Development of hyphenated micro-analytical methods for trace metal fractionation and their application to environmentally relevant solid matrices

vorgelegt von

BSc (Hons.) Chem., MSc Chem. Nigeria

Modupeola A. Jimoh

Von der Fakultät II - Mathematik und Naturwissenschaften

der Technischen Universität Berlin

zur Erlangung des akademischen Grades

Doktorin der Naturwissenschaften

-Dr. rer. nat.-

genehmigte Dissertation

Promotionsausschuss:

Vorsitzender: Prof. Dr. rer. nat. Christoph van Wüllen

Berichter/Gutachter: PD Dr. habil. Wolfgang Frenzel

Berichter/Gutachter: Prof. Dr. rer. nat. Thorsten Ressler

Berichter/Gutachter: Prof. Dr. rer. nat. Jörn Müller

Berichter/Gutachter: Dr. habil. Jürgen Mattusch (Umweltforschungszentrum Leipzig-Halle)

Tag der wissenschaftlichen Aussprache: 14. März 2006

Berlin 2006

D83

Was hilft aller Sonnenaufgang,

wenn wir nicht aufstehen."

Georg Christoph Lichtenberg, deutscher Physiker und Schriftsteller (1742-1799)

List of publications

1) V. Müller, M. Jimoh and W. Frenzel: Neuartiges mikroanalytisches System für die In-line Extraktion von Schwermetallen in festen Umweltproben. GIT-Labor. (2003) **8** 821-823

2) M. Jimoh, W. Frenzel, V. Müller, H. Stephanowitz and E. Hoffmann: Development of a Hyphenated Microanalytical System for the Investigation of Leaching Kinetics of Heavy Metals in Environmental Samples. Anal. Chem. (2004) **74** 1197-1203

3) M. Jimoh, W. Frenzel and V. Müller: Microanalytical flow-through method for the assessment of bioavailability of toxic metals in environmental samples. Anal. Bioanal. Chem (2005) **381**(2), 438-444

4) M. Miro, M. Jimoh and W. Frenzel: A novel dynamic approach for automatic microsampling and continuous monitoring of metal ion release from soils exploiting a dedicated flow-through microdialyser. Anal. Bioanal. Chem (2005) **382** 396-404

5) W. Frenzel, M. Jimoh and M. Miro: Development and application of hyphenated microanalytical systems for on-line and in-situ fractionation studies and investigations of leaching kinetics of heavy metals in solid samples of environmental interest using atomic spectrometric detection.
Chem. Anal. (2005) **50**, 279- 299

Zusammenfassung

Seit vielen Jahren ist bekannt, dass das spezifische Verhalten von Spurenmetallen in der Umwelt von deren physiko-chemischen Zustandsformen (Oxidationsstufe, Bindungspartner, Komplexierungsgrad) abhängig ist. Dies bezieht sich auf Essentialität und Toxizität für Lebewesen, Mobilität innerhalb der diversen Umweltkompartiments und die Bioverfügbarkeit z.B. für Pflanzen.
Zahlreiche Methoden sind in der Vergangenheit entwickelt worden, die eine Identifizierung und Quantifizierung unterschiedlicher Verbindungen, Formen oder Phasen, in der ein Element vorkommt, ermöglichen. Allerdings sind diese meist in instrumenteller Hinsicht und bezüglich Zeit und Arbeitseinsatz aufwendig.
Ziel der vorliegenden Arbeit war die Entwicklung von Techniken, die eine Fraktionierung von Spurenelementen mit geringem Aufwand ermöglichen. Dabei standen Aspekte der Automatisierung im Vordergrund. Ein weiterer Gesichtspunkt war die Erhöhung der Aussagekraft der gewonnen Ergebnisse durch den Einsatz kontinuierlicher Extraktionstechniken gegenüber der bislang fast ausschließlich verwendeten Batch-Verfahren.

In der vorliegenden Arbeit wurden neue Techniken entwickelt, die die Freisetzung von Schwermetallen aus festen Proben unter kinetisch kontrollierten Bedingungen kontinuierlich erfassen und die Quantifizierung unter veränderten Extraktionsbedingungen nahezu in Realzeit ermöglichen. Dazu wird das Probenmaterial in kleine Kartuschen (typischerweise 5-80 mg Einwaage) gepackt, die in ein kontinuierliches Fließsystem integriert werden. Für die in-line Detektion wurde die AAS (Flammen und Graphitofentechnik), sowie die ICP-OES und ICP-MS eingesetzt. Durch Verwendung verschiedener Extraktionsmittel wurde die Mobilisierbarkeit von Schwermetallen untersucht und aus den Ergebnissen Rückschlüsse über die Bioverfügbarkeit getroffen.
Verschiedene Elemente innerhalb eines Probenmaterials (Matrix) ergaben unterschiedliche Extraktionsmuster und für verschiedene Matrices wurde eine unterschiedliche Extrahierbarkeit für die untersuchten Elemente gefunden. So wurden aus Pflanzenmaterialien die Elemente Ni, Cd, Zn und Cu meist bereits mit Wasser nahezu vollständig eluiert, wohingegen die Elemente Pb, Fe und Al nur einen sehr geringen wasserlöslichen Anteil aufwiesen, mit verdünnter Säure aber bereits gut extrahierbar waren. Beim Vergleich verschiedener Matrices zeigte sich eine Abhängigkeit der Extrahierbarkeit der Metalle von der Morphologie, der Probenvorbehandlung und der Komplexität der Probenzusammensetzung. So waren in Böden und Sedimenten Cu und Zn meist in der säurelöslichen Fraktion vorhanden, was auf eine unterschiedliche Bindungsform gegenüber Pflanzenmaterialien hindeutet.
Durch verschiedene instrumentelle Konfigurationen der verwendeten Fließsysteme (unidirektionaler und rezirkulierender Fluss, stopped-flow Technik) konnten die Extraktionsbedingungen so verändert werden, dass sowohl schnell als auch langsam ablaufende Extraktionsprozesse verfolgt werden konnten.

Zusammenfassung

Eine Qualitätskontrolle der entwickelten Verfahren wurde durch Analysen von verschiedenen kommerziellen Referenzmaterialien durchgeführt. Dabei zeigte sich in vielen Fällen eine gute Übereinstimmung aber auch das Defizit einer unvollständigen Charakterisierung der Referenzmaterialien durch die Hersteller. Die entwickelten Verfahren wurden mit bislang üblichen und auch international standardisierten Extraktionsschemata (z.B. 3-stufige BCR Extraktion, DIN-Methode für Resorption) verglichen. Aufgrund des operationellen Charakters aller Fraktionierungsmethoden kann eine Übereinstimmung allerdings nicht erwartet werden. In vielen Fällen zeigten sich jedoch gut vergleichbare Ergebnisse. Versuche zur Präparation von Materialien für Fraktionierungsexperimente mit bekannter Spezieszusammensetzung wurden ebenfalls durchgeführt. Wenngleich die dabei erzielten Ergebnisse als nicht zufrieden stellend beurteilt werden müssen, markieren sie einen Weg zur verbesserten Qualitätskontrolle von Fraktionierungstechniken.

