and vegetation samples would confirm the suitability of the procedure for this type of environmental analysis.

15.3a Evaluation of Pressurised microwave extraction procedure.

This investigation showed that the use of pressurised microwave digestion, utilising the USEPA procedure yielded lower target metal concentrations than the open digestion method proposed in chapter 4. The reagent used in the USEPA procedure is nitric acid whereas aqua regia was used in the open digestion method. The pressurised microwave equipment used was not fitted with a temperature recorder, therefore it was not possible to determine the maximum temperature generated within the vessels. It is possible that temperature and pressure values achieved could have resulted in the formation of complex oxides within soil samples which prevented the release of a proportion of the metal into solution.

Detectable concentrations of copper were obtained from some vegetation samples using the pressurised microwave procedure whereas these metals were not detected in vegetation samples extracted using open digestion. Higher concentrations of zinc were also extracted from vegetation by the pressurised microwave process. The converse case occurred with lead concentrations in plants and it is proposed that copper and zinc are accumulated in similar cell tissues of the plants and lead is accumulated else where, i.e. more copper and zinc are released from plant tissue because of the effect of pressure rather than prolonged heating.

More extensive examination of pressurised microwave extraction using aqua regia and possibly longer processing times are required, however, if the processing time is extended then there seems little point in incurring the extra expenditure, unless higher concentrations of metal are released. Pressurised microwave extraction should also be carried out on a certified reference material to determine the relative accuracy of the process.

15.4 Assessment of Analytical Procedures

EDAX techniques were not satisfactory for use in environmental analysis where metal concentrations were below 1000 ppm. The natural heterogeneity of soils and vegetation also limit the effectiveness of this technique. Where metal concentrations are expected to be above 1000 ppm this technique does present the analyst with

relatively easy preparation and quick analysis of substrate. It is however unlikely that plants would accumulate more than 1000 ppm of copper lead and cadmium and still be around to be examined!

Ion chromatography as carried out using the Dionex 600 system, whilst having the advantage of being able to detect all four target metals simultaneously, did not produce consistent or in some cases discernable results due to interference from other components of the analyte matrix.. The manufacturers have suggested the addition of a chelating process or the use of solid phase extraction before analysis but this was considered unsatisfactory since extra processing would lead to further loss of precision and accuracy.

The concentration values obtained from FAAS and ICP-AES were generally very close and statistically the techniques exhibited no significant difference. The documented detection limits for ICP-AES were however much lower than those for FAAS and during this investigation the precision of ICP-AES was better than FAAS. It was expected that ICP-MS would have been at least as precise and accurate as ICP-AES with the added benefit of mass spectrometry being able to separate the metals detected into different oxidation states. ICP-MS determinations on this occasion were unsatisfactory as it would appear that the zinc calibration standard was contaminated.

It was therefore recommended that ICP-AES be used wherever possible and this institution have now acquired such an instrument.

15.5a Heavy metal dispersion, dilution and bioaccumualtion at Parys Mountain

Notable heavy metal concentrations were detected adjacent to the mine workings at Parys Mountain as well as near the harbour at Amlwch where smelting was carried out in the nineteenth century; metal and ore were also loaded onto ships for onward transportation. Dispersion of copper, lead and zinc had occurred downwind of the mine workings at Parys Mountain north eastwards to the coast. Dilution of the cations relative to distance from the workings was demonstrated and the general acidity of soil in the area determined. It was proposed that the acidity in the soil would aid

further dispersion of the target metals by bioaccumulation in plants and through higher trophic levels. Accumulation of copper lead and zinc in vegetation was demonstrated in relation to soil concentration, accumulation reducing with distance from the workings.

Partitioning of copper, zinc and lead was highlighted between florets of *Calluna vulgaris* and woody sections of the plant, estimated to be five years old. It is suggested that this section of the plant be used for future investigations into bioaccumulation of these heavy metals. Analysis of cow faeces collected from two of the downwind sites and a control site shows accumulation of small concentrations of copper which is probably due to accumulation of essential plant copper rather than as a result of pollution. Zinc concentrations in cow faeces however appear to be related to soil concentration.

Water courses appeared to be currently unaffected by metal dispersion downwind of the workings probably due to the effect of intensive agricultural activity in the land upstream from these sampling sites. A stream close to the workings exhibited a low pH value together with high concentrations of copper, lead and zinc, suggesting that the stream was part of an acid mine drainage system which appears to discharge in the direction of Almlwch harbour. Boult et al (1994) demonstrated that AMD was evident in the Afon Goch, a stream rising from the south side of Parys Mountain with similar pH values to the stream sampled at site 6, however lead was not detected in Afon Goch.

Copper lead and cadmium were not detected in any of the *Cladonia* sp. samples tested which suggests that atmospheric dispersion from Parys Mountain has not been noteworthy in recent times, therefore the metal concentrations found in the soil samples along the transect were deposited during the long working life of the mine.

The period during which the mine was working, together with the smelting carried out near the harbour, would have had major effects on the health of the population of

Amlwch. Persistence and bioavailability of heavy metals in soil continues to be a threat to animal and possibly human health.

15.5b The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada

Britannia Mine presented a series of point and diffuse sources of heavy metals and a series of mechanisms capable of dispersing them into the surrounding biosphere. Dispersion and bioaccumulation from spoil heaps, roads and open pits due to leaching and atmospheric dissipation was proved to be considerable especially during the warmer months.

The abandoned town site (Mount Sheer) was similarly polluted with copper, zinc and lead, due to the likely use of spoil from the open pits and underground tunnels for foundation work and road ways. Mount Sheer was a company town and all facilities including shop and hospital were company owned. Food supplies were brought in from Vancouver by boat, however it is possible that miners and there families grew vegetables in gardens surrounding their houses in which case copper lead and zinc would have been ingested due to accumulation in the vegetables.

Medical archives if available would be worth studying although those available at the Britannia Mine Museum, as in the case of Parys Mountain, Anglesey, UK, relate only to accidents and fatalities caused by physical mining activities.

Earlier concerns of pollution of Britannia Creek have been addressed by the installation of a plug in the 2700 portal preventing the discharge of acid mine drainage and samples collected from the creek confirm that contamination has essentially ceased although this has been diverted underground and is still discharging into Howe Sound.

Although persistence and continued bioaccumulation of heavy metals in the vicinity of Britannia mine has been demonstrated, investigation further up the valley would possibly demonstrate the physical causes and effects of dispersal of pollutants from

the open pits. The provincial government, in conjunction with the University of British Columbia have proposed that an environmental monitoring centre be set up near the current site of the museum but until then access to the area is severely restricted.

15.5c Heavy metal concentrations in the vicinity of Lavender Pit, Bisbee, Arizona, USA.

Lavender Pit, near Bisbee, Arizona presents a massive point source of copper and associated heavy metals for subsequent dispersion in the surrounding biosphere. The southerly to south westerly prevailing winds have carried ore particles generated by mining operations in the Pit into the town and beyond, especially into the narrow confines of Brewery Gulch. Additionally, previous smelting operations and present day spoil heaps have contributed to the dispersion of heavy metals in the area.

The concentration of heavy metals in the soil in the vicinity of Lavender Pit was influenced by the distance from the Pit and reduces with distance down wind. The ratio of Cu, Zn, Pb and Cd varied according to distance from the Pit but variation in concentration were recorded between each site. The control initial site chosen did not reflect the concentrations expected but anthropological factors may have influenced results obtained for both soil and vegetation.

Bioaccumulation of Cu, Zn and Pb was demonstrated by leaves of two species of trees and the trophic level accumulation of the target metals would probably lead to accumulation by grazing animals. The consumption of grazing animals reared in the area in the past as well as ingestion of dust then and during the present day would affect the concentration of heavy metals in humans and subsequently the health of residents living in the vicinity of the workings. . It is probable that water supplies in the town of Bisbee and surrounding communities are also affected by leaching from the spoil heaps and ore bodies remaining in the immediate area.

The hospital near Bisbee operated during the mining era was of course company owned and records are currently not available. A search at the Arizona Public

Records Office, Phoenix where all records of death are now registered, showed that since 1902, deaths from metal poisoning had not been recorded in Cochise County. Deaths from 'consumption' had been recorded; the rate of such deaths did not however differ from other counties in Arizona. A close examination of the cemetery failed to produce any evidence of high incidences of death in members of the population below the age of 60 at any period since the town was founded.

16.5d Mission mine Arizona

This location is more extensive than Lavender Pit at Bisbee Arizona where evidence was found of major atmospheric dispersion down wind of the workings, despite the fact that ore extraction ceased there in 1974. Accumulation by trees at the sample sites is also a factor at Bisbee.

Mining practices have been changed substantially in the last thirty years and extensive use of water is used to prevent the generation of large amounts of dust. The USEPA (2004) value for airborne toxic release of 2050 kg in 2002 is relatively low for such a large operation and when dispersed downwind would have had a minimal effect on the metal concentration of the surrounding land. Airborne releases have however been only been progressively reduced and accumulation since the start of operations here in 1961 could be considerable. This study demonstrated that dispersion is greatly reduced when compared with Lavender Pit as Bisbee although the sampling sites were not, by necessity, precisely in the direction of the prevailing winds. Back ground concentrations of the target metals as exhibited at the control site are only marginally lower or similar to other sites away from the complex itself.

Further investigation of soil and plant metal concentration should be undertaken in a north easterly (down wind) direction from the working but this will entail obtaining permission to enter the reservation from the tribal council in Sells, AZ.

The analysis of faeces from mammals collected in the vicinity of mine workings both here and at Parys Mountain as suspected proved to be of little use as a monitor of bioaccumulation. Examination of human tissue such as hair and nails should be

undertaken as well as an assessment of the impact on human health again with the help of the tribal council.

16.5e Cyprus oranges and olives

Analysis of data recorded for the samples from Limni and the Akamas peninsular indicated that partitioning of the metals detected followed a similar sequence within the olive tree, with the flesh of the olive accumulating the highest concentration of copper and lead. Zinc concentrations varied within respective tissues depending on the collection site, but leaves accumulate more zinc than most other tissues. Seed samples collected at both sites failed to accumulate copper or lead but contained a small concentration (6.4 – 7.1 mg kg^{-1}) of zinc which may constitute an essential, therefore normal, concentration for this metal. Pyatt (2001) recorded a similar seed protection mechanism in two different plant species on Cyprus. The orange samples exhibited accumulation copper and zinc concentrations higher at Limni than on the Akamas peninsular, as expected. Examination of the accumulation factors for copper at both sites shows that a higher proportion of the soil concentration is accumulated at the Akamas site, which may be a function of the speciation of the copper metal in the soils or a result of a form of homeostasis which is limiting the total concentration of copper in the various tissues of the plants. This phenomenon should be further investigated.

16.6 Effects of pH on mobility of metals

This study produced some interesting results, illustrating that soil pH does affect theoretical metal release when samples are subjected to sequential extraction procedures. Examining the results in terms of percentage recovery revealed that lead is the most mobile element when examined by this type of extraction system. This type of soil examination when combined with the proposed expression of accumulation factor in chapter 12 should lead to an increase in knowledge regarding metal speciation and accumulation. This chapter in the research produced an array of topics for future investigation.

Single stage extraction using EDTA demonstrated that the pH of soil affects the quantities of metals released and that this technique, for some target metals, removes a proportion of step 1 and step 2 in the SES procedure and for other metals more than step 1 and step 2 combined. Therefore during this limited investigation, there appears to be little correlation between the two techniques and in both studies there is no quantitative link to actual phyto-accumulation.

Laboratory experiments could be designed to determine more clearly how pH effects the actual rate of uptake by plants of copper zinc, lead and cadmium from contaminated soil. The effects of humic acid and organic matter content of soil could also be determined together with the relationship of plant uptake to SES and EDTA data obtained from the soils in use.

15.7 Examination of data from metalliferous mine workings relating to bioaccumulation.

Partitioning of bioaccumulated heavy metals occurred in three plant species examined however metal concentrations in annual and perennial tissues varied from species to species. This research indicates that distribution of metals within plants is not related to soil pH or to the concentration of the metal in the soil and that a similar extent of partitioning takes place in the same species whether they are located in a contaminated site or in a control site.

The concentration of bioaccumulated metals in plant tissues can exceed the concentration of those metals in the surrounding soil, particularly in the case of zinc. Zinc concentrations within plants tend to be of similar magnitude in the same species regardless of metal concentration in the soil.

The use of the proposed accumulation factor illustrates the proportion of heavy metal accumulated from the soil and suggests that homeostatic processes are being carried out by the plants examined, preventing accumulation of toxic levels. The data collected in Cyprus also suggests that seeds were protected from accumulations of toxic metals hence ensuring viability in the future.

15.8 Conclusion

The extraction procedure developed for use as a standard operating procedure proved robust throughout the research and was suitable for the digestion of spoil, soil, plant and bone tissue. The procedure produced precise and accurate results especially later in the project when handling techniques had improved. Analysis of the resultant extract was satisfactory on FAAS, ICP-AES and ICP-MS instrumentation. ICP-AES was eventually adopted as the preferred method of analysis since multi-element determinations can be undertaken. ICP-MS would be necessary if a different oxidation state (speciation) of metal ions was required. The limitations of EDAX and ion chromatography techniques for environmental analysis was established.

The field work demonstrated that atmospheric transport of metal pollution had occurred during mining operations at the four sites, regardless of climate, however recent best practice at the currently operating Mission Mine in Arizona seems to have prevented atmospheric dispersion of copper and associated metals into the surrounding biosphere. The research has adequately demonstrated that the metalliferous pollution is persistent and continues to be bio-accumulated in plants and probably succeeding trophic levels with resultant effects on the health of animals and humans. The difficulty of obtaining medical records relating to possible metal poisoning in mine workers and populations in the surrounding areas prevents further exploration of this area of research, however analysis of archaeological remains could indicate accumulation due to ingestion of metals. The toxicity of metals to plants, animals and man has been known for centuries, though it is probable that toxic effects due either industrial or environmental exposure were frequently not recognised or diagnosed correctly at the time (Thornton and Abrahams, 1984) is the probable course of the lack of information on this aspect of mining, or is this being generous?

The accumulation of metals in plants from contaminated soils in the case of copper, lead and cadmium tend to be related to the concentration of the metal ions in the soil. Zinc, however, seems to be subject to limiting processes such as homeostasis, resulting only minor differences between plants in polluted and unpolluted soils.

Partitioning of metals within plants was interesting in that some annual plant tissues such as flowers and leaves accumulate higher concentrations of the especially phytotoxic metals (copper, lead and cadmium) than any other tissue examined, whereas seeds acquire the lowest concentrations of these metals. This suggested that the annual tissues are heavy metal tolerant and possibly accumulate preferentially to protect the seeds.

The bioavailability of heavy metals in soil was dependant upon factors such as organic matter content, conductivity, pH and climate and the use of sequential extraction procedures highlighted the differences between the availability of each metal at various stages of the procedure. Alkaline soils appeared to have greater availability of the target metals in acid and reducing conditions whereas acid soils released higher concentrations of these metals under oxidising conditions. A more detailed examination of these processes and their relationship to single extraction procedures currently used for agricultural soil analysis, together with actual accumulation by plants, needs to be undertaken. The use of the novel term 'percentage accumulation factor' relating concentration of metals in soil to concentration accumulated requires showed possible homeostatic limitation of accumulation but again this subject requires further investigation.

Chapter 17.

Future Work

17.1 Preparation of some plant tissues

The preparation of some plant tissues, notably woody sections, bark, seeds and catkins as well as cactus tissues present problems relating to large variation in particle size which in turn could lead to lack of precision on subsequent analysis. Further work needs to be carried out to rectify the fragment/particle size problem.

17.2 Microniser as a grinding method for soil and vegetation samples

The use of a microniser could improve precision and reduce processing time for the preparation of soil and vegetation samples, however an extended evaluation would have to be carried out to confirm the suitability of such a procedure.

17.3 Pressurised microwave digestion.

Further critical evaluation of this method needs to be undertaken using aqua regia and extended heating period to ascertain that the process has distinct advantages over the hotplate digestion method used throughout this research.

17.4 Mine Workings

The sites in the UK, British Columbia and Arizona require further investigation into possible mammalian accumulation and particularly at Mission Mine, possible human accumulation of heavy metals on the adjacent tribal lands.