Im zweiten Teil der Arbeit wurde das Konzept der Mikrodialyse, das weite Verbreitung für die in-situ Bestimmung wichtiger Komponenten in der Neurophysiologie gefunden hat, für die Metallfraktionierung in Bodenproben eingesetzt. In diesem Zusammenhang wurden unterschiedliche Dialysesonden konstruiert und hinsichtlich ihrer Funktion getestet. Die Einflüsse operationeller Parameter auf die Dialyserate und Ansprechzeit wurden ermittelt und Untersuchungen zum Membrantransport verschiedener Elemente bei unterschiedlichen chemischen Bedingungen angestellt. Es wurde zudem eine in-line Kopplung mit der Graphitofen-Atomabsorptionsspektrometrie konfiguriert, mit der die Freisetzung von Spurenmetallen aus Bodenproben quasi kontinuierlich studiert werden kann.
In Modellexperimenten mit einer synthetischen Bodensäule wurden in der Natur vorkommende Freisetzungsprozessen wie Saurer Regen, versehentliche Kontamination, Fallout, Sickerwasser durch Kompost simuliert und die dabei erhaltenen Ergebnisse interpretiert.

Keywords: Schwermetallfraktionierung, Sequentielle Extraktion, Fließsystem, Mikrokartusche, Extraktionskinetik, Mikrodialysesonde, in-situ monitoring

Abstract

It is a proven fact that the mobility, bioavailability, toxicity or essentiality and fate of an element depends on the form in which it occurs, rather than its total concentration. Exposure to some forms of an element may be harmless, while other species of the same element may be toxic, carcinogenic or mutagenic.

The direct speciation in solids is limited to substances which have been identified, are stable enough to be isolated in the original form and for which sensitive methods are available. Fractionation methods are the preferred alternative and usually involve some form of chemical extraction scheme. Furthermore, fractionation allows assessment of mobility and (bio) availability with respect to a particular matrix and experimental conditions can be selected to mimic those of the natural environment.

The objective of the present thesis was to develop methods of gaining enhanced information about the forms of metal binding (species) in solid environmental matrices with emphasis on the improvement (e.g. automation potential) and extension of existing fractionation (extraction) methods.

Classical sequential extraction methods are based on the principle of selective dissolution of predefined phases and analysing the released metals with an element specific detector. However these schemes which are equilibrium based (meaning that the reagent has to be in contact with the solid for prolonged times, normally 8-16 hrs) suffer generally the problems of non-selectivity of reagents used, re-adsorption and re-distribution. Kinetic methods on the other hand give enhanced information about the behaviour of metal forms during leaching and other processes. Continuous extraction methods are more suitable for risk assessment studies since the environmental processes they simulate are also dynamic in nature.

In the present work, an in-line (flow through) system hyphenated with atomic spectrometric detectors for studying the kinetics of metal leaching has been developed. By the incorporation of micro-cartridges filled with 5-80 mg of solid samples (dried and pulverized as well as fresh and chopped) into the conduits of a flow system and appropriate selection of the liquid flowing through the cartridge, information about the degree of leaching and in particular of the kinetics of the leaching process were obtained in almost real time thereby allowing judgement on bioavailability to be made. Different metals within the same sample showed different leaching behaviour allowing the classification in two groups, Ni, Cd, Zn, Cu exhibited fast leaching kinetic and occurred mostly in the water soluble fraction of plant samples while the behaviour of Pb, Fe and Al was different, occurring mostly in the acid soluble fraction. Between matrices however, a different pattern was noticed, which also depended on the morphology and complexity of the matrix. Cu and Zn although fast leaching, were mostly in the acid soluble fraction in soil, indicating different binding from that in plants. Since fast leaching metals are more accessible (labile) while slow leaching metals are less accessible, an insight into the long term release of the different metals in solid samples of environmental importance was gained.

The developed methods were optimised to accommodate the study of fast as well as slow kinetics by designing a continuous in addition to a re-circulating manifold. Analytical characteristics of the flow systems were evaluated.

Quality assurance was achieved by analysis of various commercial reference materials. Good agreement was obtained in general, but the lack of suitable standards with adequate analytical characterisation by the manufacturer was evident. The design of laboratory internal reference material tailored to represent different forms of metal binding was therefore embarked on. Preliminary results of analysis of these materials were promising.

Application of the microanalytical system to real samples utilising internationally recognised schemes then followed. The standard BCR three stage sequential extraction scheme was applied in the developed micro analytical system for the assessment of bioavailable fraction of 13 elements in soil and other samples. An in-line four stage nitric acid scheme was utilised in the determination of metals in different matrices of environmental concern exploiting in-line detection by FAAS, ICP-AES and ICP-MS. Finally, the system was used for the in-line translation of the DIN method for the assessment of bioaccessibility. Results of the different schemes were compared and interpretations according to risk assessment made.

The last part of the work concerned the role and applicability of microdialysis as a separation and sampling technique to metal fractionation. Dialysis and by extension microdialysis, is one of the oldest separation methods based on the discrimination of substances according to molecular weight. Due to the success of microdialysis for the in-situ monitoring of target substances in neurological fluids, the in-situ fractionation of metals in soil was aimed. Since the method is non-invasive (i.e. the diffusion process does not disturb the equilibria naturally present in the systems), there is no depletion of metals in the vicinity of the probe. Thus it should be possible to differentiate different forms according to distribution and diffusion properties while upholding sample integrity.

For these studies, a microdialysis probe was constructed for the in-situ sampling of metal ions from soil in a laboratory-made soil column and hyphenated with ETAAS for in-line quantification. After full characterisation of the probe, the response to small changes in the environment was ascertained. Application of the system for in-situ study of the leaching of metals from soil was also performed. Further environmental situations like acid rain, accidental spillage, fallout, seepage through solid waste or compost and stimulus-response scenarios could be effectively simulated using the developed system.

Keywords: heavy metal fractionation, sequential extraction, flow-through system, microcolumn, leaching kinetics, microdialysis probe, in-situ monitoring

My mum and in loving memory of my father

Acknowledgements

Praise be to God, the Lord of the Worlds

I am most grateful to Dr. Wolfgang Frenzel for the kind supervision of the work. Always to be remembered are the many meetings, words of encouragement and all.

Special thanks to Dr. Hoffmann and Ms. Stephanowitz ISAS, Berlin for making it possible to analyse the samples at the ICP-MS.

The Management and Staff of AZBA GmbH are thanked for the funding and support that made the project a success. Mrs Grunack and Ms Klewe are worth mentioning for their help at the ICP-AES and other jobs but especially the collegial atmosphere at the trace element laboratory. Thanks to the students on Internship especially Mrs. Kath and Miss. Trier for their contributions to the experimental work.

I am grateful to the staff and colleagues at the institute for their ready help and support in the course of my work, most particularly Betty, Carmen, Manuel, Michael and Tatjana for the helpful discussions and tea. Manuel's assistance with the microdialysis work is gratefully acknowledged. For the fabrication of the micro cartridges, holders, and countless contraptions used over the years, the workshop staff, especially Thomas is gratefully acknowledged. Mr. Steinbrecher is thanked for his support with the instruments. His being the all round man saved many a situation.

Praise be to God for my husband and the children for their patience, understanding and great love and sacrifice in all these years that I have not been my usual self. Finally, thanks to my mother for everything.

Contents

All substances are poisons; there is none which is not a poison.
The right dose differentiates a poison and a remedy." –Paracelsius

Introduction

The industrial developments of the 19th and early 20th century were accompanied by the problems of toxic waste management and metal emission into the environment. Indeed most cases of pollution to date are anthropogenic in nature [1].

Heavy metals (i.e. metals with a density of 5 g/cm^3 and higher) are ubiquitous, occurring naturally at about 0.01% in the earth's crust. There is no clear cut demarcation between essentiality and toxicity of the metals, since functionality depends largely on the concentration. Thus an element may be essential in a particular organism, while being toxic at the same or higher concentration in other organisms. Also, it may be that the elements like Hg, Pb and Tl for which no essentiality is proven at the moment, may still be found needed at a yet to detect level in the future. As a general classification however, it is taken that few elements are considered essential (e.g. Fe, Zn, Cu, Co), some are known to be toxic (e.g. Cr, Cd, Hg) while others display toxic properties at high enough concentration.

Analysis of soil for essential metals necessary for plant growth and for fertiliser application has long been routinely carried out [2,3]. However it was the incident at the Minamata Bay and the cadmium poisoning in Japan [4] that brought awareness on the drastic effects of trace metal pollution and the need for monitoring.

Early methods of analysis were mostly colorimetric, using organic reagents like dithizone to develop colour which could then be matched against standards. The results of such tests were qualitative to semi- quantitative. The Second World War and the demands of the industry provided the stimulus and resources for the development of instrumentation for chemical analysis [5]. Thus the advent of instrumentation like the atomic absorption spectrometer in the mid 1950's, revolutionalised trace metal analysis. Lower limits of detection (ppm range) were possible and quantitative results obtained. State of the art analysis by means of instrumental techniques based on completely different principles is possible today from the wide range of instrumental techniques available resulting in an increasing confidence in analysis even at picogram levels of detection. The direct analysis of trace constituents in solids however is limited to methods like x-ray methods [6,7], neutron activation analysis, electron microscopy [8] and laser ablation technique coupled to an element selective detector [9]. Here, the problems of calibration, standards and reference materials occur. Furthermore, x-ray techniques require sample in dry and crystalline form, which limits their application. Also, where the surface of the material is sampled for direct analysis, sample inhomogeneity would be a problem. Thus instrumental methods which receive the sample in liquid form are the preferred alternative.

Sample preparation techniques therefore involve some form of sample homogenisation and transformation into liquid form. Depending on the method of analysis, instrument selectivity and sensitivity, further steps to isolate the analyte(s) or remove matrix interference, cleanup, or preconcentrate could be necessary. The more complex and lengthier the preparation steps, the higher the risk of errors. Thus sample preparation has been the Achilles heel of trace metal analysis to date. A lot of research has been devoted to the development of simple, rapid yet effective sample preparation methods, for instance, employing microwave [10] and ultrasonic energy [11] to speed up the dissolution processes. Furthermore, a trend is seen towards extraction and leaching techniques as opposed to total decomposition of sample matrix, since for most metals sufficient information e.g. for environmental risk assessment can be obtained using shorter, environmentally friendlier (less toxic) pseudo total digestion methods.

The last decade or so has seen less of the total determination attitude as it has become obvious that the total concentration of metals does not deliver information as to the actual behaviour, effects and long term fate of metals in the environment, biological systems and relating to health and nutrition.

It is widely agreed that the metabolism, bioavailability, (eco) toxicity or essentiality, lability and fate of a metal or metalloid all depend on the form in which it occurs in the respective media, as opposed to the total concentration in which it is present. Exposure to some forms of an element may be harmless, while other species of the same element may be toxic, carcinogenic or mutagenic.

For instance, it is known that chromium (VI) is more toxic and taken up more readily in organisms than chromium (III) [12], inorganic forms of arsenic are toxic while organic forms like arsenobetain or arsenosugars common in fish are not. The toxicity of the three oxidation states of mercury differ considerably, the metallic form being associated with CNS damage, while the mercurous form is less toxic. Organo mercury compounds (e.g. methyl mercury) are well known for their toxicity; being bio-accumulated and transferred through the food chain. A meaningful risk assessment thus requires differentiation between metal forms.

In the biomedical field, the action of both therapeutic and diagnostic metal chelating agents in humans is dependent on the binding forms in which they occurs. Cis-platin is more effective than the trans counterpart as anti-tumour agent [13]. Metal transport in biological systems is species dependent; tranferrin for instance has binding sites for Fe^{3+}. In the presence of dietary strongly binding phytates and polyphenols, iron is rendered unavailable for uptake by the mucousal cell iron transporter. The presence of ascorbic acid however enhances uptake [14]. The efficacy of mineral food supplements is therefore dependent on the binding form as well as dietary and other host factors since the mineral must first be in an absorbable form in the lumen of the intestine before the individual can respond.

In soil and other geological matrices, the (bio) availability of a metal depends on its binding form and association within the matrix. However, several geochemical factors like pH, redox conditions, moisture content, organic matter content and cation exchange capacity (CEC) [15, 16] are important.

The history and complexity of the sample- ageing, as well as mineral structure also play a role in metal bioavailability.

Accordingly, emphasis has been placed on the development of appropriate instrumental techniques capable of determining the various forms of trace quantities of elements in solid (particulate), liquid and gaseous (aerosol) matrices. In chemical analysis, the quantitative and qualitative determination of specific chemical species is long established, especially for non-metallic species (e.g. the different forms of nitrogen: organo nitrogen compounds, NO_3^-, NO_2^-, NH_4^+ and NH_3). However, much of the recent effort in analytical chemistry has focused on the speciation of metals because of their functionality as part of biologically important enzymes and co-factors, and their detrimental effects in the environment.

Speciation can be defined as the identification and quantification of the binding forms of an element. The International Union of Pure and Applied Chemistry (IUPAC) [17] further defines fractionation as the process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

While chemical speciation or speciation analysis deals with the determination of individual species, fractionation or operational speciation involves the determination of elements associated with predefined phases, pools or fractions in the matrix. This is usually done by attacking the particular phase with a selective reagent, thereby releasing metals associated for analysis. A fraction thus describes the group of species in a sample which can be isolated from the matrix due to special properties it possesses.