17.5 Metal speciation, soil properties and bioaccumulation

The importance of metal speciation, soil contents, pH and conductivity needs a considerable amount of investigation to determine the mechanisms which affect

leaching of heavy metal ions into the environment and subsequent bioaccumulation by plants. Laboratory experiments could be designed to correlate single and sequential stage extraction with actual accumulation in a variety of plants together with the effects of pH on this mechanism. The introduction of statistical procedures such as factor analysis could also aid the understanding of the subject.

17.7 Development of the use of percentage accumulation factor

Percentage accumulation factor developed during this research should be further examined with a view to determining its relevance to environmental research and attempts made to quantify the factor in terms of types of vegetation most useful as bioaccumulators and therefore useful for land reclamation purposes.

References

Alloway, B.J. 1999. Land Contamination and Reclamation. In *Understanding our Environment* Ed. Harrison, R.M. Royal Society of Chemistry. Cambridge.

Amlwch Industrial Heritage Trust. 1998. Parys Mountain Copper Mines Industrial Heritage Trail. Menter Mon. Llangefni.

Amlwch Industrial Heritage Trust, 2002. Porth Amlwch A Brief History. Menter Mon, Llangefni.

Anonymous, 1997. Water resources in the Tucson Basin: Institutional Resources, AR&S 195a-11. The University of Arizona. Tucson.

Arias, M., Nuñez, A., Barral, M.T. and Díaz-Fierros, F. 1998. Pollution potential of copper mine spoil used for road making. Sci. Total Environ. 221, 111-116.

Bacon, J.R., Hewitt, I.J. and Cooper, P. 2004. Origin of lead associated with different reactive phases in Scottish upland soils: ans assessment made using sequential extraction and isotope analysis. J. Environ. Monit. **6**, 766 – 773.

Balkau, F. 1999. Abandoned Mine Sites Problems Issues and Options. Presented at Berlin II Roundtable on Mining and the Environment. 24 November.

Bari, A., Rossi, A., Minciardi, M.R., Troiani, F. and Piervittori, R. 2001. Analysis of heavy metals in atmospheric particulates in relation to their bioaccumulation in explanted *Pseudevernia furfuracea* thalli. Environ Monit Asses. 69(3), 205 -220

Barry, K.I., Grout, J.A., Levings, C.D., Nidle, B.H. and Piercey, C.E., 2000. Can. J. Fish.Aquat. Sci. 57, 2032 - 2043.

Bech, J., Poschenreider, C., Llugany, M., Barcelo, J., Tobias, F. J., Barranzuela, J.L. and Vasquez, E.R. (1997). Arsenic and heavy metal contamination of soil and

vegetation around a copper mine in Northern Peru. The Science of the Total Environment. 203. 83 -91.

Bellis, D., Cox, A.J., Staton, I., McLeod, C.W. and Satake, K. (2001). Mapping airborne lead contamination near a metals smelter in Derbyshire, UK: spatial variation of Pb concentration and 'enrichment factor' for tree bark. Journal of Environmental Monitoring. **3**(5), 512 – 514.

Bellis, D.J., Satake, K., Tsunoda, K. and McLeod, C.W. 2003. Environmental monitoring of historical change in arsenic deposition with tree bark pockets. J Envir Monit , 5(4): 671 – 674.

Boon, G.T., Bouwman, L.A., Bloem, J. and Rőmkens. 1998. Effects of copper-tolerant grass (*Agrostis calillaris*) on the ecosystem of copper contaminated arable soil. Environ.Toxicol.Chem. **17**, 1964 – 1971.

Boult, S., Collins, D.N., White, K.N. and Curtis, C.D. 1994. Metal transport in a stream polluted by acid mine drainage – the Afon Goch, Anglesey, UK. Environ. Pollut. **84**, 279-284

Britannia Mine Remediation Project., 2002. Background on Pollution and Remediation Issues. Ministry of Water Land and Air Protection. Government of British Columbia, Victoria.

Brito, J.A.A., McNeill, F.E., Chettle, D.R., Webber, C.E. and Vaillancourt, C. (2000). Study of relationship between bone lead levels and its variation in time and the cumulative blood level index in a repeated bone lead survey. Journal of Environmental Monitoring. **2**. (3). 271 - 276.

Brockman, C.F. 1979. Trees of North America. Golden Press. New York. pp108 – 109

Brown, D.E. 1994. Biotic Communities: SW United States and NW Mexico. University of Utah Press. Salt Lake City.

Brown, R.D. 1981. Geology and Geomorphology. In Climbs in the Derwent Valley. Ed. Ballard, J. and Marshall, E. British Mountaineering Council. Manchester. pp. 9-11

Bruno, P., Caselli, P., de Gennaro, G., Ielpo, P. and Traini, A. 2000. Analysis of heavy metals in atmospheric particulate by ion chromatography. Journal of Chromatography A. **888**. (1-2). 145 - 150.

CCME, 2002. Canada Environmental Quality Guide Lines. Available at http://www.ccme.ca/assets/pdf/e1-06.pdf
Accessed 26 March 2004.

Charlesworth, S.M. and Lees, J.A. 1999. The distribution of heavy metals in deposited urban dusts and sediments, Coventry, England. Environ. Geochem. Health. **21**(2), 97-115.

Cid, B.P., Alborés, E., Gómez, E.F. and López, E.F. 2001. Metal fractionation in olive oil and urban sewage sludges using the three stage BCR sequential extraction method and microwave single extractions. Analyst, 126, 1304-1311.

Clark, M.W., Walsh, S.R. and Smith, J.V. (2001). The distribution of heavy metals in an abandoned mining area: a case study of Strauss Pit, the Drake mining area, Australia: implications for the environmental management of mine sites. Environmental Geology. **40.** (6). 655 -663.

Clarke AG. 1997 The Atmosphere. In: Harrison RM, editor. Understanding our Environment. An Introduction to Environmental Chemistry and Pollution. 2nd Edition. The Royal Society of Chemistry, Cambridge, pp. 5 – 51.

Clarke, A.G. 1997. The Atmosphere. In Understanding our Environment. Ed. Harrison, R.M. The Royal Society of Chemistry. Cambridge. pp. 16 - 52.

Community Bureau of Refence. 1990. The certification of the content of N, P, S and Cl in beech Leaves (CRM 100 – *Fagus silvatica*) and Al, Ca, Mg, Zn, N, P, S and Cl

in spruce needles (CRM 101 – *Picea abies*). Report EUR 12680 EN. Luxembourg. Commission of the European Communities.

Community Bureau of Reference. 1994. Certification of total contents (mass fractions) of Cd, Co, Cu, Mn, Hg and Ni and the aqua regia soluble fractions of Cd, Pb, Ni and Zn in a light sandy soil. CRM142R. Report EUR 15283 EN. Luxembourg. Commission of the European Communities.

Cottenie, A., Camerlynck, R., Verloo, M. and Dhaese, A. 1979. Fractionation and determination of trace elements in plants, soils and sediments. Pure and Appl. Chem. **52**, 45 – 53.

Cox, R.M. and Hutchinson, T.C. 1980. Copper tolerance in *Deschampsia caespitose*. New Phytol. 64, 631.

Crowder, A. 1991. Acidification, metals and macrophytes. Environ. Pollut. **71**, 171-203

Curtis, L. 2004. Metalliferous pollution in an archaeologically important site in Anglesey, North Wales, U.K. Unpublished MPhil thesis, Nottingham Trent University, Nottingham.

Davidson, C.M., Duncan, A.L., Littlejohn, D., Ure, A.M. and Garden, L.M. 1998. A critical evaluation of the three-stage BCR extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially-contaminated land. Analyt. Chimica Acta. **363** (1), 45 – 55.

De Gregori, I.., Fuentes, E., Rojas, M., Pinochet, H. and Potin-Gautier, M. 2003. Monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non impacted by mining activities from three regions of Chile. J. Environ Monit, 5, 287 – 295.

De Koe, T. 1994. *Agrostis castellana* and *Agrostis deticulata*on heavy metal and arsenic rich enriched sites in NE Portugal. Science of the Total Environment. 145. 103 – 109.

DEFRA 2002. Contaminated Land Exposure Assessment (CLEA 2002). UK Department for Environment, Food and Rural Affairs, London.

Del Rio, M., Font, R., Almela, C., Velez, D., Montoro, R. and De Haro Bailon, A. (2002). Heavy metals and Arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the Aznalcollar mine. J. Biotechnol. 98. 125 - 137.

Domingo, J.L. 1994. Metal induced developmental toxicity in mammals. A review. J. Toxicol.Environ. Health. **42**, 123-141.

Ellis, D.V. and Hoover, P.M., 1990. Mar. Poll. Bull. 21(10), 477 - 480.

Ernst, W.H.O. (1993). Geobotanical and biogeochemical prospecting for heavy metal deposits in Europe and Africa. In *Plants as biomonitors. Indicators of heavy metals in the terrestrial environment*. Ed. Merkert, B. VCH Publishers Inc. New York.

European Commission. 2001. Report No. EUR 19775 EN. The certification of the extractable contents (mass fractions) of Cd, Cr, Cu, Pb, and Zn in freshwater sediment following a sequential extraction procedure. BCR-701. Office for Official Publications of the European Communities. Luxembourg.

Evdokimova, G.A. and Mozgona, N.P. 2003. Restoration of properties of cultivated soils polluted with copper and nickel. J. Environ. Monit., **5**(4), 675 - 680

Filgueiras, A.V., Lavilla, I. and Bendicho, C. 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. J. Environ. Monit. **4**, 823 – 857.

Forstner, U. 1995. Land contaminated by metals - global scope and magnitude of the problem. In *Metal Speciation and Contamination* of Soil. Ed. Allen, H.E., Huang, C.P., Bailey, G.W. and Bowers, A.R. C.R.C. Press Inc. Florida. pp. 3-21.

Francaviglia, R.V. 1982. Copper Mining and Landscape Evolution. The Journal of Arizona History. **23** (3). 267 – 298.

Gabriel, I.E and Patten, T.. 1994. Distribution of copper smelter emissions in south eastern Arizona using honey mesquite as a biomonitor. Water, Air and Soil Pollution. (72) 67 – 87.

Gale, N.L., Adams, C.D., Wixson, B.G., Loftin, K.A. and Huang, Y.: 2004, Lead, zinc, copper and cadmium in fish and sediments from the Big River and Flat River Creek of Missouri's Old Lead Belt. Environ Geochem Health **26**, 37-39,

Golder Associates Ltd., 2002A. Water Treatment Pilot Plant Open Day. In Britannia Mine Remediation News Letter. Issue 2, 1, Vancouver

Golder Associates Ltd, 2002B. Britannia Creek Fan Preliminary Site Investigation Britannia Beach, BC. Prepared for BC Environment.

Goodarzi, F., Sanei, H., Labonte, M. and Duncan W.F. 2002. Sources of Lead and Zinc associated with metal smelting activities in the Trail area, British Columbia, Canada. J Environ Monit 2002; 4(3): 400 – 407.

Goodhew, P.J. and Humphreys, F.J. 1992. Electron Microscopy and Analysis. 2nd Edition. Taylor and Francis Ltd., London.

Groet, S.S. 1980 Biological indicators of atmospheric zinc dispersal and deposition. In *Zinc in the Environment: Part 1. Ecological cycling.* Ed. Nriagu, J.O. John Wiley and Sons Inc. New York. pp. 161 – 192.

Gunderson, P., Olsvik, P.A. and Steinnes, E. 2001. Variations in Heavy Metal Concentrations and Speciation in Two Mining-polluted Streams in Central Norway. Environ Toxicol Chem, **20**. (5). 978 – 984

Gupta, S.K., Vollmer, M.K. and Krebs, R. 1996. The importance of mobility, mobilisable and pseudototal heavy metal fractions in soil for three-level risk assessment and risk management. Sci. Total Environ. **178**, 11 – 20.

Hagen, M., 2001. Britannia Marine Sediment Contamination: Interim Status of Knowledge and Next Options. Prepared for Environment Canada. Ottawa.

Heikens, A., Peijnenburg, W.J.G.M. and Hendriks, A.J. Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Pollut. 113, 385 – 393.

Heinrichs, W.E. 2000. The Pima Mine Story. Western Economic History Center, Marana, AZ.

Hewitt, C.N. and Allott, R. 1997. Environmental Monitoring Strategies. In Understanding our Environment: An Introduction to Environmental Chemistry and Pollution. Ed.Harrison, R.M. The Royal Society of Chemistry. Cambridge. pp. 229

Hilbk-Kortenbruck, K.F., Noll, R., Winjens, P., Falk, H. and Becker, C. 2001. Analysis of heavy metals in soils using laser-induced breakdown spectrometry combined with laser-induced fluorescence. Spectrochimica Acta Part B. **56** (6), 933 – 945.

Hoenig, M., Baeten, H., Vanhentenrijk, S., Vassileva, E. and Quevauviller, 1998. P. Critical discussion on the need for an efficient mineralization procedure for the nalysis of plant material by atomic spectometric methods. Analyt. Chimica Acta, **358**, 1, 85 – 94.

Hu, H. 2002. Human Health and Heavy Metals Exposure. In Life Support: The Environment and Human Health. Ed. McCally, M. MIT Press, Cambridge, USA.

Hur, J., Schautman, M.A. and Yin, S.: 2004, Effects of organic ligands and pH on the leaching of copper from brake wear debris in model environmental solutions. J. Environ. Monit., **6**, 89 – 94.

Ilha, M.R.S., Loretti, A.P. and Barros, S.S. 2001. Spontaneous poisoning of sheep by *Senecio brasiliensis* (Asteraceae) in southern Brazil. Pesquilla Vetinaria de Brasilia. **21**. 3. 123 – 138.

Ipolyi, I., Brunori, C., Cremisini, C., Fodor, P., Macaluso, L. and Morabito, R. 2002. Evaluation of performance of time-saving extraction devices in the BCR three-step sequential extraction procedure. J. Envir. Mon. 4. 541 – 548.

Jenkins, D.A., Johnson, D.B. and Freeman, C.: 2000, Mynydd Parys Cu-Pb-Zn Mines:minerology, microbiology and acid mine drainage. In Environmental Minerology: Microbial interactions, Anthroprogenic Influences, Contaminated Land and Waste Management. Ed.L.S. Cotter-Howells, Cambell, E. Valsami-Jones, and M. Batchelder. Mineralogical Society, London. pp. 161-179.

Jong, T. and Parry, D.L. 2004. Heavy metal speciation in solid phase materials from bacterial sulphate reducing bioreactor using sequential extraction procedure combined with acid volatile sulphide analysis. J Environ. Monit. **6**, 278 – 285.

Kearney, J.: 2003. Anglesey Mining plc Annual Report 2003. Anglesey Mining plc. Amlwch.

Kheboian, C. and Bauer, C.F. 1987. Accuracy of selected extraction procedures for metla speciation in model aquatic sediments. Anal. Chem. **59**, 1417 – 1423.

Koptsik, S., Koptsik, G., Livantsova, S., Eruslankina, L., Zhmelkova, T. and Vologdina, Zh. 2003. Heavy metals in soils near the nickel smelter: chemistry, spatial variation and inpacts on plant diversity. J. Environ. Monit. **5**(3), 441-450.

Koucky, F.L. and Steinberg, A. 1982. The ancient slags of Cyprus. In *Early metallurgy in Cyprus 4000 – 500BC.* Ed. Muhly, J.D, Maddin, R. and Karageorghis, V. Pierides Foundation, Nicosia. 117 – 137.

Kozlov, M. V., Haukioja, E. and Kovnatsky, E. F. 1998. Uptake and excretion of nickel and copper by leaf-mining larvae of *Eriocrania semipurpurella* (Lepidoptera: Eriocraniidae) feeding on contaminated birch foliage. Environmental Pollution. 108. 303 – 310

Krachler, M., Mohl, C., Emons, H. and Shotyk, W. 2002. Analytical procedures for the determination of selected trace elements in peat and plant samples by inductively coupled plasma mass spectrometry. Spectro Chimica Acta Part B 57, 1277 – 1289.

Kruckeberg, A.L. and Wu, L. 1992. Copper tolerance and copper accumulation of herbacious plants colonising inactive californian copper mines. Ecotoxicology and Environmental Safety. 23. 307 - 319.

Lavkulich, L.M. 2002. Soil and water resources research of relevance to mining. Presented at Workshop Seminar, September 2002. University of British Columbia, Vancouver, BC, Canada.

Leaming, G.F. 1998. The Story of Mining in Bisbee. Free Geos Library. Marana, Arizona.

Levings, C.D. and McDaniel, N., 1973. Biological observations from the submersible Pisces IV near Britannia Beach, Howe Sound, B.C. Fish and Marine Service Tech. Report No. 663. Government of British Columbia, Victoria.