Although speciation and fractionation are often used interchangeably, the main difference between the two techniques lies in the fact that in speciation analysis a true identification of definite species is sought while in fractionation, the total metal content of an operationally defined fraction is obtained. Consequently, the former relies on the integrity of sampled species while in the latter the species are converted into other forms during operation.

Both techniques can yield different but complementary information on the sample/metal species sought.

For appropriate legislation and control (monitoring) to be effected, suitable rapid routine methods of species analysis must be available. As at yet, only few regulations take the species concentration into account with allowable levels in water, food etc. A look through the literature shows that there is a need for information such as on the molecular interactions of species at phase boundaries (e.g. to combat arsenic pollution in Bangladesh), long and short term effects of metal species on the environment (e.g. mobility, bioavailability, bioaccessibility studies), and common (tolerable) levels of metal species in the various environmental or ecological compartments (for legislative and monitoring purposes). Since ultra-trace levels of species are being targeted, hyphenated instrumentation with sufficiently low detection limits is necessary.

The present work therefore set out to provide methods for the rapid assessment of metal forms in solid environmental matrices with the following objectives:

a) Development of microanalytical flow through extraction systems suitable for screening solid samples for bioavailable metal content,

b) Hyphenation of the systems to various atomic spectroscopic instruments for in-line, real time monitoring of leaching and other processes,

c) Gaining additional kinetic information, not otherwise obtained with the existing fractionation methods.

Finally, due to the success of dialysis as a separation technique and the advantages of in situ monitoring over grab sampling (non-invasive, preserving analyte species information, no need for laboratory transport and storage with the attendant risks), a method for the in situ sampling and in-line monitoring of metal ions in environmental compartments was sought. This resulted in the

d) Development of a microdialysis probe hyphenated to ETAAS for studying metal forms in environmental compartments. Applications of the developed method include the on site and in- situ

   i. monitoring of metal leaching and by extension metal sorption,

   ii. evaluation of remediation and

   iii. assessment of metals available for plant uptake, etc.

1 Heavy metals

The occurrence, impact and analysis of heavy metals in the environment are discussed in this chapter. Heavy metals are a persistent group of pollutants that have become the subject of analysis due to their toxic effects on the environment. A few examples are given below. A chronological review of methods of trace heavy metal analysis in the past century is attempted. Increasing awareness of the effect of metals (e.g. through toxicological studies) and the resulting development of sensitive instrumental methods have led to better control and international legislation. The analysis of solid environmental matrices, which is the focus of this work, entails some form of sample preparation that makes the sample amenable to instrumental end analysis. Indeed the rapid developments in instrumentation for trace analysis have not been augmented by the slow pace of developments in this field. An overview of latest trends in sample preparation methods is also presented.

1.1 Occurrence, usage, impact

Heavy metals occur naturally in the earth's crust in trace quantities (below 1000 mg/kg) and generally have a density greater than 5g/cm^3. Some are essential trace and micronutrients for micro organisms, plants and animals (chromium, copper, iron and selenium, as metalloproteins in enzymes, etc.) while others are toxic even in trace quantities, entering biological systems mainly by ingestion (in particulate, solid form or wet deposition) or inhalation (as gaseous vapour).

Anthropogenic (artificial) sources account for most of the pollution that has occurred to date, mainly through metallurgy (mining, smelting, refining), energy production (reactors, battery production, and power plants), waste incineration and other industrial wastes (waste water, fly ash, *flue gas*, automobile exhaust). The emitted metals, in gaseous (aerosol), particulate, aqueous or solid forms are deposited onto plants, soil, or transported into the ground water and other environmental compartments.

Since the publication of the book Silent Spring [18], focus and attention has been given to the impact of human activity on the environment. However it was the events at Minamata Bay (methyl mercury poisoning) and the Cd poisoning, with the accompanying Itai-itai (meaning *pain-pain*) disease that focused attention on the adverse effects and bioaccumulation problems of trace toxic metals. The Itai-itai disease came about as a result of irrigation of rice fields with waste effluents from a nearby factory containing CdS. At the beginning, no adverse effects were apparent, but as the fields were drained ready for harvesting, the Cd was converted to bioavailable forms, taken up by the plants and the cause of much misery to the population. The mining waste incident in Donana, Spain [19] and the heavy metal spill in Romania [20] are recent reminders of the devastating effects of metal pollution. The

main pathways of pollution to humans are through inhalation (atmospheric pollution), ingestion (water, soil, plant, food pollution) and to a lesser degree dermal contact (e.g. occupational pollution). Thus pollution control involves the monitoring of metals in air, soil and water environments, with special attention to possible sources of entry of pollutants into the food chain. Soil is a particularly important source as well as a sink for heavy metal pollution. Major sources of heavy metals to soil include discarded manufactured goods in scrap yards and landfills, sewage sludge application, and the agricultural use of pesticides and fertilisers.

Long and short term risk assessments thus require the study of transport mechanisms, fate (metal binding and interactions) as well as the (eco) toxicological effects of heavy metals in the environment. More often than not, the toxicity depends on the concentrations at which the metal occurs. Thus a metal may be essential at a low level, but toxic at higher concentrations.

The analysis of food related, soil and other samples for essential and trace metals has been routinely carried out for decades and a plethora of methods exist. International standard methods [21], reference methods [22] and national regulatory methods [23] have been established. These methods are all based on total content determination. Further research soon showed that the behaviour and effects of metals in the environment depend to a larger extent on the species in which they occur. Accordingly, methods which discriminate between inorganic/organic forms, between oxidation states such as Fe(II)/Fe(III), Cr(III)/Cr(VI) have been described [e.g. 24]. Also, methods exploiting the physico-chemical differences of metal forms were developed, e.g. between soluble forms, volatile components e.g. metal hydrides and organometals. Analysis of arsenicals, selenium and mercury compounds was also based on the different reactivities and toxicity of the different binding forms of these metals in environmental matrices.

Further studies on metal behaviour at the molecular level, at interfaces between various environmental compartments are therefore still required.

1.1.1 Need for measurement, control and legislation

The adverse effects of pollutants on organisms have most often been recognised through their lethal impact. Heavy metals are especially of interest because they are persistent, can be accumulated and bio-transformed. Prevention of pollution thus involves the control of industrial production processes, the main source of heavy metals, through legislation and monitoring. The United States Environmental Protection Agency (US EPA) included 13 metals on their priority pollutant list viz. Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl and Zn. Ground water serves as a direct source of potable and irrigation water. The quality is therefore an important requirement to prevent contamination to aquatic and terrestrial food chains and upkeep human health.

The WHO health based guideline limits for the concentration of metals in drinking water compiled from [25] are given in Table 1.1.