Luckey, T.D. and Venugopal, B. 1977. Metal Toxicity in Mammals: 1. Physiological and chemical basis for metal toxicity. Plenum Press, New York.

Luyssaert, S., Mertens, J and Raitio, H. 2003. Support, shape and number of replicate samples for tree foliage analysis. J Environ Monit , 5: 500 – 504.

Ma, L.Q., Komar, K.M, Tu, C. Zhang, W.Cai, Y. and Kennelly, E.D. 2001. A fern that hyperaccumulates arsenic. Nature. 409. 579.

Maggs, R., Mann, C. and Brooker, J. 2001. Monitoring of Lead, Arsenic, Cadmium, Nickel and Mercury around Industrial Sites. Report prepared for DEFRA by Stanger, Science and Environment. pp 65 - 67.

Maisto, G., Alfani, A., Baldantoni, D., De Marco, A and De Santo, A.V. 2004. Trace metals in the soil and in *Quercus ilex* L. leaves at anthropic and remote sites of the Campania region of Italy. Geoderma 122, 269-279.

Maiz, I., Esnaola, M.V. and Millan, E. 1997. Evaluation of heavy metal availability ib contaminated soils by a short sequential extraction procedure. Sci. Total Environ. **206**. 107 – 115.

Maiz, I., Arambarri, I., Garcia, R. and Millan, E. 2000. Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. Environ. Pollut. **110**, 3 – 9.

Malawska, M and Wilkomirska, B. 2001. An analysis of soil and plant (*Taraxacum officinale*) contamination with heavy metals and polycyclic aromatic hydrocarbons (PAHs) in the area of a railway junction Ilawa Glowna, Poland. Water Air Soil Pollut. **127**, 339 – 349.

Market, B. 1993. Instrumental analysis of plants. In Plants as Biomonitors. Ed Market, B. VCH Publishers Inc. New York. 65 - 103.

Martin, J.S. 1998. Chronic Copper Poisoning in Sheep. Ontario Ministry of Agriculture and Fisheries. Ottawa.

Martinez, G.M.L., Carlosena, Z.A., Miniategui, L.S., Lopez, M. P. and Prada, R.D. 1999. Analysis of metals in marine sediments by microwave extraction and flame, hydride generation and cold vapour atomic-absorption spectrometry. Analytical Letters. **32** (1), 139 – 157.

Massadeh, A.M. and Snook, R.D. 2002. Determination of Pb and Cd in road dusts over the period in which Pb was removed from petrol in the UK. J. Environ. Monit., **4** (4), 567 – 572.

Mathys, W. 1980. Zinc Tolerance of plants. In *Zinc in the Environment. Part 2 Health Effects.* Ed. Nriagu. John Wiley and Sons. New York. 415 – 437.

McGrath, D. 1998. Use of microwave digestion for estimation of heavy metal content of soils in a geochemical survey. Talanta. **46** (3), 439 – 448.

Mierzwa, J., Sun, Y.C., Chung, Y.T. and Yang, M.H. 1998. Comparative determination of Ba, Cu, Fe, Pb and Zn in tea leaves by slurry sampling eletrothermal atomic absorption and liquid sampling inductively coupled plasma atomic emission spectrometry. Talanta, **47** 5, 1263 – 1270.

Miller, J.C. and Miller, J.N. 2000. Statistics and Chemometrics for Analytical Chemistry. 4th Edition. Pearson Education Ltd., Harlow.

Miller, W.J. and Neathery, M.W. 1980. Manifestations of zinc abnormalities in animals. In Zinc in the Environment. Ed. Nriagu, J.O. John Wiley and Sons, New York. pp 61 – 70.

Mills, C. 2003. The Former Britannia Mine, Mount Sheer/Britannia Beach, British Columbia.
Available at http://technology.infomine.com/enviromine/ard/Case%20Studies/Britannia.htm
Accessed 12 May 2003.

Moeller, A., Ambrose, R. F. and Que Hee, S.S. 2001. A comparison of techniques for preparing fish fillet for ICP-AES multielemental analysis and the microwave digestion of whole fish. Food Additives and Contaminants.**18** (1), 19 – 29.

de Mora, S.J.: 1999. The Oceanic Environment. In Understanding our environment. Ed. R.M. Harrison . Royal Society for Chemistry, Cambridge. pp. 150 – 153.

Murphy, A.P., Courdert, M. and Barker, J. 2000. Plants as biomarkers for monitoring heavy metal contaminants on land fill sites using sequential extraction and inductively coupled plasma atomic emission spectrophotometry, (ICP-AES). J.Environ.Monit. 2, 621 – 627.

Pederson, M. B., Kjaer, C. and Elmegaard, N. 2000. Toxicity and bioaccumulation of copper in Black Bindweed (*Fallopia convolvulus*) in relation to bioavailability and age of soil contamination. Arch. Environ. Contam. Toxicol. 39, 431 – 439.

Petecost, A. 1999. Analysing Environmental Data. Pearson Education Ltd, Harlow, U.K. pp. 84-98.

Pérez, G. and Valiente, M. 2005. Determination of pollution trends in an abandoned mining site by application of a multivariate statistical analysis to heavy metals fractionation using SM&T-SES. J. Environ. Monit. 7, 29-36.

Polkowska, M.H., Danko, B., Dybczynski, R., Koster, A.A. and Bode, P. 2000. Effect of acid digestion method on cobalt determination in plant materials. Analyt. Chimica Acta, **408** 1, 89 – 95.

Pueyo, M., Rauret, G., Luck,D., Yli-Halla, M., Mutau, H., Quevauviller, Ph. and Lopez-Sanchez, J.F. 2001. Certification of the extractable contents of Cd, Cr. Cu. Ni. Pb and Zn in a freshwater sediment following a collaboratively tested and optimised three step sequential extraction procedure. J. Environ. Monit. **3** (2), 243 – 250.

Pyatt, F.B. 1999. Comparison of Foliar and Stem Bioaccumulation of heavy Metals by Corsican Pines in the Mount Olympus Area of Cyprus. Ecotox Environ Safety, 42: 57 – 61.

Pyatt, F.B. 2001. Copper and lead bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in sites contaminated as a consequence of extensive Ancient mining activities in Cyprus. Ecotoxicology and Environmental Safety. 50, 60 - 64.

Pyatt, F.B. and Birch, P. 1994. Atmospheric erosion of metalliferous spoil tips: some localised effects. Polish J. Environ. Stud. **4**, 351 – 353.

Pyatt, F.B. and Grattan, J.P. 2001. Some consequences of ancient mining activities on the health of ancient and modern human populations. J. Public Health Medicine. **23**(3), 235 – 236.

Pyatt, F.B. and Grattan, J.P. 2003. Fen and Loch sediment geochemistry: climatic and environmental changes over 12,000 years on the western seaboard of Britain – EDMA approaches and solutions. Variazioni climatico-ambientali e impatto sull,uomo nell'area circum mediterranea durente l'olocene. Ed. C.A. Lavadie and F. Ortolant. 203 – 214. Centro Universitario Europeo per i beni Culturali, Ravello, Italy.

Pyatt, F.B., Grattan, J.P., Lacy, D., Pyatt, A.J. and Seaward, R.D. 1999. Comparative effectiveness of *Tillandsia usneoides* L. and *Parmotrema praesorediosum* (NYL.) Hale as Bio-indicators of atmospheric pollution in Lousiana (U.S.A.). Water,Air and Soil Pollution. 111, 317 – 326.

Pyatt, F.B., Gilmore, G., Grattan, J.P., Hunt, C.O. and McLaren, S. 2000. An imperial legacy? An exploration of the environmental impact of ancient metal mining and smelting in Southern Jordan. Journal of Archaeological Science. 27, 771 - 778.

Pyatt, F.B., Pyatt, A.J., Walker, C., Sheen, T. and Grattan, J.P. 2005A. The heavy metal content of skeletons from an ancient metalliferous polluted area in southern Jordan with particular reference to bioaccumulation and human health. Ecotox. Environ. Safety. **60**, 295 – 300.

Qin, F., Shan, X. and Wei, B. 2004. Effects of low-molecular-weight organic acids and residence time on desorption of Cu, Cd and Pb from soils. Chemosphere. **57**, 253 – 263.

P. Quevauviller, P., Lachica, M., Barahona. E., Gomez, A., G. Rauret, G., Ure, A. and Muntau, H. Certified reference material for the quality control of EDTA- and DTPA-

extractable trace metal contents in calcareous soil (CRM 600). Fesenious' J. Anal. Chem. **360**, 505 – 511.

Raber, P.A. 1984. Organization and development of early copper metallurgy in the Polis region, western Cyprus. PhD thesis, Pennsylvania State University.

Radofevic, M. and Bashkin, V. N. 1999. Practical Environmental Analysis. Cambridge. The Royal Society of Chemistry. pp. 296-298.

Rapp, G., Jr. 1982. Native Copper and the beginning of smelting: chemical studies. In *Early metallurgy in Cyprus 4000 – 500BC.* Ed. Muhly, J.D, Maddin, R. and Karageorghis, V. Pierides Foundation, Nicosia. 33 – 38.

Rawlins, B.G., Webster, R. and Lister, T.R. 2003. The influence of parent material on topsoil geochemistry in Eastern England. Earth Surf. Process. Landforms. 28, 1389-1409.

Reinecke, A. J., Reinecke, S. A., Musilbono, D. E. and Chapman, A. (2000). The transfer of lead (Pb) from earth worms to shrews (*Myosorex varius*). Arch. Environ. Contam. Toxicol. 39, 392 – 397.

Rowell, D.L. 1994. Soil Science. Methods and Application. Longman Scientific and Technical. Harlow. pp. 285-286

Rowlands, J.R. 1966. Copper Mountain. 2nd edition. Anglesey Antiquarian Society Llangefni.

Salt, D.E., Smith, R.D. and Raskin, I. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Mol. Biol. **49**, 643-668

Ščančar, J., Milačič, R. and Horvat, M. 2000. Comparison of various digestion and extraction procedures in analysis of heavy metals in sediments. Water, Air, Soil Pollut. **118**(1-2). 87 – 99.

Scwitzguébel, J.P., van de Lelie, D., Baker, A., Glass, D. and Vangronsveld, J. 2002. Phytoremediation: European and American trends. Successes, obstacles and needs. J.Soils Sediments. **2**, 91-99

Shallari, S., Schwartz, C., Hasko, A. and Morel, J.L. 1998. Heavy meatsl in soils and plants of serpentine and industrial sites of Albania. Science of the Total Environment. 209. 133 - 142.

Sharenberg, W and Ebeling, E. 1996. Distribution of heavy metals in a woodland food web. Bulletin of Environmental Contamination and Toxicology. 56. 389 - 396.

Shotyk, W. 1996.Peat bog archives of atmospheric metal deposition: geochemical evaluation of peat profiles, natural variations in metal concentrations and metal enrichment factors. Environmental Review. 4, 149 - 183.

Shparyk, Y.S. and Parpan, V.I. 2004. Heavy metal pollution and forest health in the Ukrainian Carpathians. Environ Pollut. 130, 55 – 63.

Slack, R. 2000. Lead Miners Heyday. Ron Slack. Chesterfield. pp. 29

Smith, J.G. and Rongstad, O.J. 1982. Small mammal heavy metal concentrations from mined and control sites. Environ. Pollut. Ser. A. 28, 121-134.

Sparks, D.L. 1995. Environmental Soil Chemistry. Academic Press Inc. San Diego, California. pp. 56-57.

Streit, B and Stumm, W. 1993. Chemical properties of Metals and the Process of Bioaccumulation in Terrestrial Plants. In Plants as Biomonitors. Ed. Markert, B. VCH Verlagsgegeellschaft. Weinheim. pp. 31 – 62.

Tack, F .M.G. and Verloo, M.G. 1999. Single extractions versus sequential extraction of heavy metal factions in reduced and oxidised dredged sediments. Chem. Speciat. Bioavail. **11**(2), 43 – 50.

Tessier, A., Cambell, P.G.C. and Bisson, M.:1979, Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry **51**, 844-851

Thompson, J.A.J. and McComas, F.T., 1974. Fish. Res. Bd. Can. Tech. Rep. No.437.

Thornton, I. and Abrahams, P. 1984. Historical records of metal pollution in the environment. In: *Changing Metal Cycles and Human Health.* Ed. Nriagu, J.O. Springer Verlag, Berlin.

Towne, D.C. 2000. Ambient Ground Water Quality of the Douglas Basin: An ADEQ 1995-1996 Baseline Study. Arizona Department of Environmental Quality, Phoenix.

Tingi, U., Reilly, C. and Patterson, C. 1997. Determination of manganese and chromium in foods by atomic absorption spectrometry after wet digestion. Food Chemistry. **60**. (1). 123 – 128.

Tokalıoğlu, Ş, Kartal, Ş and Birol, G. 2003. Comparison of three sequential extraction procedures for partitioning of heavy metals in car park dusts. J.Environ. Monit., **5**(3), 468 - 476.

Ure, A.M. 1996. Single extraction schemes for soil analysis and related applications. Sci. Total Environ. **178**, 3 – 10.

URS. 2002. Britannia contaminated site investigation. Fan area remediation planning document Britannia Mine site, British Columbia. Prepared for BCMinistry of Water, Land and Air Protection. Vancouver.

USEPA. 1997. Water supply, waste water collection and treatment project for city of Naco, Sonora, Mexico. Prepared by Science Applications International Corporation, Denver.

USEPA. 1998. SW 846 Test Methods for Evaluating solid wastes. Physical /Chemical methods. National Technical Information Service. Springfield, VA., USA.

USEPA. 2004. TRI Explorer report for Asarco Inc Mission complex (zip code 85629) year 2002. Available at www.epa.gov

Vassilev, A., Schwitzguébel, J-P., Thewys, T., van de Lelie, D. and Vagronsveld, J. 2004. The use of plants for remediation of metal-contaminated soils. TheScientificWorldJOURNAL. **4**, 9-34.

Veinott, G., Sylvester, P., Hamoutene, D., Anderson, M.R, Meade, J. and Payne, J. 2003. State of the marine environment at Little Bay Arm, Newfoundland and Labrador, Canada, 10 years after a "do nothing" response to a mine tailings spill. J. Environ. Monit. 5, 626 – 634.

Walker, D.J., Clemete, R. and Bernal, M.P. 2004. Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste. Chemosphere. **57**, 215 – 224.

Wallingford, G.W. and Simkins, C.A. 1977. Copper for organic soils. University of Minnesota Agric. Ext. Service. Folder 374.

Warkentin, D. 2000. Taking out the Trash at the old Britannia Mine Site. BC's Green Party News. **49**. Summer.

Wasilewska, M., Goessler, W., Zischka, M., Maichin, B. and Knapp, G. 2002. Efficiency of oxidation in wet digestion procedures and influence from the residual organic carbon content on selected techniques for determination of trace elements. J. Analyt. Atomic Spectroscopy. **17** (9), 1121 – 1125.

Weber, J. and Karczewska, A. 2004. Biochemical processes and the role of heavy metalsin the soil environment. Geoderma. **122**, 105 – 107.

Whalley, C. and Grant, A. Assessment of phase selectivity of the BCR sequential extrction procedure for metals in sediment. Anal. Chim. Acta. **291**, 287 – 295.

West, S., Charman, D.J., Grattan, J.P. and Cherburkin, A.K. 1997. Heavy metals in halocene peats from South West England: detecting mining impacts and atmospheric pollution. Water Air and Soil Pollution. 100. 343 - 353.

Wilson, B. 2002. The evolution of water composition in the Trent basin. Undergraduate BSc(Hons) project. The Nottingham Trent University. Unpublished.

Wilson, B, Braithwaite, A and Pyatt, F.B. 2005A. An evaluation of procedures for the digestion of soils and vegetation from areas with metalliferous pollution. Toxicol. Environ. Chem. *In press.*

Wilson, B, Lang, B. and Pyatt, F.B. 2005B. The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada. Ecotoxicol. Environ. Safety. 60, 269 – 276.

Xiao-Quan, S. and Bin, C. 1993. Evaluation of sequential extraction for speciation of trace metals in model soil containing natural minerals and humic acid. Anal. Chem. **65**, 802 – 807.

Yaron, B., Calvet, R. and Prost, R. 1996. Soil Pollution. Processes and Dynamics. Springer-Verlag. Berlin. pp. 21-22.