Table 1.1: Guidelines and maximum permitted levels of heavy metals in drinking water

Element	Limit (mg/l)	Remark
Aluminium	No limit set	[a]
Antimony	0.02	
Arsenic	0.01 (P)	
Cadmium	0.003	
Chromium	0.05 (P)	For total chromium
Copper	2	Staining of laundry and sanitary ware may occur below guideline value
Iron	No limit set	Not of health concern at concentrations normally observed in drinking-water, and taste and appearance of water are affected below the health-based value
Lead	0.01	
Manganese	0.4 (C)	
Mercury	0.001	For total mercury (inorganic plus organic)
Molybdenum	0.07	
Nickel	0.02 (P)	
Uranium	0.015 (P, T)	Only chemical aspects of uranium addressed
Zinc	No limit set	Not of health concern at concentrations normally observed in drinking-water, May affect acceptability of drinking-water

P = provisional guideline value, as there is evidence of a hazard, but the available information on health effects is limited;

T = provisional guideline value because calculated guideline value is below the level that can be achieved through practical treatment methods, source protection, etc.;

C = concentrations of the substance at or below the health-based guideline value may affect the appearance, taste or odour of the water, leading to consumer complaints.

[a] Owing to limitations in the animal data as a model for humans and the uncertainty surrounding the human data, a health-based guideline value cannot be derived; however, practicable levels based on optimization of the coagulation process in drinking-water plants using aluminium-based coagulants are derived: 0.1 mg/litre or less in large water treatment facilities, and 0.2 mg/litre or less in small facilities

Few legislative directives however address the concentration limits in terms of metal forms. This should improve when simple and reliable analytical methods suitable for routine control analysis and the appropriate data for legislation become abundant. The EU through its Speciation 21 network (1998-2000) provided a forum for the cooperation of legislative bodies with researchers and industrial workers aimed at improvement of written standards and EC regulations. Recommendations of the penultimate meeting [26] are summarised as follows: There was a general demand for the

Rapid, simple, robust, inexpensive methods for species analysis, allowing regular monitoring

Further development of coupled techniques and in-situ methods especially for solids and

Provision of certified reference materials

Current international and governmental activities (in terms of legislation, promotion of scientific research, waste management etc.) aimed at making the environment safer can be obtained from United Nations Environmental Programme, UNEP (www.unep.org), United States Environmental Protection Agency, USEPA (www.epa.gov.org), European Commission, EC (www.europa.eu.int) and German Environmental Protection Office, (Umweltbundesamt ,UBA) (www.uba.de) websites.

1.1.2 Excursus: Bioavailability and Bioaccessibility

In order to relate the measured concentration of contaminant in the outer environment to that portion which is relevant to biological systems (i.e. biologically available and picked up in food chains), the terms bioavailability and lately bioaccessibility have been introduced.

Bioavailability is traditionally defined in ecotoxicological terms as the part of a contaminant pool available for uptake by organisms from the environment. Toxicologically however, bioavailability is defined as that portion of absorbed chemical which reaches the target organ. Nonetheless, the bioavailable amount of any substance is almost always less than the total measured amount in the environment. An exception would be the bioaccumulation of substances in organisms.

The concept of bioavailability has in recent times been expanded to include the availability of metals to tissues within organisms once inside the organisms [27]. In order to describe the potential toxicity of contaminants exclusively in humans on ingestion, the term bioaccessibility has been introduced. This has led to some confusion amongst the various fields.

In their paper, Ruby et al. [28] defined the absolute bioavailability as that fraction of ingested element that is absorbed into systemic circulation. The term “relative bioavailability” was used to describe the bioavailability of the element(s) in solid matrix e.g. mine waste or soil relative to that dissolved in water. Finally, the term “bioaccessibility” was used to define the fraction of total element that dissolves in the stomach and is available for absorption during transit through the small intestine.

Some compounds are so tightly integrated into the soil that they may stay bound and be excreted with the soil, never entering into the circulatory system where they could cause harmful effects. This pool would, according to toxicologists, fall into the bioaccessible fraction. The fraction of bioavailable element will therefore be less than the bioaccessible fraction due to incomplete uptake of solubilized form in the small intestine.

Kramer and Ryan [29] also used the term *bioaccessibility* to define the maximum fraction of contaminants in soil systems that can be recovered from a model of the physiological composition and digestion conditions of the human digestive tract. This view was also held by Chu and Beauchemin [30].

The term oral bioavailability has been used to describe both the bioaccessible fraction determined by physiologically based extraction technique (PBET) and in vivo method measuring metal levels in the major organs and urine, faeces, and blood measured in Sprague Dawley rats [31]. In contrast, Oomen et al. [32], believe oral bioavailability can be seen as the result of four steps: (1) soil ingestion; (2) mobilization from soil during digestion, i.e., bioaccessibility; (3) transport across the intestinal epithelium; and (4) first-pass effect.

The foregoing discussion would imply that the correct designation of mobile fraction in SES and other extraction schemes used to date by environmentalists is actually the (bio) accessible and not the bioavailable fraction [33]. However an opposite light is shed in the dark tunnel with the treatise by

Peijnenburg et al. [34], who define the bioaccessible fraction as a part of the entire bioavailable portion.

Prediction of metal behaviour in soil

Several models for the sorption of metals based on the classical Langmuir or adsorption isotherms fail to describe the situation involving complex matrices like soil. Chemical extraction (fractionation) methods show that metals exhibit different behaviour depending mostly on the soil characteristics and composition. The availability of metals for biota depends mostly on the pH but is also influenced by redox potential Eh, magnitude and kind of organic matter (OM) present, cation exchange capacity CEC, soil texture and other factors [35]. The well accepted view that heavy metals are released favourably at lower acidic pH conditions does not hold in cases where the soil is highly buffered e.g. high carbonate content. Solubility of metals could also increase at higher pH due to the binding to dissolved organic matter (DOM) which dissolves at higher pH. Predicting the availability of oxyanions like arsenate or phosphate is even more difficult. Generally, sorption of arsenate/arsenite decreases with increasing pH [36]. However, certain components of SOM (such as fulvic acid) tend to complex As, thereby making it more soluble and hence bioavailable [37]. On the other hand, humic acids can contribute more to the retention of As in acidic environments than do clays and some metal oxides, thereby lowering its ultimate bioavailability.

In conclusion, the bioavailability and mobility of heavy metals in the environment depend on the species in which they occur. This on the other hand depends on several factors like pH, carbonate content, natural organic matter, morphology of matrix- particle adsorption etc. This implies that no single universal method has been evolved to predict accurately the bioavailability of metals in all types of soil or sediment matrices talk less of the generality of solid environmental matrices. The proper analysis of raw data on pollutant concentrations must take into account the varying availabilities of the different pollutants and the potential variation of the bioavailable amounts with space and time for correct inferences to be made and legislative steps taken. It remains, in my view, to continue to foster methods that allow the rapid screening of the multitude of samples on an individual basis like the method developed in this thesis for metal fractionation.