Yukselen. M.A. and Alpaslan, B.: 2001, Leaching of metals from soil contaminated by mining activities. J. Hazardous Mater. **B87**, 289 – 300.

Zalasiewicz, J. 2000. The relationship of sedimentation, diagius, volcannen and mineralization at Parys Mountain, Anglesey. Proposed research topic. Leicester University.

www[1] – http://www.ap.pwp.blueyonder.co.uk/sbhist.httm. Accessed 14 November 2002.

www[2] - http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3051.pdf Accessed 25 January 2005

www[3] www.bcmuseumofmining.org/history.html (accessed 15 September 2004)

www[4] http://www.weatherbase.com/weather/weather.php3?s=547227 (accessed 13 September 2004)

www[5] http://asarco.com/mission.html (accessed 13 September 2004)

www[6] http://www.elmhurst.edu/~chm/vchembook/330copper.html (accessed 13 September 2004

www[7] http://www.geology.ucdavis.edu/~cowen/~GEL115/115CH4.html (accessed 20 January 2005)

www[8] http://www.llansadwrn-wx.co.uk/gwynt/windstats.html#dir (accessed 12 February 2005)

Appendix

Published Work

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Ecotoxicology and Environmental Safety 60 (2005) 269–276
Ecotoxicology and Environmental Safety
http://www.elsevier.com/locate/ecoenv

The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada

Bob Wilson,[a,*] Brennan Lang,[b] and F. Brian Pyatt[a]

[a] *Interdisciplinary Biomedical Research Centre, Faculty of Science and Technology, The Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK*

[b] *Department of Mining Engineering, The University of British Columbia, 2329 West Mall, Vancouver, Canada V6T 1Z4*

Received 7 July 2003; received in revised form 29 March 2004; accepted 8 April 2004

Available online 25 May 2004

Abstract

Britannia Mine in British Columbia, Canada, is a major source of copper and other heavy metal pollutants, which enter the sea at Howe Sound. This investigation aims to determine whether there is sufficient Cd, Cu, Pb, and Zn, in the terrestrial environment surrounding the mine to suggest that dispersion and subsequent bioaccumulation has occurred in the past and continues. Samples of spoil, vegetation, and water were collected in January 2003 from areas at sea level and up to an altitude of 790 m. The samples of soil and vegetation were digested with aqua regia. The subsequent extracts and water samples were analyzed using flame atomic absorption spectrophotometry. The target metal concentrations were found to be much higher on the hillside spoil tips than on the seashore where the ore concentrators had formerly been located. The dispersion of heavy metals into the surrounding biosphere is discussed and further investigations into dispersion and partitioning within organisms including humans are suggested.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Copper; Heavy metals; Pollution; Dispersion; Bioaccumulation; Britannia Mine

1. Introduction

Britannia Mine is situated above the settlement of Britannia Beach, some 50 km north of Vancouver on Highway 99, also known as the Sea to Sky Highway. The nearest large town is Squamish, 11 km north of the site.

The mine at Britannia Beach operated from 1898 until 1974 and in the 1920s was said to be the largest copper mine in the British Commonwealth (URS, 2002). Between 1905 and 1974 the mine produced 47.8 million tonnes of ore (Mills, 2003). Secondary products were Zn, Ag, Au, Cd, and Pb (URS, 2002)

The site is currently considered to be one of the worst sources of water pollution in North America (Warkentin, 2000). The mine covers an area of some 28 km^2 and consists of a series of tunnels and some open-cast mining. The mine originally commenced on a hill at an elevation of 1400 m above sea level and now extends below sea level. Five open pits were also created near the summit of the hill. A series of portals exit onto the hillside at different altitudes and aspects, but all have waste rock dumps (spoil tips) nearby, eventually washing down into a series of creeks. When the mine was abandoned, acid mine drainage (AMD) became a major problem, issuing from several portals and a submerged drain extending into Howe Sound. Britannia Creek, which collected AMD from Jane Creek and Mineral Creek, was also a source of discharge into Howe Sound. A concrete plug is reported to have closed off AMD from the 2200 level portal (Britannia Mine Remediation Project, 2002) and AMD from the mine is being discharged via the submerged drain. Dr. John Meech of the UBC Department of Mining estimates that at least 1 tonne of copper is discharged into the Sound each day (personal communication, 2002).

The provincial government has obtained reparations from various previous owners of the mine to carry out remedial work and currently employs several

*Corresponding author. Fax: 44-1158483384.
E-mail address: bob.wilson@ntu.ac.uk (B. Wilson).

0147-6513/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2004.04.005

consultants who are determining the extent of the remediation required to make the site safe. They have identified many areas of environmental concern (AEC), the majority of which center on the Fan Area, where the old concentrators were sited. This area now supports some 300 individuals who live in either old mine housing or trailers. The site owners when the samples were collected were Copper Beach Estates, however currently, responsibility for the area appears to have been assumed by the Provincial Government. It is clearly in their interest to have the site declared safe to allow further commercial development of the land.

Fig. 1 illustrates the extent of the site. The old town site (Mt. Sheer) has been leveled and the area has now been invaded by *Alnus* spp. with some early growth of *Picea sitchensis*, the usual successional sequence in western Canada after clear cutting of lumber. The prevailing winds are either northerly or southerly up and down Howe Sound and have little effect in the valleys on either side of the mine. The area is classified as a coastal wet climatic zone with high levels of precipitation most of the year but with very warm summers. Precipitation in winter regularly falls as snow above about 300 m.

Much work has been carried out examining the effect of AMD into the Sound and the effects of some 40,000 tonnes of tailings (from the concentrators) that were discharged from 1902 to 1974 into Howe Sound (Hagen, 2001). Such examinations range from underwater surveys from diving vessels (Levings and McDaniel, 1973) to the effects of the local sea water on transplanted rainbow trout (Barry et al., 2000) and estimates of benthic organism reduction around the Sound (Ellis and Hoover, 1990). There has been no assessment of the dispersion, accumulation, and partitioning of copper and associated metals into the surrounding biosphere. Local residents have expressed concern over health issues for many years, although such concerns have been refuted by the various consultants employed by the provincial government. Hagen in 2001, noted that groundwater and upland influences will need to be studied. It should be noted that a pilot water-treatment plant has been installed in the local primary school to allay concerns regarding the health of children in the area (Golder Associates, 2002a).

2. Materials and methods

Samples of spoil (waste rock) were collected in January 2003 near the entrances of the 2200 level and 2700 level tunnels and from the fan area adjacent to the seashore. Foliage from small spruce trees (*Picea sitchensis*) was collected from the spoil area at the 2200 level and the seashore. Samples were collected from the same aspect and height above the ground for each tree. Trees in a similar condition of health were selected. Water samples were collected from Britannia Creek above and below the confluences with Jane Creek and from Jane Creek just above the confluence with Britannia Creek. The sample collection sites are shown in Fig. 1:

Site No. 1. Shore, just above high tide mark.
Site No. 2. 2200 level, adjacent to the portal entrance.
Site No. 3. 2700 level, adjacent to the portal entrance.
Site No. 4. Britannia Creek, above the confluence with Jane Creek.
Site No. 5. Jane Creek, adjacent to the gauging station.
Site No. 6. Britannia Creek, below the confluence with Jane Creek.

Spoil samples were collected using a clean stainless steel trowel. The initial 5 mm of soil in an approximately 400 cm^2 area was scraped off and discarded. Approximately 200 g of soil was then collected to a maximum depth of 50 mm and placed in a clean polythene bag. Foliage samples were collected using clean vinyl examination gloves and clean stainless-steel secateurs where necessary and placed in clean polythene bags. Leaf samples are taken from the same species of tree, of similar height and from the same aspect. All solid samples were triple bagged to prevent damage and contamination during transport to the laboratory. Water samples were collected in previously acid-washed and cleaned HDPE bottles. Single samples of each substrate were taken from each site on this occasion.

3. Experimental procedure

After being returned to the laboratory, spoil samples were air-dried in a closed cabinet for 8 days and then sieved through a clean 2-mm stainless-steel mesh. The sieved material was then placed in an oven for 3 h at 60°C, cooled, and ground in a previously acid-washed mortar and pestle (West et al., 1997), and the resultant dust was stored in a cleaned HDPE bottle for analysis.

Foliage samples were thoroughly washed and rinsed in deionized distilled water and air-dried overnight and the needles were separated from the stems by hand using clean examination gloves. The needles were then placed in an oven at 105°C for 3 h (Radojevic and Bashkin, 1999) and, after cooling, were ground for 5 min in a cleaned mortar and pestle before transfer to a cleaned HDPE bottle.

When returned to the laboratory, water samples were filtered through Millipore 0.45-μm nitrocellulose filters into cleaned HDPE bottles and 0.5% of 70% HNO_3 was added before storage in a refrigerator at 4°C.

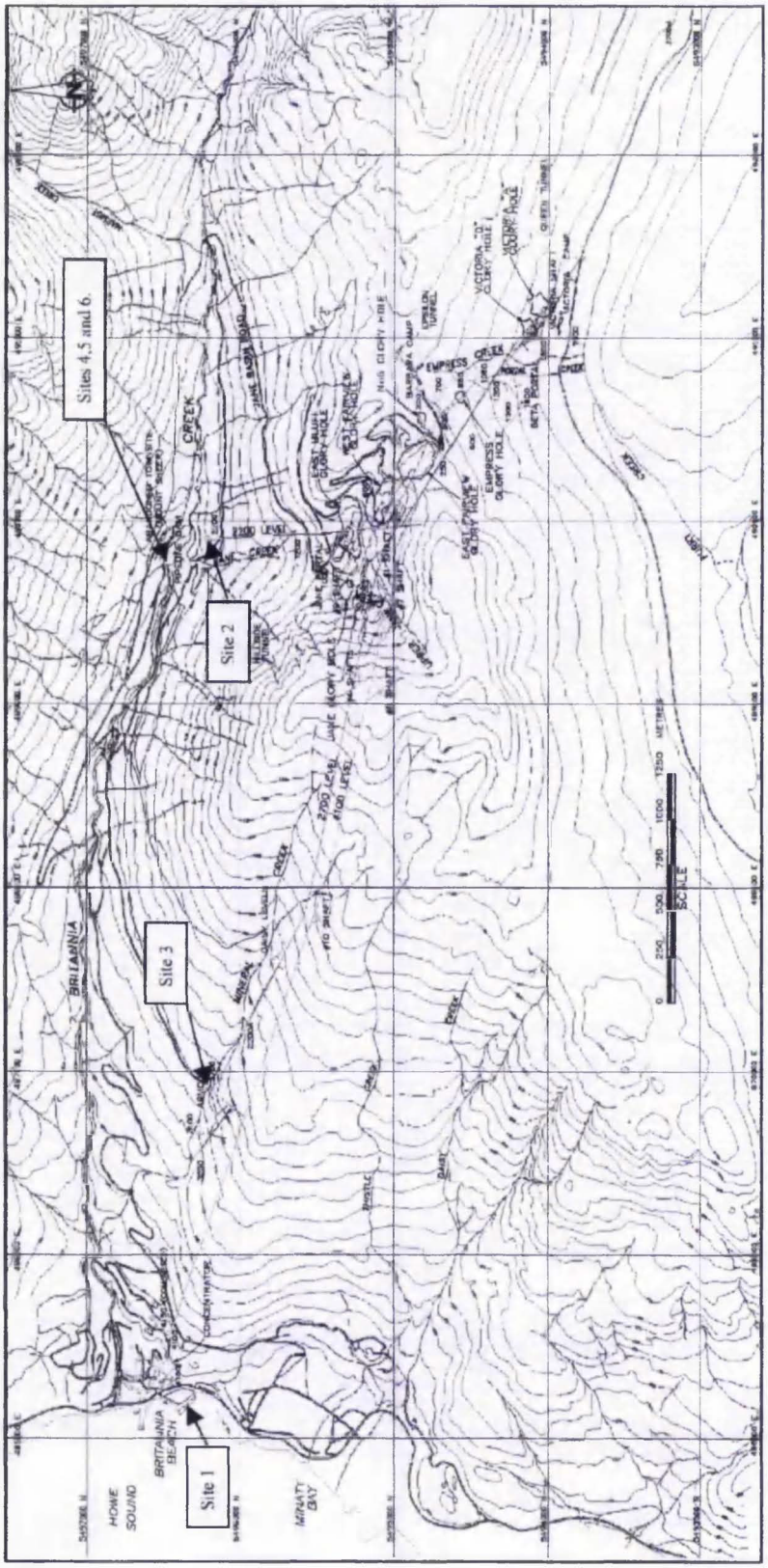


Fig. 1. Position, layout, and sample sites of Britannia Mine, British Columbia, Canada.

Table 1
Wavelength and calibration ranges used for each element

Element	Wavelength (nm)	Calibration range (ppm)
Cd	228.8	0.05–0.5
Cu	324.7	0.2–2.0
Pb	217.0	0.5–5.0
Zn	213.9	0.1–1.0

The following reagents were obtained from Fischer Chemicals Ltd., Loughborough, Leicestershire, UK: 70% nitric acid, certified grade; 32% hydrochloric acid, certified grade. Deionized distilled water (maximum conductivity $0.2\,\mu s\,cm^{-2}$) was prepared using a Purite Still Plus, standard model, fitted with a D339 Deioniser column and a C340 Carbon polisher column.

Aqua regia made by adding 150 mL of 36% hydrochloric acid to 50 mL of 70% nitric acid in a 500-mL conical flask (Radojevic and Bashkin, 1999) was used to digest the soil and foliage samples.

A quantity of 1 g of each spoil sample was weighed into a 50-mL conical flask, 14 mL of aqua regia was added, and the flasks were left in a fume cupboard overnight. The flasks were then gently boiled on a hot plate for 90 min and cooled. A quantity of 4 mL of aqua regia was added and the flask returned to the boil for a further 30 min. After cooling, the resultant mixture was filtered through Whatman 541 filter paper into a 50-mL volumetric flask and washed through with 0.1 M HNO_3. The filtrate was made up to the mark with 0.1 M HNO_3 and the solution was stored in cleaned HDPE bottles in a refrigerator at 4°C.

Due to the bulk density of ground foliage and the fact that vegetation often accumulates much higher concentrations of heavy metals than the soil in which it grows (Pyatt, 2000), 0.5 g of each sample was added to a 50-mL conical flask and digested exactly like the spoil samples.

Ten replicates of each sample of soil, foliage, and water were digested/filtered and stored in a refrigerator for subsequent analysis

All samples were diluted where appropriate with 0.1 M HNO_3 and analyzed using a Perkin–Elmer Model 1100 flame atomic absorption spectrometer, fitted with an impact bead, using appropriate blanks. Wavelengths and calibration standards used for each element are shown in Table 1. If the initial absorption value of each replicate extract was in excess of the upper calibration standard for the target metal under examination, appropriate dilution of the sample was undertaken using 0.1 M nitric acid. The resultant dilution ratio was included in the final metal concentration calculation.

4. Results

Tables 2–5 illustrate the concentrations of Cd, Cu, Pb, and Zn determined in each replicate, analyzed together with the mean and other statistical information.

In all cases where Pb was detected, noisy signals were encountered, especially where lower concentrations were present. Generally, however, the results show good reproducibility when related to similar results obtained in this area of environmental analysis.

The values in Table 2 show that Britannia Beach shore spoil exhibits significant concentrations of Cu and Zn, with lower concentrations of Pb and only traces of Cd. Britannia Mine 2200 level spoil contains large concentrations of Cu, Zn, and Pb with significant concentrations of Cd.

Table 3 shows that spoil at the 2700 level portal contains lower concentrations of Cu and Zn than the spoil at the 2200 level but a higher concentration of Pb. Cd was not detected in this spoil sample.

The spruce needles collected at the shore site contained significant concentrations of Zn and traces of Cd. The spruce needles collected at the 2200 level portal, illustrated in Table 4, exhibit concentrations of Zn similar to those collected by the shore; however, traces of Cu were detected but Cd was not detected.

Target metals were not detected in water from Britannia Creek above the confluence with Jane Creek. Table 5 shows, however, that Jane Creek contains small concentrations of Cu and Zn and traces of Cd. Samples taken from Britannia Creek below the confluence with Jane Creek (BC2L series,) contain only traces of Cu and low concentrations of Zn.

Fig. 2 illustrates the variation in metal concentrations in the spoil samples. The 2200 level spoil has the highest concentration of Cu, Zn, and Cd, whereas the sample of 2700 level spoil exhibits the highest Pb concentration.

5. Discussion

All spoil samples collected in January 2003 contained significant concentrations of Cu, Zn, and Pb (see Fig. 2). The spoil around the 2200 level portal contains relatively high proportions of all target metals and the 2700 level spoil contains the highest concentration of Pb. Cadmium, however, was not detected in the spoil from the 2700 level. Surprisingly, the lowest concentration of target metals detected was in the sample collected on the seashore at the edge of the fan area. The fan area was the site of the concentrators in the past and one would have expected a high concentration of Cu in particular in this area. Much of the analysis by visiting consultants has been carried out in this area (Golder Associates, 2002b; URS, 2002). The sample from this site was taken from an area close to the high tide mark

Table 2
Metal concentrations in replicates of dried sample of spoil from shore and 2200 level, Britannia Mine, British Columbia, Canada

Metal concentrations, Britannia Mine samples	Conc. Cu ($mg\,kg^{-1}$)	Conc. Zn ($mg\,kg^{-1}$)	Conc. Pb ($mg\,kg^{-1}$)	Conc. Cd ($mg\,kg^{-1}$)
Brit Mine shore spoil				
CM1/1	94.282	88.069	9.715	Trace
CM1/2	96.734	86.286	6.340	Trace
CM1/3	94.282	84.503	13.091	Trace
CM1/4	93.056	86.286	16.466	Trace
CM1/5	96.734	82.696	16.466	Trace
CM1/6	95.508	82.696	9.715	Trace
CM1/7	94.282	82.696	16.466	Trace
CM1/8	93.056	84.503	23.216	Trace
CM1/9	95.508	82.696	26.596	Trace
CM1/10	95.508	79.130	23.216	Trace
Mean	94.895	83.9561	16.1287	—
Standard deviation	1.324231	2.53889	6.647328	—
Relative SD %	1.39	3.02	41.21	—
95% CI ±	0.947	1.816	4.755	
Brit. Mine 2200 level spoil				
BB22S/1	1530.0	1092.4	580.31	3.0387
BB22S/2	1458.8	1081.7	532.82	3.7990
BB22S/3	1565.7	1077.1	470.01	3.1706
BB22S/4	1482.1	1064.9	422.27	3.3282
BB22S/5	1481.5	1088.1	395.14	2.9906
BB22S/6	1702.6	1022.4	431.91	3.5184
BB22S/7	1480.4	1064.2	472.23	4.0772
BB22S/8	1783.2	1024.4	452.37	3.2886
BB22S/9	1590.4	1062.7	372.12	3.9936
BB22S/10	1630.8	1066.0	386.94	3.7005
Mean	1570.55	1064.39	451.612	3.49054
Standard deviation	107.867	23.9815	65.71126	0.38885
Relative SD %	6.87	2.25	14.55	11.12
95% CI ±	77.16	17.15	47.00	0.278

and may be subject to splashing with subsequent accelerated leaching of metals. Core sampling methods in this area have been used in the past (Golder Associates, 2002b) and higher values of Cu, Zn, and Pb were recorded. It would appear that metal concentrations in this area vary since some areas have been overlaid with gravel, earth, etc. The values recorded from core samples taken in February 2001 (Golder Associates, 2002b) in the same area are, however, much higher, suggesting that additional leaching by splashing had taken place.

The dispersion of metals from these spoil heaps must be considerable due to heavy rainfall causing general erosion and dissolution. All of the roadways and railways were constructed using spoil consequently further dispersion in the region must have taken place. Thompson and McComas (1974) recorded Cu concentrations of $1300\,\mu g\,g^{-1}$ in the sediment in Howe Sound, collected in the vicinity of Britannia just after the mine closed. This gave rise to concern and according to Hagen (2001), this has now been reduced to below $300\,\mu g\,g^{-1}$. The concentration in 2200 level spoil collected at the surface of the spoil heap was determined as $1570\,mg\,kg^{-1}$ in January 2002.

The samples of spoil collected near the 2200 level portal contain copper, lead, and zinc concentrations in excess of concentrations outlined by CCME (2002) for all classifications of subsequent use (Table 6). Similarly, spoil from near the 2700 level portal contains copper and lead concentrations in excess of permitted values; however, zinc concentrations exceed only agricultural and residential/parkland permitted values.

The analysis of spruce needles collected from the spoil tips adjacent to the 2200 and 2700 portals showed significant concentrations of Zn only. This may well be a normal concentration for this species, since it is an essential trace element. This could be verified by comparison with samples collected away from the immediate area of the workings, probably in the next valley north and at a similar altitude. Markert (1993), concluded that temporary seasonally conditioned differences in concentration exercise a greater influence for individual elements. Comparisons of target metal concentrations in samples collected in summer during the growing season would also confirm or otherwise this hypothesis. Traces of Cu were recorded in the spruce needles collected at the 2200 level. The trees sampled on this occasion were apparently very

Table 3
Metal concentrations in replicates of dried samples of 2700 level spoil and spruce leaves from shore at Britannia Mine, British Columbia, Canada

Metal concentrations, Britannia Mine samples	Conc. Cu ($mg\,kg^{-1}$)	Conc. Zn ($mg\,kg^{-1}$)	Conc. Pb ($mg\,kg^{-1}$)	Conc. Cd ($mg\,kg^{-1}$)
Brit. Mine 2700 spoil				
BB27S/1	443.09	245.02	606.22	—
BB27S/2	401.94	221.16	585.12	—
BB27S/3	436.96	209.79	631.27	—
BB27S/4	379.12	232.45	661.94	—
BB27S/5	381.70	214.00	646.81	—
BB27S/6	395.05	209.54	593.70	—
BB27S/7	403.95	220.45	677.40	—
BB27S/8	378.67	215.40	692.86	—
BB27S/9	411.55	227.21	697.39	—
BB27S/10	438.49	208.60	715.41	—
Mean	407.052	220.362	650.812	
Standard deviation	24.96679	11.70945	45.83154	
Relative SD %	6.13	5.31	7.04	
95% CI ±	17.86	8.44	32.78	
Brit. Mine shore spruce				
CV4/1	—	13.459	—	Trace
CV4/2	—	13.459	—	Trace
CV4/3	—	15.296	—	Trace
CV4/4	—	12.069	—	Trace
CV4/5	—	12.764	—	Trace
CV4/6	—	11.375	—	Trace
CV4/7	—	12.069	—	Trace
CV4/8	—	11.375	—	Trace
CV4/9	—	12.069	—	Trace
CV4/10	—	12.764	—	Trace
Mean		12.6699		
Standard deviation	—	1.18304	—	—
Relative SD %	—	9.34	—	—
95% CI ±	—	0.846	—	—

Table 4
Metal concentrations in replicates of dried samples of spruce needles from the 2200 level and water extracted from Britannia Creek above the confluence with Jane Creek, Britannia Mine, British Columbia, Canada

Metal concentrations, Britannia Mine samples	Conc. Cu ($mg\,kg^{-1}$)	Conc. Zn ($mg\,kg^{-1}$)	Conc. Pb ($mg\,kg^{-1}$)	Conc. Cd ($mg\,kg^{-1}$)
Brit Mine 2200 spruce				
C225/1	Trace	12.548	—	—
C225/2	Trace	12.548	—	—
C225/3	Trace	11.826	—	—
C225/4	Trace	13.271	—	—
C225/5	Trace	13.271	—	—
C225/6	Trace	12.548	—	—
C225/7	Trace	13.271	—	—
C225/8	Trace	13.271	—	—
C225/9	Trace	12.548	—	—
C225/10	Trace	11.826	—	—
Mean		12.6928		
Standard deviation		0.569972		
Relative SD %		4.49		
95% CI ±		0.408		

young specimens (shore site 2 m high, 2200 site 3 m high); therefore any bioaccumulation would be in initial stages. A further consideration is that the outer bark of a trunk is more enriched by heavy metals than the inner bark. The least accumulation is in trunk wood. The advantage of analyzing bark and twigs as opposed to leaves is their permanence throughout the year (Ernst, 1993).

Table 5
Metal concentrations in replicates of samples of water taken from Jane Creek and Britannia Creek after the confluence with Jane Creek, Britannia Mine, British Columbia, Canada

Metal concentrations, Britannia Mine samples	Conc. Cu ($mg\,L^{-1}$)	Conc. Zn ($mg\,L^{-1}$)	Conc. Pb ($mg\,L^{-1}$)	Conc. Cd ($mg\,L^{-1}$)
Jane Creek				
BJ3/1	0.2745	0.5917	—	Trace
BJ3/2	0.2244	0.5724	—	Trace
BJ3/3	0.2745	0.5724	—	Trace
BJ3/4	0.2494	0.5724	—	Trace
BJ3/5	0.2996	0.6016	—	Trace
BJ3/6	0.2745	0.5651	—	Trace
BJ3/7	0.2996	0.5917	—	Trace
BJ3/8	0.2494	0.5724	—	Trace
BJ3/9	0.2996	0.5724	—	Trace
BJ3/10	0.2996	0.5917	—	Trace
Mean	0.27451	0.58038		
Standard deviation	0.026437	0.012412		
Relative SD %	10.4	2.14		
95% CI ±	0.0189	0.040089		
Brit. Creek < Jane Creek				
BC2L/1	Trace	0.0610	—	—
BC2L/2	Trace	0.0464	—	—
BC2L/3	Trace	0.0610	—	—
BC2L/4	Trace	0.0684	—	—
BC2L/5	Trace	0.0684	—	—
BC2L/6	Trace	0.0684	—	—
BC2L/7	Trace	0.0757	—	—
BC2L/8	Trace	0.0537	—	—
BC2L/9	Trace	0.0610	—	—
BC2L/10	Trace	0.0684	—	—
Mean		0.06324		
Standard deviation		0.008508		
Relative SD %		13.4		

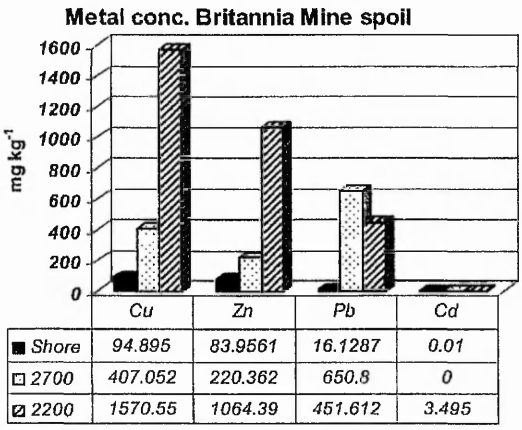


Fig. 2. The concentration of target metals in spoil samples collected at Britannia Mine, British Columbia, Canada.

Target metal concentrations in the creek waters were low, with only the water in Jane Creek registering Cu and Zn. The plugging of the 2200 portal with an underground structure would seem to have prevented major outflow of mine drainage into Jane Creek and subsequently into Britannia Creek. It should be noted, however, that the water samples were collected in January; with snow around the 2200 level, consequently, the water temperature would be on the order of 3–4°C. Work carried out in the UK in 2002 by Wilson et al. (unpublished thesis) demonstrated that the concentration of ions in river and stream water reduced rapidly with lower temperatures. Summertime concentrations of heavy metals in Britannia Creek are expected to be higher.

Table 6
Environmental quality criteria in Canada for contaminated sites (CCME, 2002)—concentrations given in $mg\,kg^{-1}$

Metal	Agricultural	Residential/Parkland	Commercial	Industrial
Cadmium	1.4	10.0	22.0	23.0
Copper	63.0	63.0	91.0	91.0
Lead	70.0	140	260	260
Zinc	200	200	360	360

6. Conclusions

Britannia Mine presents a series of point and diffuse sources of heavy metals and a series of mechanisms capable of dispersing them into the surrounding biosphere. The dispersion of heavy metals, particularly copper as a result of AMD entering the creeks, appears to have been greatly reduced by plugging operations within the mine. However, dispersion and possible bioaccumulation from spoil heaps, roads, and open pits due to leaching and atmospheric dissipation must be considerable especially during the warmer months. The abandoned town site (see Fig. 1) would be useful to investigate in an attempt to determine the extent of heavy metal accumulation. Vegetation and animal matter from this area could also yield important information relating to heavy metal dispersion.

The areas surrounding the open pits, such as East Bluff, Victoria A, and Victoria Camp, together with Empress Creek, should also be investigated. Medical archives, if available, would be worth studying, although the authors suspect that, as in the case of Parys Mountain, Anglesey, UK, the only reports will be of accidents and fatalities caused by physical mining activities (Rowlands, 1981).

Britannia Mine would therefore be an ideal site for further research and would assist in the construction of a model relating the age of metalliferous workings to their effect on organisms, including humans and the pedosphere.

References

Barry, K.I., Grout, J.A., Levings, C.D., Nidle, B.H., Piercey, C.E., 2000. Can. J. Fish. Aquat. Sci. 57, 2032–2043.

Britannia Mine Remediation Project, 2002. Background on Pollution and Remediation Issues. Ministry of Water Land and Air Protection, Government of British Coluimbia, available at http://wlapwww.gov.bc.ca/sry/p2/britannia/background.htm (Accessed 29 April 2003).

CCME, 2002. Canada Environmental Quality Guide Lines, available at http://www.ccme.ca/assets/pdf/e1-06.pdf (Accessed 26 March 2004).

Ellis, D.V., Hoover, P.M., 1990. Mar. Pollut. Bull. 21 (10), 477–480.

Ernst, W.H.O., 1993. Geological and biochemical prospecting for heavy metal deposits in Europe and Africa. In: Markert, B. (Ed.), Plants as Biomonitors—Indicators of Heavy Metals in the Terrestrial Environment. VCH, New York, pp. 107–126.

Golder Associates Ltd., 2002a. Water Treatment Pilot Plant Open Day. Britannia Mine Remediation News Letter, Issue 2, p. 1.

Golder Associates Ltd., 2002b. Britannia Creek Fan Preliminary Site Investigation Britannia Beach, BC. Prepared for BC Environment.

Hagen, M., 2001. Britannia Marine Sediment Contamination: Interim Status of Knowledge and Next Options. Prepared for Environment Canada.

Levings, C.D., McDaniel, N., 1973. Biological observations from the submersible Pisces IV near Britannia Beach, Howe Sound, BC. Fish and Marine Service Tech. Report No. 663.

Markert, B., 1993. Instrumental analysis of plants. In: Markert, B. (Ed.), Plants as Biomonitors—Indicators for Heavy Metals in the Terrestrial Environment. VCH, New York, pp. 65–103.

Mills, C., 2003. The Former Britannia Mine, Mount Sheer/Britannia Beach, British Columbia, available at http://technology.infomine.com/enviromine/ard/Case%20Studies/Britannia.htm (Accessed 12 May 2003).

Pyatt, F.B., 2000. Ecotoxicol. Environ. Saf. 50, 60–64.

Radojevic, M., Bashkin, V.N., 1999. Practical Environmental Analysis. The Royal Society of Chemistry, Cambridge, UK.

Rowlands, J.R., 1981. Copper Mountain Second Edition.. Anglesey Antiquarian Society, Llangefni.

Thompson, J.A.J., McComas, F.T., 1974. Fish. Res. Bd. Can. Tech. Rep. No. 437.

URS, 2002. Britannia contaminated site investigation. Fan area remediation planning document Britannia Mine site, British Columbia. Prepared for BC Ministry of Water, Land and Air Protection, Vancouver.

Warkentin, D., 2000. BC's Green Party News 49, Summer.

West, S., Charman, D.J., Grattan, J.P., Cherburkin, A.K., 1997. Water Air Soil Pollut. 100, 343–353.

ELSEVIER

Journal of Archaeological Science 32 (2005) 897–901

Journal of Archaeological SCIENCE

http://www.elsevier.com/locate/jas

The chemistry of tree resins and ancient rock paintings in the Niah Caves, Sarawak (Borneo): some evidence of rain forest management by early human populations

F.B. Pyatt[a,*], B. Wilson[a], G.W. Barker[b]

[a] *Interdisciplinary Biomedical Research Centre, School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK*

[b] *McDonald Institute for Archaeological Research, University of Cambridge, Downing Street, Cambridge CB2 3ER, UK*

Abstract

Research concerning the Niah Caves located in Sarawak, Borneo, has demonstrated human occupation of south-east Asia from c. 50,000 years ago until the present. Elaborate paintings, believed to date from the first millennium AD, in which a red colouration predominates, occur in an adjacent cave system. Analysis of the paint revealed it to be comparable with a red resin produced by a native leguminous tree and a resin produced by trees in Australia where caves containing predominantly red paintings are also to be found. Potential cultural/technological linkages between Sarawak and Australia are suggested. Our findings additionally contribute to evidence for the antiquity and complexity of rain forest management in south-east Asia.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Cave painting; Chemistry; Leguminous trees; Resin; Sarawak; Niah

1. Introduction

Harrisson [8] located a human skull in the Niah Great Cave which is located in Sarawak, Borneo and Brothwell [7] described the skull as that of an adolescent human which he considered comparable to that of modern Tasmanians; however, Wu Xinzhi [16] considered that the affinities were closer to human material obtained from mainland Asia.