1.2 Determination of metals

The establishment of base levels of metals in various environmental compartments is necessary in order to recognise the occurrence of contamination and pollution. The occurrence, impact and control of heavy metals in the environment was treated in the last subchapter. The common methods of determination are discussed here. Solid samples pose an additional challenge for trace metal analysis since most methods require the sample in liquid form. A brief discussion of sample preparation techniques commonly employed and improvements to date is also provided.

1.2.1 Instrumentation for trace metal analysis

Traditionally, development in trace analysis has been aimed at the quantitative recovery or isolation of analytes (sample preparation) and highly sensitive determination of the total amount of the element in the sample (instrumental detection limits).

The most commonly used atomic spectrometric methods are flame atomic absorption spectrometry (FAAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). FAAS is the most common technique since it is relatively cheap and sensitivity can be enhanced by on-line coupling of a pre-concentration step as described in many FI-FAAS analytical methods [e.g.38, 39]. The versatility of flow injection based methods in metal speciation is discussed in Chapter two (section 2.1.2.1 Flow methods for metal speciation). Plasma source atomic spectrometry is gaining ground as the most sensitive and multi-elemental detector [40]. The argon based plasma is amenable to aqueous aerosol as well as continuously flowing liquid sample introduction. Highly organic content is however not well tolerated by the plasma.

Direct solid sample introduction requires additional laser ablation, electrothermal vaporisation, slurry or other solid sampling techniques. An overview of the use of lasers in atomic spectroscopy including laser ablation, the use of electro-thermal vaporisation (ETV) techniques for the sampling of solids and introduction of the vapour into the plasma directly as well as direct solid insertion techniques is given in many books and reviews[e.g. 41, 42, 43, 44].

While aqueous samples do not pose much problem, the analysis of solid samples remains difficult in several ways:

- The analyte must be brought in a form appropriate for end detection i.e. liquid form. This usually calls for a dissolution and isolation step
- The preservation of sample integrity must be weighed against sample homogeneity and

- Matrix interference (complex matrices) must be removed by suitable sample treatment where selective methods of analysis are not available.

Classical metal analysis, depending on the method chosen, therefore involves some form of sample collection, handling (cleaning, drying, homogenisation), preparation (matrix decomposition, enrichment/ preconcentration /derivatisation and cleanup) before instrumental end determination and subsequent data evaluation. The determination of total metal burden gives an indication of the immediate risk of pollution. Of all the analytical steps leading to end determination, sampling and sample preparation are critical factors for correct assessments to be made.

1.2.2 Sample preparation

Few instrumental methods allow the direct analysis of solid samples, but these are quite expensive and not readily available in routine laboratories. The goals of sample preparation are thus to isolate the analytes of interest and to improve the selectivity, detectability, reliability, accuracy and repeatability of the analysis.

Isolation may involve the destruction of sample matrix, although non-destructive methods are being preferred. Commonly used isolation techniques in analysis of trace metals in environmental samples range from total digestion, i.e. the decomposition of organic matrix and/or solubilisation of inorganic components [45], partial digestion involving the extraction or leaching of analytes from the sample matrix using dilute acids [46] to non-destructive chelation [47] and derivatisation [48] methods. Besides analyte extraction, sample preparation often includes cleaning procedures for complex high matrix loaded samples. Frequently, pre-concentration of the analytes is also required, to a level that can be determined by the analytical method.

Risks of sample loss and contamination are major problems in multi-step sample pre-treatment methods.

1.2.2.1 Sample collection and handling

The way a sample is handled depends on its physico-chemical properties, the aim of analysis and chosen method of determination. Sampling should be representative of the original natural condition, although this is a problem with biomatrices, where the presence of hot particles must be considered [49]. Furthermore, the question of sample representativeness is probably interesting in a highly heterogeneous substrate like a refuse depot for instance, where there is a large variety of solid matter to be sampled. However, there are cases where the local distribution of the analytes is of primary interest, for instance in the spatial distribution of metals in a roadside area, the distribution of elements in the various organs of a biomonitor organism. Also, where a study of the distribution of analyte in the various parts of a population (e.g. leaves, root, stems) is the focus of analysis, the heterogeneity of

the sample must be upheld. Otherwise, the reproducibility of analysis can be enhanced by improving the homogeneity of the samples used.

Homogeneity is usually effected by particle size reduction (grinding, milling) and mixing. This process however is known to alter the distribution of chemical species in the sample matrix and expose areas of the matrix to contact with reagents, which would otherwise have been non-accessible. Further, in the collection of biological samples, adequate attention must be given to the effects of physical parameters of the individuals, climatic and other external factors as well as stress on sampling.

Thus the sampling strategy chosen would determine to a great extent whether the analysis is to be successful or not.

1.2.2.2 Sample digestion

Sample digestion procedures serve to bring the analytes into a form compatible with the analytical detection method. Since the metals of interest are usually present in trace quantities in an organic or inorganic matrix, care must be taken to completely solubilise and release the analytes of interest from the matrix. Thus appropriate sample digestion is a crucial prerequisite to accurate determination. The type of digestion procedure chosen would depend on the element to be analysed, its level in the matrix, type of matrix and the analytical method to be used.

Common wet digestion procedures involve the use of some oxidising acid (or mixtures thereof) like concentrated nitric acid, sulphuric acid, aqua regia, hydrogen peroxide, perchloric or even hydrofluoric acid in a suitable vessel with heating possibly under pressure and/or some form of extraneous energy (ultrasonic, microwave). Depending on the technique, 0.1 to 1g of homogeneous sample is digested using either single or a mixture of acids. Total digestion thus defines the total burden of pollutants, while the DIN S7 [50] method is considered to define the mobilisable fraction under oxidising conditions. Disadvantages of these methods include the hazards of the use of corrosive acids and noxious fumes produced. The question of disposal of reagent wastes must also be considered.

Dry ashing and fusion techniques have been less widely accepted in trace analysis than closed vessel techniques, due to problems of contamination, loss of analytes due to volatilisation or retention on vessel surface [51].

These classical digestion methods generally require a great deal of operator attention, skill and experience in order to gain accurate and precise results. As a consequence, sample preparation is often regarded as the weak link in sample analysis, and an area which provides much scope for development. The following describe the main developments in sample dissolution techniques used to date.