In addition to the "Deep Skull", Harrisson [8] also found evidence of human habitation and burials spanning the last 40,000 years. Barker et al. [1–4], noted that between about 3000 BC and 800 AD, the West Mouth area of the cave was used for diverse burials including "extended, flexed, and within jars, of people using Neolithic pottery.... A series of boat burials in Kain Hitam was dated to the latter part of this "Neolithic" funerary activity, in the first millennium AD, the cave paintings being assumed to be associated with these."

Dramatic cave paintings (Fig. 1) are located in Kain Hitam (the "Painted Cave"), which is situated high within a limestone island, and here approximately 65 meters of cave walls are adorned with paintings of "dancing human" figures and "curved" boats with "high prows and sterns"; the cave floor has scattered death ships with associated bones, beads, porcelain and stoneware sherds. The latter indicate the medieval period as a possible *terminus ante quem* for the age of the boat burials and wood from the boats has been dated to c. 2000 BP [9]. Hence the general assumption is that the boat burials are between 1000 and 2000 years old, and the Kain Hitam paintings are likely to be of a similar age.

* Corresponding author. Tel.: +44 115 848 3302; fax: +44 115 848 6680.

E-mail address: brian.pyatt@ntu.ac.uk (F.B. Pyatt).

0305-4403/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jas.2005.01.010

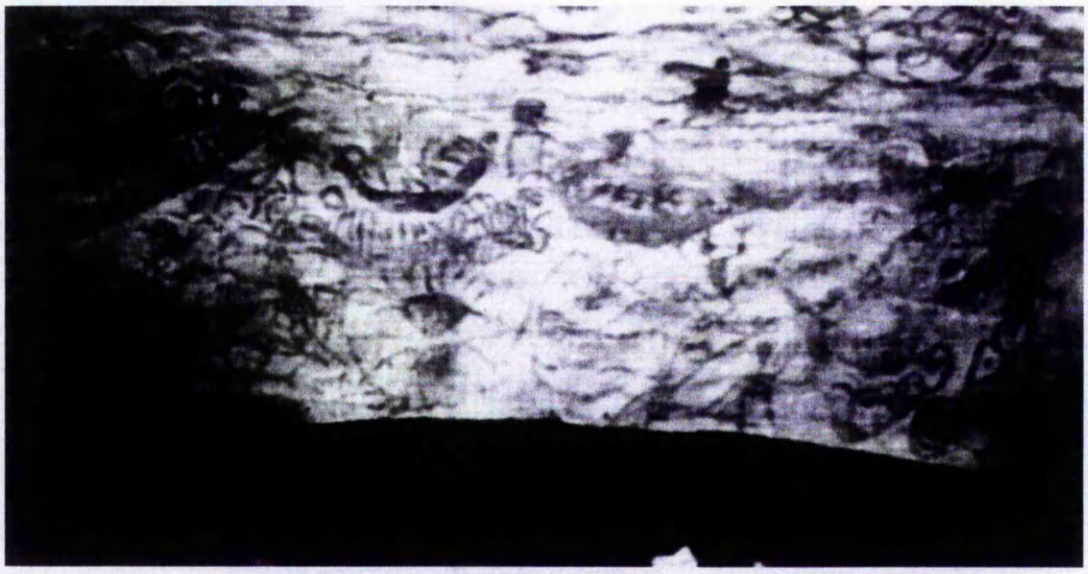


Fig. 1. Cave paintings in Kain Hitam depicting "humans" and "boats".

The dramatic paintings are dominated by distinctive red colourations which are not, following research in our laboratories, the result of the application of haematite to the limestone cave walls. In order to identify the material used for the painting, one of the team (FBP) devoted time during one of his research visits to Sarawak in an attempt to identify the material(s) which could be employed, by ancient human artists, as paint. Eventually, after prolonged field sampling, and painting trials, material obtained from trees of *Pterocarpus indicus* Willd. was found to fit the important initial requirements including colour, availability, texture and practicality of application to limestone substrates. It was considered that the resin was sufficiently coloured with organic vegetable dye allowing use without further pigmentation as has been reported in some rock painting techniques found in Australia [5].

P. indicus is a deciduous tree which attains heights of c. 32 m in the rain forest of the Niah area; importantly it is a member of the Leguminosae and as such possesses the ability to "fix" nitrogen. The trunks are generally fluted and buttressing occurs. The large crowns initially are ascending and subsequently arch over and may droop at the ends. Rojo [13] reported on the wide distribution of this rapidly growing species; it has a western limit in southern Burma and an eastern limit in the Solomon Islands.

List and Horhammer [10] and Little and Wadsworth [11] considered the chemistry of this tree and noted the occurrence of pterocarpin and pterostilben homopterocarpin, prunetin (prunusetin), formonoetin, isoliquiritigenin, p-hydroxyhydratropic acid, pterofuran, pterocarpol, and β-eudesmol. A complex array of chemicals is hence present in this leguminous tree as indeed is the case in most arboreal resins.

2. Materials and methods

Small samples of paint from the "Painted Cave" (from outside the protected, fenced in, main series), were collected with the permission of the Sarawak Museum, transported and subsequently analysed by infrared spectroscopy in our laboratories at Nottingham Trent University; indeed, Beridge et al. [6] noted that infrared spectroscopy can be employed to identify probable sources of paint through the use of data bases and also to compare samples to ascertain whether a common origin is feasible. Samples of paint flakes and tree resin were separately ground in a clean laminar flow cabinet and the resultant material transferred to clean dry sample vials employing the procedure described by the Infrared Spectroscopy Committee of the Chicago Society for Coatings Technology [15]. The vials were agitated for 5 min and 2 mg accurately sub-sampled; the contents were further ground with 300 mg of dried potassium bromide. The resultant powder was transferred to a clean dry die and placed within a hydraulic press. A vacuum was employed for 2 min without pressure and then 9 tons pressure was applied for a further 2 min whilst under vacuum. The pressure was quickly released and reapplied for 30 seconds. The disc was eventually carefully extracted and inserted into a Perkin Elmer 1710 Infrared Fourier Transform Spectrophotometer.

3. Results and discussion

The results indicate that the spectrum produced by the resin collected from *P. indicus* (Fig. 2) and that from paint fragments obtained from the cave (Fig. 3) were

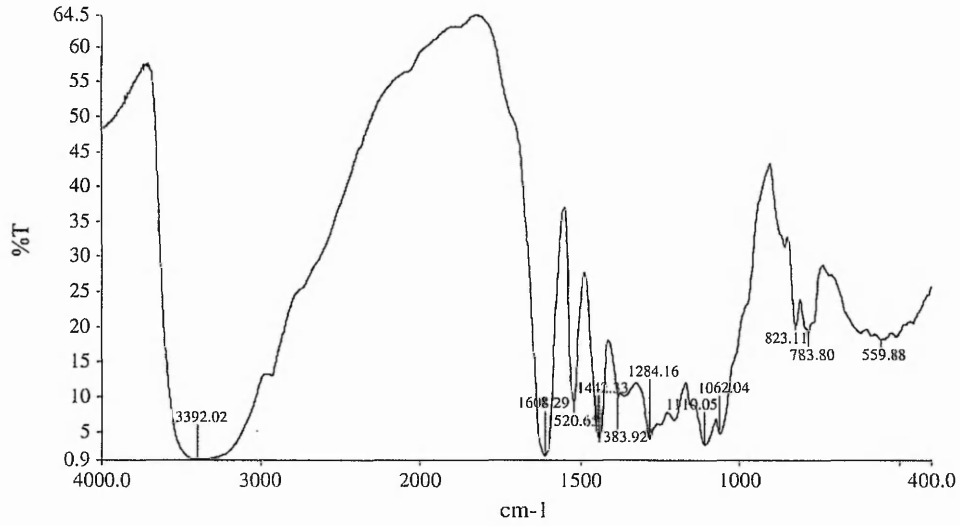


Fig. 2. The "fingerprint" section (between wave no. 1800 and 400) infrared spectrum obtained from fresh Sarawak tree resin.

indeed very similar. However the resin sample examined was collected from the tree in 2003, whereas the paint sample examined was reputed to be 1000–2000 years old. Advanced oxidation due to ageing and degradation of the paint film, due to the presence in the cave of ammonia [12] may have substantially altered the chemical composition of the film. This together with fragments of limestone, removed with the paint, has produced a less definite spectrum than that obtained from the resin. Nevertheless, the fit suggests that resin obtained from *P. indicus* was the probable source of the paint utilised in the Kain Hitam paintings. We tentatively suggest that members of ancient human populations were tapping trees of *P. indicus* to obtain the resin which was freshly applied to the absorbent limestone walls of Kaim Hitam ("Painted Cave"). We further speculate that any planting of young trees in close proximity to the cave area for the provision of fresh "paint" would have markedly affected the nitrogen status of the soil and thence the associated species which would survive indicating that early human populations were essentially modifying portions of the forest ecosystem in this area of Sarawak.

Thus tree planting by humans in ancient times potentially could have had enormous repercussions on the ecology of the rain forest; such a system possesses a pedosphere which is typically nutrient deficient with most of the nutrients held in the biomass of the standing crop. Leaching under the prevailing abiotic environmental conditions could be profound. Any form of

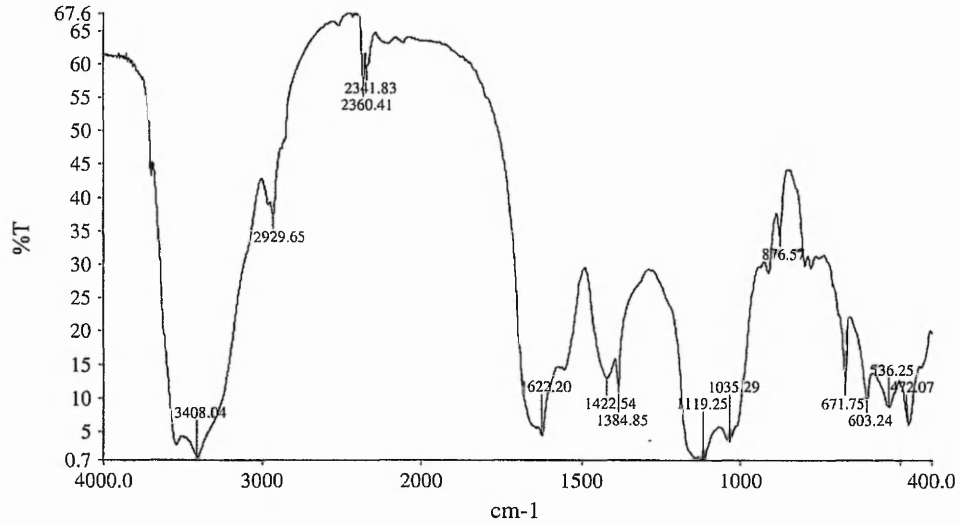


Fig. 3. Comparable section of infrared spectrum obtained from Kain Hitam ("Painted Cave") paint flakes.

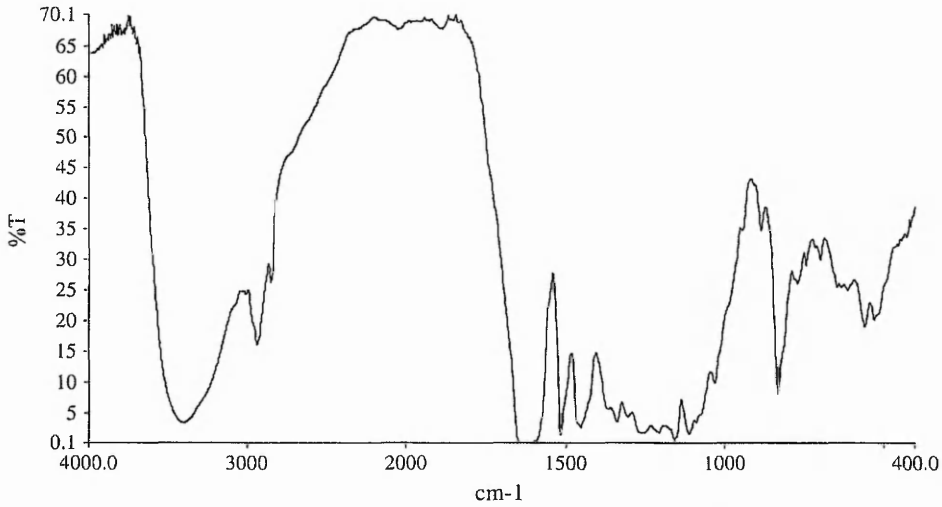


Fig. 4. Spectrum for *Xanthoria* sp. (collected from Australia).

"agriculture", for example transplantation of young leguminous plants (or the planting of seeds) of *P. indicus*, to more convenient/readily accessible locations, would have modified the nitrogen input into the pedosphere and thence affected other rain forest species, both dominant and subordinate, present in the area. Furthermore, the planting of trees/saplings of *P. indicus* would probably have involved the removal of other species to facilitate the successful establishment of the resin bearing trees by, for example significantly reducing the element of competition for abiotic factors such as light.

It may further be noted that the distinctive colour and texture of the applied paint are comparable to those found by one of the authors (FBP) in aboriginal paintings located in north Queensland, Australia. Infra red spectra (Fig. 4) associated with resins produced by *Xanthoria australis* (grass tree) growing in Australia, are very similar to the *P. indicus* spectrum. Resin from *Xanthoria* spp. was used commercially by Europeans from the mid 19th century as a coloured varnish or binder for pigmented paints and is known as gum accroides or grass tree resin [14].

Similar methodologies/practices may be evident in Australia; possibly indicating an early event of "technology transfer" associated with the migration of humans. This must of course remain highly speculative at this stage as we do not yet possess material from aboriginal paintings for chemical fingerprinting. Furthermore it should be noted that there exists a possibility of "technology transfer" in either direction; indeed there may even have been some convergence in earlier times.

Acknowledgements

The Niah Cave Project has been funded principally by the Arts and Humanities Research Board of the U.K. whose generous support is acknowledged, as well as the support of the British Academy's Committee for Southeast Asian Studies, and we additionally acknowledge financial support to BW from the Higher Education Funding Council of England (RAE). We are grateful to the Chief Minister's Department of Sarawak for permission to undertake the fieldwork in Niah, and the staff of Sarawak Museum for their continued support and encouragement. We also thank the Sarawak Biodiversity Centre (Kuching) and Sarawak Museum for permission to take the resin and paint samples respectively and export them for subsequent detailed analysis, and Dr. K. Pearce of the National Parks and Wildlife Division of the Sarawak Forest Department for confirming the identification of *Pterocarpus indicus* Willd. We thank the other team members for their support and friendship. We thank Ryan Rabett for providing information concerning some of the Harrisson papers.

References

[1] G. Barker, H. Barton, P. Beavitt, S. Chapman, M. Derrick, C. Doherty, L. Farr, D. Gilbertson, C. Hunt, W. Jarvis, J. Krigbaum, B. Maloney, S. McLaren, P. Pettitt, B. Pyatt, T. Reynolds, G. Rushworth, M. Stephens, The Niah Caves Project: preliminary report on the first (2000) season, Sarawak Museum Journal 55 (76) (2000) 111–149.

[2] G. Barker, D. Badang, H. Barton, P. Beavitt, M. Bird, P. Daly, C. Doherty, D. Gilbertson, I. Glover, C. Hunt, J. Manser, S. McLaren, V. Paz, B. Pyatt, T. Reynolds, J. Rose, G. Rushworth, M. Stephens, The Niah Cave Project: the second (2001) season of fieldwork, Sarawak Museum Journal 56 (77) (2001) 37–119.

[3] G. Barker, H. Barton, M. Bird, F. Cole, P. Daly, D. Gilbertson, C. Hunt, J. Krigbaum, H. Lewis, L. Lloyd-Smith, J. Manser, F. Menotti, V. Paz, P. Piper, B. Pyatt, R. Rabett, T. Reynolds, M. Stephens, M. Trickett, P. Whittaker, The Niah Cave

Project: the third (2002) season of fieldwork, Sarawak Museum Journal 57 (78) (2002) 87–179.
[4] G. Barker, H. Barton, P. Beavitt, M. Bird, P. Daly, C. Doherty, D. Gilbertson, C. Hunt, J. Krigbaum, H. Lewis, J. Manser, S. McLaren, V. Paz, P. Piper, B. Pyatt, R. Rabett, T. Reynolds, J. Rose, G. Rushworth, M. Stephens, Prehistoric foragers and farmers in south-east Asia: renewed investigations at Niah Cave, Sarawak, Proceedings of Prehistoric Society 68 (2000) 147–164.
[5] R.G. Bednarik, Rock Art Science: The Scientific Study of Palaeoart, Brepols Publishers n.v, Turnhout, 2001.
[6] A. Beridge, T. Fung, D. Macdougal, Use of infrared spectroscopy for characterization of paint fragments, in: B. Caddy (Ed.), Forensic Examination of Glass and Paint, Taylor and Francis, London, 2001.
[7] D. Brothwell, Upper Pleistocene human skull from Niah Caves, Sarawak, Sarawak Museum Journal 9 (15–16) (1960) 323–350.
[8] T. Harrisson, The caves of Niah: a history of prehistory, Sarawak Museum Journal 8 (12) (1958) 549–595.
[9] T. Harrisson, Niah Caves Progress Report to 1967, Sarawak Museum Journal 15 (30–31) (1967) 95–97.
[10] P.H. List, L. Horhammer, Hager's Handbuch der Pharmazeutischen Praxis, vols. 2–6, Springer-Verlag, Berlin, 1969.
[11] E.L. Little Jr, F.H. Wadsworth, Common trees of Puerto Rico and the Virgin Islands. Agric. Handbook, 249, USDA, Washington, DC, 1964.
[12] F.B. Pyatt, Potential effects on human health of an ammonia rich atmospheric environment in an archaeologically important cave in southeast Asia, Occupational and Environmental Medicine 60 (2003) 986–988.
[13] P. Rojo, Pantropic speciation of *Pterocarpus* (Leguminosae-Papilionaceae) and the Malesia-Pacific species, Forestry Abstracts 3 (1) (1977) 19–32.
[14] L.E. Sayre, A Manual of Organic Material Medica and Pharmacognosy, fourth ed., P Blakiston's Son and Co, Philadelphia, 1917.
[15] The Infrared Spectroscopy Committee of the Chicago Society for Coatings Technology, An Infrared Spectroscopy Atlas for the Coatings Industry, Federation of Societies for Coatings Technology, Philadelphia, USA, 1980.
[16] Wu Xinzhi. The origin and dispersal of anatomically modern humans in east and southeast Asia, in: T. Akazawa, K. Aoki, T. Kimura (Eds.), The Evolution and Dispersal of Modern Humans in Asia, Hokusen-sha, Tokyo, 1992, pp. 373–378.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 1]
(GandB)Glec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

Toxicological & Environmental Chemistry, Month?? 2005; ?(?): 1–10

Taylor & Francis
Taylor & Francis Group

An evaluation of procedures for the digestion of soils and vegetation from areas with metalliferous pollution

BOB WILSON[1], ALAN BRAITHWAITE[2], & F. BRIAN PYATT[1]

[1]*Interdisciplinary Biomedical Research Centre, School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, UK, and*
[2]*School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, UK*

(Received 8 October 2004; revised 8 February 2005; in final form 19 April 2005)

Abstract

Various digestion or extraction processes are available for the determination of trace metals in environmental samples using diverse analytical techniques. Our research examines the most efficient and reproducible methods of digestion utilising readily available laboratory equipment, reagents and instrumentation. Extraction procedures reported in over 80 recent research works were considered and the most common methods were critically evaluated. Thus nitric acid, nitric acid/perchloric acid, aqua regia, nitric acid/hydrogen peroxide and microwave-assisted methods were investigated using soil and leaf samples collected adjacent to three old metalliferous workings. Analysis was carried out using Flame Atomic Absorption Spectrometry and the results were evaluated. Differences in efficiency of extraction relative to specific metals and different substrates are highlighted and recommendations made for a digestion procedure suitable for environmental samples. Aqua regia produced the most accurate, efficient and reproducible results; we consider methods of improving both of these factors and sample preparation. Our recommended methodology has applications in environmental surveys, environmental geology, environmental toxicology, geochemistry and provides a means of obtaining meaningful and reliable results for the determination of metals present in soils, minerals and vegetation.

Keywords: *Analysis, digestion, heavy metals, soil, procedural evaluations, vegetation*

Introduction

The accurate determination of concentrations of specific cations is essential in both environmental and toxicological research. This article seeks to determine the most efficient and pragmatic method of digesting vegetable and mineral samples prior to trace metal analysis; the use of standard laboratory equipment was a prerequisite.

Correspondence: Dr Bob Wilson, Interdisciplinary Biomedical Research Centre, School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, UK. Tel: +44 (0)115 848 35 65. Fax: +44 (0)115 848 33 84. E-mail: bob.wilson@ntu.ac.uk

ISSN 0277-2248 print/ISSN 1029-0486 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/02772240500165570

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 2]
{Band B}Gtec/GTEB-116540.3d (GTEC) First Proof GTEB-116540

The common and reliable technique, Flame Atomic Absorption Spectrometry (FAAS) was selected to analyse the resultant extracts.

Many papers have been produced that consider extracting suitable analytes from soil, vegetation or animal tissue samples, popular procedures are Wet Digestion [1] and Wet Ashing [2]. All of the methods, however, use an acid or a combination of acids or a combination of acids and oxidizing agents. Additionally open digestion on a hot plate [3], reflux extraction at atmospheric pressure [4] and microwave-aided digestion in either open [5] or closed vessels [6] or simply closed pressure vessels [7] have been extensively used.

An examination of 84 publications concerned with heavy metal determinations in soil (and sediments), vegetation and animals produced the following data relating to extraction methods: open digestion in a conical flask (55%), microwave-assisted closed pressure vessel (31%), analytical methods not requiring extraction (12%) and one method utilizing reflux digestion [4].

The combination of reagents employed within each of these methods varied, however, HNO_3 and its effectiveness has been reported by Wasilewska et al. [8] when digesting organic materials in pressurized equipment and by Bellis et al. [3] for open digestion of tree bark. Open and closed digestion procedures used HNO_3 > aqua regia > HNO_3/H_2O_2 > $HNO_3/HClO_4$.

In microwave-assisted pressure digestion the most frequent combinations were HNO_3 (39%), aqua regia (17%) and HNO_3/H_2O_2 (11%).

It can be argued that the use of hydrofluoric acid in extraction procedures is not essential from an environmental or ecological perspective since metals associated with the original inorganic material, especially in the case of silicate species do not achieve the mobility required for bioaccumulation [9]. Thus, we decided to only use methods, which would be suitable for use in general laboratory fume cupboards and to consider the open method of digestion.

Ipolyi et al. [10], investigated accelerating the extraction processes in a three-stage sequential extraction method. One of the ideas investigated was to employ a standard laboratory microwave oven. Significant reductions in extraction time for particularly the acid phase were reported and it was suggested that microwaves significantly influenced efficiency. We decided to investigate the use of microwave digestion with nitric acid.

In our research spoil (waste rock) from an old copper mine at Parys Mountain, Anglesey, UK, spoil and leaves of *Corylus avellana* from an old lead mine at Snail Beach, Shropshire, UK, and needles from *Pinus sylvestris* collected near an old lead mine at Black Rocks, Derbyshire, UK were examined. Certified standards of soil and *Picea abies* needles were employed as quality-assured materials and the accuracy determined within the preparation, extraction and analytical processes.

2

Sampling collection and preparation

Spoil samples were collected using a clean stainless steel trowel. The initial 10 mm of soil in an approximately 400 cm^2 area was scraped off and discarded. Approximately 200 g of soil was then collected to a maximum depth of 50 mm and placed in a clean polythene bag. Samples were triple bagged to prevent transit-damage and transported to the laboratory; they were air dried for seven days in a drying cabinet at 25°C and placed in an oven for 3 h at 105°C. After cooling, the samples were passed through a 2-mm sieve and ground for 5 min in a clean pestle and mortar [11] before transfer to clean HDPE storage bottles.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 3]
{Gandl}Gtec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

Leaf and needle samples, collected by hand using vinyl examination gloves, were thoroughly washed with distilled water and rinsed with deionised distilled water. The leaves and needles were air dried overnight and then dried at 70°C for 24 h [2] in a drying cabinet. After cooling the dried leaves and needles were ground for 5 min in a pestle and mortar, and stored in HDPE sample bottles. For control purposes 7 g of the CRM 101 Spruce Needles certified sample was also dried at 105°C for 3 h and ground in a pestle and mortar for 5 min before being transferred to an HDPE bottle.

Digestion and extraction procedure

The reagents 70% Nitric acid, certified grade; 32% hydrochloric acid, certified grade; 70% perchloric acid, certified grade; 30% hydrogen peroxide, certified grade were all obtained from Fischer Chemicals Ltd, Loughborough, Leicestershire, UK.

Deionised distilled water (maximum conductivity 0.2 $\mu s\,cm^{-2}$) was prepared using a Purite Still Plus, standard model, fitted with a D339 deioniser column and a C340 carbon polisher column.

Certified copper, lead, cadmium and zinc FAAS standards obtained from Reagecon, Shannon Free Zone, Shannon, County Clare, Ireland.

Certified materials CRM142R Light Sandy Soil and CRM 101 Spruce (*Picea abies*) were obtained from the Commission of European communities, Community Bureau of Reference.

Dried, ground soil 0.5 g (±0.02 g) and 0.25 g (±0.01 g) of dried ground vegetation were used and the final solution, from each extraction, was made up to 25 mL with 0.1 M HNO_3 for analysis. The larger sample size for soils was chosen because several workers including Scharenberg and Ebeling [12], and Pyatt [13] have demonstrated that plant leaves can accumulate higher concentrations of heavy metals than the soil in which the trees grow by a factor of at least two. The lower bulk density of dried vegetative material relative to that of soil was also a factor.

All glassware were immersed in 10% v/v nitric acid for 24 h and then washed thoroughly with deionised distilled water before use [14] and all extractions were carried out on a hotplate in a suitably equipped fume cupboard.

(1) *Extraction with concentrated nitric acid* [2,15]
The substrate was weighed into a 50 mL conical flask and 7.0 mL of 70% v/v HNO_3 was added. The flask was carefully swirled to mix the reactants and simmered gently for 90 min. After cooling a further 2.5 mL of 70% v/v nitric acid added and the beaker gently simmered for a further 30 min.

(2) *Extraction with aqua regia* [2]
Aqua regia was made by carefully adding 150 mL of 32% v/v HCl to 50 mL of 70% v/v HNO_3 in a 500 mL conical flask.

The extraction process was carried out exactly as method 1 above, substituting aqua regia for 70% v/v HNO_3.

(3) *Extraction with HNO_3/H_2O_2 combination* [16]
The prepared substrate was weighed into a 50 mL conical flask and 7 mL of 70% v/v HNO_3 was added. The flask was carefully swirled and the mixture boiled gently for 90 min. After cooling 1 mL of 30% v/v H_2O_2 was added and the mixture gently heated. After the effervescence had subsided, a further 1 mL of H_2O_2 was added and the mixture heated gently for 30 min.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 4]
(Gamil B) Gtec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

(4) *Extraction with $HNO_3/HClO_4$* [17]
The substrate was weighed into a 50 mL conical flask. Seven millilitres of 70% v/v HNO_3 was added and heated for 1 h at 100/110°C. After cooling, 2.0 mL of 70% v/v $HClO_4$ was added and the mixture reheated to 110°C for 30 min or until brown fumes were emitted.

(5) *Extraction with HNO_3 assisted by microwaves* [10]
The substrate was weighed into a 50 mL conical flask and 7 mL of 70% v/v HNO_3 was added. The flask was placed in a microwave oven and exposed to 270 W for 20 min.

After cooling 5 mL of 0.1 M HNO_3 was added to each flask and the mixture filtered through a Whatman 451 filter paper, washed through with 0.1 M nitric acid into a 25 mL graduated flask and made up with 0.1 M nitric acid. After thorough mixing the solution was transferred to HDPE bottles. Seven replicates of each soil and vegetation sample were processed for each extraction method and all replicate extractions were stored in a refrigerator at 4°C until required for analysis.

3

Analysis

Analysis was carried out using a Perkin-Elmer Flame Atomic Absorption Spectrometer Model 1100 fitted with an impact bead and appropriate hollow cathode lamps. Analytical conditions, sensitivity and linear range used were as recommended by the manufacturer.

Calibration solutions were made up from 1000 mg L^{-1} certified standards and within the recommended linear ranges.

Dilution factors for the extracts were determined by trial and error, the final dilution ratio producing absorption values within the linear range.

Evaluation of open microwave/nitric acid digestion was carried out on samples of Snail Beach spoil and hazel leaves only. It was considered sufficient to evaluate these extractions for indications of improvements in extraction of the metals in question before proceeding with other samples.

Relative standard deviation

Table I shows the mean relative standard deviations obtained for each target metal from each extraction method and each substrate. Having determined the relative standard deviation for each metal in each of the six substrates by each extraction method, the mean relative standard deviation was calculated for each metal.

Relative standard deviation is a measure of the relative error encountered and can be used as a measure of precision when determining concentrations of varying magnitudes [18].

Table I. Mean relative standard deviation for each target element using different extraction reagents.

	Mean relative standard deviation %			
Extraction method	Copper	Zinc	Lead	Cadmium
HNO_3	10.8	8.4	9.1	20.1
$HNO_3/HClO_4$	7.2	5.4	9.9	14.9
Aqua regia	10.2	6.3	9.4	11.8
HNO_3/H_2O_2	17.5	7.0	10.2	11.3

*** T&F (2005 Style) [3.6.2005-2:42pm] [1–10] [Page No. 5]
{Band8}Gtec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

Accuracy

The quality assurance standards used for the extraction analysis were the certified materials CRM 142R and CRM 101.

The accuracy values, shown in Table II, were obtained using the formula:

$$\text{Accuracy } A\% = 100 - \left[\frac{(x_{QA} - x_A)}{x_{QA}}\right]100$$

where x_{QA} is the certified value, x_A is the analytical result.

Results

The figures below indicate the average concentration obtained from each extraction process. The error bars, where applied, indicate upper and lower 95% confidence intervals (CIs).

In all figures the methods indicated are $1 = HNO_3$, $2 = HNO_3/HClO_4$, $3 =$ Aqua regia, $4 = HNO_3/H_2O_2$ and $5 = HNO_3$ with microwave assistance.

Figure 1 indicates that aqua regia digestion extracts more copper than the other processes and has the lowest 95% CI variance for all metal extractions when used with Parys Mountain waste rock. Cadmium was not detected in Parys Mountain spoil extracts.

Concentration values in Figure 2 are $\log_{10}$ of the actual values in order to adequately show all metals within the axis scale. Nitric acid digestion yields the highest zinc concentration ($9140\,mg\,kg^{-1}$) with the lowest variance in 95% CI ($\pm 2.4\%$) for Snail Beach spoil samples.

Aqua regia digestion produces the highest average concentration of cadmium with average $65.9\,mg\,kg^{-1}$ and 95% CI variance of $\pm 9.1\%$.

Figure 3 illustrates that the highest average copper concentration for CRM 142R light sandy soil was obtained using nitric acid digestion ($61.1\,mg\,kg^{-1}$) closely followed by aqua regia digestion ($61.0\,mg\,kg^{-1}$). Cadmium in CRM 142R was not detected since the absorption signals were below the LOD, the instrument in use.

Charts illustrating analysis carried out on hazel leaves from Snail Beach and Pine needles from Black Rocks are not included since their concentrations are low and consequently 95% CI generally were high. However, aqua regia digestion extracts the highest average

Table II. Accuracy % for determination of metal concentrations in extracts as a result of four different digestion methods.