Microwave digestion

Microwave assisted digestion since its introduction in the late 70’s has revolutionised wet digestion techniques. Microwaves generated from a magnetron cause the rearrangement of molecules in dipoles

and as a result of frictional forces generated, heat is produced. Since the first work on the application of microwave energy in analytical chemistry [52], a wide field of application has evolved. The closed digestion technique involves placing the sample in a vial (or bomb), usually constructed of a fluorinated polymer, such as polytetrafluoroethylene (PTFE) or perfluoroalkoxy (PFA). After adding the digestion reagents, the bomb is tightly sealed and placed in the microwave oven for irradiation by microwave energy. During heating, pressure is built up due to the evaporation of digestion acids and the gases evolved during the decomposition of the sample matrix. This is beneficial by increasing the boiling-point of the reagents, which aids the breakdown of the sample matrix. However, the excessive build-up of pressure, especially during the digestion of samples with high organic content may cause rupture of sample vessel and subsequent loss of analyte. Ways of avoiding over-pressures include pre-digestion of samples, for example overnight at room temperature, which may be inconvenient where large numbers of samples are to be analysed. Also pressure relief valves on the digestion bomb are designed to open when the pressure becomes too high, thus maintaining safety. But here, sample losses are likely and owing to the reduction in acid vapours, the digestion may be incomplete.

Initial closed vessel domestic multimode microwave ovens have since been replaced by commercial laboratory units offering more efficient use of power, safety programming and control of the digestion by real time monitoring pressure and temperature

MW technique offers a universal method of digestion, extraction of compounds from a wide variety of environmental samples with the advantage of quick and controlled heating which greatly reduces the analysis time. However, solvents used (or sample) must be dielectric, the vessels used transparent to microwaves and heat and pressure resistant. The PTFE materials that have been employed cannot be heated to higher temperatures like the classical Tölg bombs and as such the recovery of some refractory metals in plant, soil and other complex matrices is somewhat reduced.

Reagents used have ranged from nitric acid, sulphuric acid to combinations of nitric, hydrochloric and hydrofluoric acids for the determination of heavy metals in biological samples.

1.2.3 Extraction methods for pseudo-total metal content determination

1.2.3.1 Developments in batch extractions

A look through the literature shows a general trend towards environmentally friendlier extraction techniques as opposed to the classical wet digestions utilising corrosive concentrated acids. Extraction involves the dissolution/partition of analytes from a solid sample into a fluid (extractant) which had been in direct contact (usually till attainment of equilibrium state) with the solid matrix. Enhancement of mass transfer is achieved through optimisation of sample size (reduction of particle size increases

the surface layer in contact with solvent), sample to liquid ratio, extraction time, agitation e.g. by shaking in batch (static) extractions [53].

Extraction methods have been classically carried out in the batch (static) mode, however the methods are time, reagent and sample consuming, requiring several extraction steps to achieve quantitative recovery of metals. Further, problems of loss of analyte or contamination through preparation steps could not be prevented.

Developments in the form of ultrasonic assisted and microwave assisted extraction evolved in the 1980's with a plethora of methods for the determination of metals in plant and agricultural produce and other environmental matrices [e.g. 10,54] in the literature.

1.2.3.2 Ultrasonic assisted extraction (USE)

Ultrasonic waves (acoustic vibrations with frequencies above 20 kHz) applied to the sample induce cavitation or microbubbles, which on collapse result in localised heat eruption and high pressure thereby opening the solid particle structure for solvent access. Moreover, shock waves are generated, which enhance the removal of analytes from the matrix surface. The capability of sonication lies in the intensity per volume and duration of application rather than the maximum power of the instrument in use. Although the usual ultrasonic baths take a larger number of samples simultaneously, the sonic probe has the advantage of focused waves, which makes it more effective. Ultrasound has proved to be an effective auxiliary energy for accelerating various steps of the analytical process, like slurry dispersion [55] and homogenisation [56]. Other analytical applications include the assistance of ultrasound in homogeneous systems, such as typical complex formation [57].

USE has been used for the extraction of metals (Cu, Cd, Zn, Al, Fe) from plants and soil [e.g. 58,59, 60]. The efficacy of USE for the multi element determinations by atomic spectrometry in environmental and industrial hygiene related samples has been demonstrated. Various dilute acid protocols with and without heating were applied to a wide range of standard reference materials (SRMs) as sample matrix. However, not all metals could be extracted quantitatively with these methods [61].

1.2.3.3 Microwave assisted extraction (MAE)

Microwave assisted extraction (using for instance dilute nitric acid or a combination of mineral acids) has been widely used for the determination of metals in a wide variety of biological and environmental matrices – fish [62], dust [63], soil [64], sewage sludge [65] and plant [66] and several reviews exist [67, 68,69]. The main advantages of MAE are the fast rate of extraction due to the quick heating and elevated temperatures, but also the ease of instrument operation and automation potential. In

metallurgy, MW has also found application in the enhancement of extraction of metals from ores, removal of metallic impurities etc [70]. Almost all MAE applications involve off-line procedures. Only a few approaches involving an on-line system have been published [71] and these are discussed below (see dynamic leaching).

1.2.3.4 Other methods

Other newer techniques in extraction include dynamic methods like the pressurised liquid extraction (PLE) and supercritical fluid extraction (SFE). Although used mainly for the extraction of organic pollutants, some applications of these techniques for heavy metals exist. An SFE method for the sequential extraction of heavy metals from sediment samples [72] and a pressure assisted chelating extraction (PACE) for the digestion of metals from solid matrices [47] are worth mentioning. The latter method involved the extraction of metals with EDTA under similar conditions and with the use of equipment for PLE. Advantages of the method include the speed (minutes) and the non destructive nature of the method.

Another approach to extraction is the enzymatic hydrolysis in which certain protein or lipids of the sample are hydrolysed using a particular enzyme or group of enzymes. Metals released from the break up of these macro molecules are then determined after appropriate separation, usually centrifugation.

The most commonly used enzymes to hydrolyse proteins are trypsin and pronase [73, 74]. Since the enzymes act only on certain chemical bonds, information as to the different bonding in the sample matrix may be obtained. Other advantages of the method are the avoidance of corrosive acids and mild extraction conditions (sample integrity, less risk of loss of volatile analytes). Although the hydrolysis usually take hours, considerable time saving (minutes) can be achieved in combination with ultrasound energy.

An innovative use of an “accelerated dialysis” method for the determination of persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) is worth mentioning [75]. Samples were held in a semi-permeable membrane supported by a mesh using commercial ASE equipment for regulating high pressure and temperature requirements. The method reportedly offered high recovery of analytes at much shorter time (40 min compared to 48 h of conventional dialysis).

2 Metal speciation and fractionation

Terminology

Terms used in the literature include chemical and physical speciation, speciation analysis, operational speciation, fractionation and speciation.
The IUPAC committee [17] has offered definitions to clarify the situation.
Chemical species pertain to a specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.
Speciation analysis refers to the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
Speciation of an element; speciation is the distribution of defined chemical species of an element in a system.
Fractionation on the other hand refers to the process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

The changes that occur for instance between the leaching of trace elements from soil or rock and their subsequent distribution in the aquatic environment would be better described as “species transformation”. For the purposes of clarity and simplicity, the term speciation is used in the thesis to mean the identification and quantification of chemical forms of an element while fractionation refers to the quantification of binding forms extractable under predefined conditions.