Sample	Cu mean ($mg\,kg^{-1}$)	Cu (A%)	Zn mean ($mg\,kg^{-1}$)	Zn (A%)	Pb mean ($mg\,kg^{-1}$)	Pb (A%)
CRM 142R	69.8	–	93.3	–	25.7	–
HNO_3	61.1	87.5	77.1	82.7	23.9	93.0
$HNO_3/HClO_4$	55.0	78.9	81.9	87.8	24.3	94.5
Aqua regia	61.0	87.4	79.3	85.0	24.2	94.1
HNO_3/H_2O_2	46.7	66.9	78.3	84.0	23.2	90.1
CRM 101	5.00*	–	35.3	–	2.8**	–
HNO_3	<LOD	–	26.1	73.8	<LOQ	–
$HNO_3/HClO_4$	<LOD	–	29.5	83.6	<LOQ	–
Aqua regia	<LOD	–	31.9	90.4	<LOQ	–
HNO_3/H_2O_2	<LOD	–	28.7	81.4	<LOQ	–

* Indicative values obtained by participating laboratories but not certified.
** Additional value recorded by some of the participating laboratories but not certified.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 6]
{Gand0}64ec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

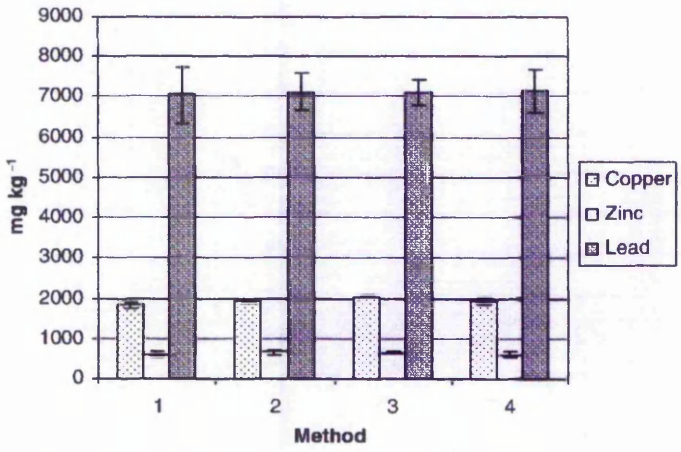


Figure 1. Metal concentration using different digestion methods in Parys Mountain spoil: 1 = HNO_3; 2 = $HNO_3/HClO_4$; 3 = Aqua regia and 4 = HNO_3/H_2O_2.

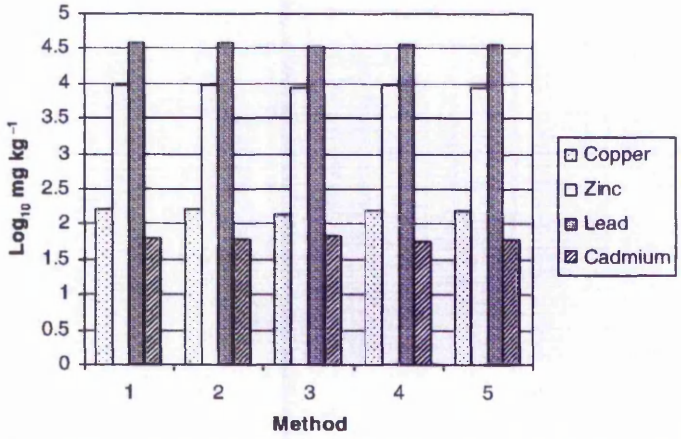


Figure 2. Log_{10} of metal concentrations using different digestion methods in Snail Beach spoil: 1 = HNO_3; 2 = $HNO_3/HClO_4$; 3 = Aqua regia; 4 = HNO_3/H_2O_2 and 5 = HNO_3 + microwaves.

zinc concentration in Scots pine needles. Lead was present in the pine needles collected at Black Rocks but in very low concentrations and Aqua regia digestion exhibits the highest lead concentration (13.5 mg kg^{-1}).

The highest zinc concentration (1970 mg kg^{-1}) in hazel leaves from Snail Beach was obtained using nitric acid/perchloric acid digestion and a 95% CI variance of ±4.04%. Aqua regia although producing the lowest zinc concentration (1810 mg kg^{-1}), resulted in the lowest 95% CI variance of ±2.81%. The highest lead concentration in hazel leaves from Snail Beach was obtained using aqua regia extraction.

Figure 4 illustrates that the highest average concentration of zinc in CRM 101 Spruce needles reference material is produced by aqua regia digestion (31.9 mg kg^{-1}) with a 95% CI variance of ±8.2%. Concentration of lead is also highest using aqua regia with a 95% CI of only ±0.46 against a mean value of 18.0 mg kg^{-1}.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1–10] [Page No. 7]
(Sandi8) Glee/GTEC-116540.3d (61EC) First Proof GTEC-116540

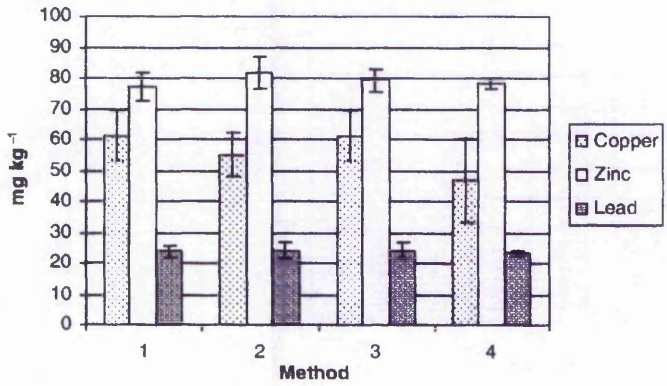


Figure 3. Metal concentrations using different digestion methods in CRM 142R light sandy soil: 1 = HNO_3; 2 = $HNO_3/HClO_4$; 3 = Aqua regia and 4 = HNO_3/H_2O_2.

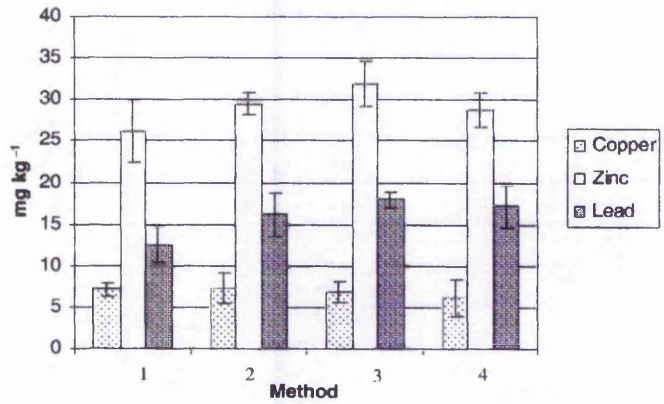


Figure 4. Metal concentrations using different digestion methods in CRM 101 Spruce needles.

Analysis of variation (ANOVA)

Two-way ANOVA was carried out comparing mean metal concentration with extraction method with each metal detected for each of the six substrates using Minitab release 13.30 [19]. The difference between the four acid digestion methods was not significant at the 5% level of significance for each of the six substrates analysed, however, microwave-assisted digestion was significantly different in the case of hazel leaves.

Discussion

The analysis of soil and vegetation samples was difficult due to the variability of the substrate under examination, homogeneity of the prepared samples being the most critical factor. The Community Bureau of Reference [20] make much of this point in the preparation and supply of their certified samples of soil and in another report [21] referring to their certified samples of Spruce (*P. abies*) needles.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 8]
(GandB)Gtec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

The generally high level of mean relative standard deviation (Table I) obtained from each series of replicates was a result of several processing factors not least of which was the nature of the samples under examination and the processing of the samples before digestion. The method used for soil and sediment preparation separated large stones etc. from the general mass by passing through a 2-mm sieve after drying, however, particles of inert base rock would still pass through to the grinding process and may or may not be ground as finely as other metal-bearing components in the matrix.

In the case of acid digestion the relative error should not exceed 4% and the error due to instrumentation, in this case FAAS, is unlikely to exceed 1%. Significant errors arising from calculation would only arise when multiplication factors of 10^6 were involved, which in these determinations would not be significant. Therefore, the major potential in this experiment were indeed as a result of sample variation.

Standard deviation (SD) was used to calculate 95% CI using a paired *t*-test, which in turn, was used to assign the error bars on the bar charts used in the result section. The use of more replicates could have reduced the value of SD and also reduced the 95% CI values.

The main factor affecting this investigation is that of errors arising from sample preparation and subsequent handling. In order to facilitate handling and storage facilities only 500 mg of soil and 250 mg of vegetation was used in each replicate. Clearly larger quantities of sample used together with subsequent larger volumes of extract would reduce the digestion and handling errors.

Despite the variation in 95% CI and relative standard deviation within each extraction method and each substrate it is proposed that meaningful observations can be made. All results for metal concentrations obtained by each extraction method were of similar orders of magnitude and the variation in relative standard deviation obtained was in the order of those obtained by other workers. Indeed Pueyo et al. [4] highlighted the problems encountered when inter laboratory studies were undertaken in an endeavour to certify polluted river sediment. As well as relative standard deviation variation between laboratories, some 40% of results were rejected because of systematic errors or inappropriate detection techniques. Analysis by X-ray fluorescence on control samples of copper mine wastes [22] illustrates large variations in concentrations observed for copper and zinc. Pederson et al. [15] obtained large SDs ($\pm 55\,mg\,kg^{-1}$ on means of $105\,mg\,kg^{-1}$) using 10 replicates for copper concentrations in *Fallopia convolvulus* shoots grown in copper-contaminated soils.

The results obtained for each sample appear to be acceptable from the information available, however, the lead concentration of Parys Mountain spoil is surprising since extraction of lead is not recorded during the active period of mining at this site [23]. All extraction methods and replicates, however, produced lead concentrations of the same order of magnitude.

Since two-way ANOVA examination determined that there was no significant difference between most extraction methods, it was decided that the criteria used for determining the most efficient extraction method would be a combination of maximum mean concentration of metal and minimum 95% CI in conjunction with accuracy determinations carried out on the reference materials.

The use of microwaves with the various digestion methods on Snail Beach spoil (Figure 2) and hazel leaves collected at Snail Beach did not improve the extraction process with the exception of cadmium in Snail Beach spoil. In all cases the relative standard deviation and 95% CI was one of the highest for that particular extraction. Additionally ANOVA analysis determined that the range of results obtained using this method was significantly different to the other four procedures.

*** T&F (2005 Style) [3.6.2005-2:42pm] [1-10] [Page No. 0]
(GandB)Gtec/GTEC-116540.3d (GTEC) First Proof GTEC-116540

The most efficient extraction method was aqua regia, which produced the highest number of maximum metal concentrations. Aqua regia was particularly effective when used to digest vegetation. When used to digest spoil from Snail Beach it was least effective except in the case of cadmium extraction.

Aqua regia digestion also exhibits higher accuracy results when compared with the other methods used. Accuracy data is not included relative to copper, lead and cadmium extractions by all reagents on CRM101 Spruce needles since the absorption values obtained for copper were below the calculated LOD and those for lead were marginally above the LOD but below the LOQ. No signal was detected for cadmium in CRM101.

Reviewing the above data demonstrates that the suitability of individual digestion methods vary according to the metal under examination and the substrate being analyzed. In the case of soils etc., metal distribution and the degree of binding to individual phases in the soil matrix varies as does the speciation of the metals within the matrix. Lead is predominantly bound to carbonates, oxides and residual components and to a lesser extent to the organic fraction. Copper is bound mainly to the residual fraction and zinc is bound to oxides and to a lesser extent carbonates and residual. Cadmium shows an almost even split between oxides and residual fractions [24]. The origin of the soil and the type of ore deposits in the area will affect the extractability of the metals under examination; however, the most successful general extractant was aqua regia.

Pyatt et al. [25] showed that metals can be accumulated in vegetation by factors 12 times greater for lead and 5.5 times greater for copper when compared to surrounding soil concentrations also root and leaf concentrations can vary considerably and by different factors from species to species. This feature of bioaccumulation processes can affect the selection of the amount of material to be digested, however, this study has shown that aqua regia is the most successful extractant for vegetation.

Conclusion

Despite the problems associated with sample collection, preparation and extraction, the use of the aqua regia digestion method outlined above as a general purpose process for the digestion of soil and vegetation samples is recommended.

Sample collection and transportation procedures adopted in this study were satisfactory, however, sample preparation and subsequent handling could be improved. The drying processes employed appear to be sufficient but the grinding process needs to be improved for both soil and vegetation samples. An extended grinding time in a pestle and mortar appears to be necessary, however, the use of a microniser or ball mill producing particle sizes <100 microns would improve homogeneity in the handling, storage and digestion processes.

The quantity of sample used for each determination should be increased to 1.0 g of soil and 0.5 g of vegetation as a minimum requirement and the quantity of reagents increased accordingly. The final volume of extraction produced would also be increased to 50 mL improving accuracy and precision by reducing processing losses. The number of replicates of each sample used in the process should be increased to a minimum of 10 to produce more meaningful statistical data as well as improving precision.

The use of microwave assistance for digestion in open conditions had no significant effect when used with nitric acid and is considered to be of little use in this type of digestion process.

Acknowledgements

Thanks are due to Dr David Crab for help with ANOVA, Dr Mike Coffey for technical support and to HEFCE (RAE) for financial support to Bob Wilson.

References

1. Tingi U, Reilly C, Patterson C. Food Chem. 1997;60(1):123–128.
2. Radofevic M, Bashkin VN. Practical environmental analysis. Cambridge: The Royal Society of Chemistry; 1999. pp ■■–■■.
3. Bellis D, Cox AJ, Staton I, Mcleod CW, Satake K. J. Environ. Monit. 2001;3(5):512–514.
4. Pueyo M, Rauret G, Luck D, Yli-Halla M, Mutau H, Quevauviller Ph, Lopez-Sanchez JF. J. Environ. Monit. 2001;3(2):243–250.
5. McGrath D. Talanta 1998;46(3):439–448.
6. Martinez GML, Carlosena ZA, Miniategui LS, Lopez MP, Prada RD. Anal. Lett. 1999;32(1):139–157.
7. Moeller A, Ambrose RF, Que Hee SS. Food Addit. Contam. 2001;18(1):19–29.
8. Wasilewska M, Goessler W, Zischka M, Maichin B, Knapp G. J. Anal. Atom. Spectrom. 2002;17(9):1121–1125.
9. van Loon GW, Duffy SJ. Environmental chemistry: A global perspective. Oxford: Oxford University Press; 2000. pp ■■–■■.
10. Ipolyi, Brunori C, Cremisini C, Fodor P, Macaluso L, Morabito R. J. Envir. Mon. 2002;4:541–548.
11. West S, Charman DJ, Grattan P, Cherburkin AK. Water, Air, Soil Pollut. 1997;100:343–353.
12. Sharenberg W, Ebeling E. Bull. Environ. Contam. Toxicol. 1996;56:369–396.
13. Pyatt FB. Ecotoxicol. Environ. Saf. 2001;50:60–64.
14. McFarland N, Bendell-Young LI, Guglielmo C, Williams TD. J. Environ. Monit. 2002;4(5):791–795.
15. Pederson MB, Kjaer C, Elmegaard N. Arch. Environ. Contam. Toxicol. 2000;39:431–439.
16. Kozlov MV, Haukioja E, Kovnatsky EF. Environ. Pollut. 2000;108:303–310.
17. Reinecke AJ, Reinecke SA, Musilbono DE, Chapman A. Arch. Environ. Contam. Toxicol. 2000;39:392–397.
18. Miller JN, Miller JC. In: Statistics and chemometrics for analytical chemistry. 4th ed. Harlow: Pearson Education Ltd; 2000. pp ■■–■■.
19. Pentecost A. Analysing environmental data. Harlow: Pearson Education Ltd; 1999. pp ■■–■■.
20. Community Bureau of Reference. Certification of total contents (mass fractions) of Cd, Co, Cu, Mn, Hg and Ni and the aqua regia soluble fractions of Cd, Pb, Ni and Zn in a light sandy soil. CRM142R, Report EUR 15283 EN, Commission of the European Communities, Luxembourg, 1994.
21. Community Bureau of Reference. The certification of the content of N, P, S and Cl in beech leaves (CRM 100 – *Fagus silvatica*) and Al, Ca, Mg, Zn, N, P, S and Cl in spruce needles (CRM 101 – *Picea abies*), Report EUR 12680 EN, Commission of the European Communities, Luxembourg, 1990.
22. Kruckeberg AL, Wu L. Ecotoxicol. and Environ. Saf. 1992;23:307–309.
23. Rowlands J, Copper mountain. Llangefni: Anglesey Antiquarian Society; 1966. pp ■■–■■.
24. Murphy AP, Courdert M, Barker J. J. Environ. Monit. 2000;2:621–627.
25. Pyatt FB, Gilmore G, Grattan JP, Hunt CO, McLaren S. J. Archaeol. Sci. 2000;27:771–778.