2.1 Speciation analysis

Speciation analysis is seen under two aspects- (i) the speciation of metals and metalloids in largely aqueous medium and (ii) the speciation of elements and bio-inorganic molecules in biological matrices. While the elemental detection method may be similar for both, the sample handling and analyte separation differs. These are discussed briefly below.

2.1.1 Sample handling, storage and preparation in speciation studies

In speciation, the aim is to isolate and quantify the species of interest in their native forms, without the risk of loss or inter-conversion. This is a challenge especially where labile species are to be analysed in complex matrices such as biological samples. Sample preparation in speciation analysis is mostly

done on-line, i.e. hyphenated to the detection instrument. Das et al. [76] provide a review of flow methods used in the preparation of aqueous samples for elemental speciation. Flow methods are treated briefly in section 2.1.2.1.

For speciation involving bio-molecules, separation may include membrane techniques, some form of chromatography or electrophoresis. The basics of sampling and sample preparation techniques for air particulates, water, soil, sediment and other environmental samples is treated in several book chapters [77,78,79] and updates found in periodical reviews of the journal of analytical atomic spectrometry.

2.1.2 Instrumentation

Instrumentation used for speciation are at most adaptations of those commonly used in trace analysis. The last decade has seen the development of an auto-speciation analyser based on multicapillary GC separation, microwave induced plasma and plasma emission detector [80].

A look through the literature shows that an overwhelming proportion of published work in the early 1980's dealt with the speciation of metals in liquid samples, mainly water.

In the early 1950s, aquatic geochemists first made the distinction between 'dissolved metal' and 'particulate metal' fractions. A simple filtering membrane of 0.45µm and a clean filtration unit allowed discrimination between the two different phases.

Electrochemical speciation methods starting from the pioneering work of Florence [81] and Florence and Batley [82] allowed the discrimination of free metal ions as opposed to colloidal or particulate bound metal. Using preconcentration with Chelex 100 ion-exchange resin and final detection by voltammetry or atomic absorption, they carefully differentiated between different metal fractions in water. The nature of metal binding in these media was also investigated either using the ligand competition methods [83] or through pseudo-polarography [84]. Electrochemical methods are versatile, allowing the selective and sensitive detection of many metals, without the need for extensive sample preparation. A recent review of application of voltammetry to speciation, not only in water but other environmental matrices is given in [85]. Drawbacks of the method include the fouling of electrode membranes, handling problems with mercury (still the dominant electrode material in voltammetric measurements) and lack of identification of the binding partners.

Spectrometric methods soon gained ground, offering the advantages of on-line coupling of the sample preparation techniques (ranging from derivatisation, separation, extraction to preconcentration and cleanup) to the non-selective yet sensitive end instrument.

The first instrumental coupling between a gas chromatograph and a flame atomic absorption spectrometer was achieved by Kolb et al. to determine alkyllead compounds in gasoline [86].

The choice of separation method depends on the nature and concentration of the analyte species, sample quantity and the required resolution. Gas chromatography, high pressure liquid

chromatography and electrophoresis (capillary electrophoresis) have been widely used. Atomic spectrometric methods are the commonly used detectors since they are easy to couple with the separation step and have sufficiently low detection limits. For instance, Günther et al. [87] used size exclusion chromatography followed by atomic spectrometry of the fractions for the determination of metallothioneins in vegetable samples. Such hyphenated methods for bioinorganic speciation have since become routine. A full report of methods in the literature is not intended here; more comprehensive reviews are available in the literature [88, 89].

For successful speciation analysis involving chromatographic separation, the species must be sufficiently thermodynamically stable and kinetically inert to avoid conversion, destruction or artefact species formation on the column. As calibration standards are unavailable for most of trace element species in living organisms, the use of molecule specific (and not element-selective) detection technique, such as ESI-MS or NMR is necessary to complete the characterization of the species detected by such hyphenated systems. Further advances in the direction of speciation of metals associated with proteins and other macromolecules in biological systems (metallomics) have seen the hybridisation/merging of separation technique, purely elemental selective instrumentation with structure identification using classical mass spectroscopy. This provides a powerful means of obtaining highly resolved information needed to distinguish between the numerous analytes that are locally present in a sample. Gomez-Ariza et al. [90] provide an overview of recent developments in techniques for metal speciation in metallomics.

Speciation analysis techniques therefore have the drawbacks of the tedious and lengthy separation processes which may not preserve the integrity of the species, (leading to conversion and /or loss of more volatile constituents) and expensive instrumentation.

The direct identification and quantification of species in the solid sample without need for matrix separation would be ideal. Direct analysis in solids has been achieved by surface analysis method using microscopy combined with x-ray techniques e.g. the use of scanning electron microscopy combined with X-ray emission (SEM-EDX) and the use of synchrotron radiation with a diversity of X-ray methods like X-ray absorption spectrometry (XAS, XAFS, XANES) and X-ray diffraction methods (XRD). This relatively new field gives information as to the elemental composition, chemical bonding, structure and electronic and magnetic properties of heterogeneous systems, thus combining sensitive elemental analysis with elemental speciation information. However, the instrumentation involved is complex and expensive [91]. Also problems of quantification like limited sensitivity and lack of suitable standards persist.

2.1.2.1 Flow methods for metal speciation

FIA since its introduction by Ruzicka in 1975 [92] as a liquid handling technique, has developed into a versatile means of sample preparation with the aim of enhancement of sensitivity and selectivity by means of incorporated derivatisation, preconcentration, extraction or separation modules. Furthermore it serves as a means of coupling the analytical steps to instrumental detection for on-line, in-line and at-line process control and environmental monitoring assays. The application of flow injection analysis in the determination of metals and especially metal speciation in various aqueous environmental matrices has been widely described. Several books [93, 94, 95], monograph [96] and reviews [73, 97, 98, 99, 100] on this subject abound in literature. Advantages offered include ease of manipulations, automation potential, high sample throughput, low reagent and sample consumption, high precision and less risk of contamination or loss of analyte. An in-depth discussion will not be made here. Rather, a few exemplary works serve to illustrate the potentials of this technique.

FIA preconcentration methods usually involve use of mini columns containing suitable chelating resin or other solid sorbents [101, 102, 103]. For instance, a microcolumn field sampling (MFS) technique was proposed in which water samples are processed in flow systems at the sampling site and trace elements of interest are immobilized on minicolumns. The minicolumns are then returned to the laboratory and directly inserted into a FI system for on-line elution and quantitative analysis [104]. Recently, the use of a tubular reactor was described for the on-line pre-concentration of iron where the iron species were adsorbed directly to the walls [105].

Membrane separation techniques include the on-line dialysis of chloride ions prior to potentiometric detection [106], use of gas diffusion unit to selectively analyse gaseous components like NH_3 [107] for ammonium determination.

Metal speciation in solid matrices is even more complicated. Solid environmental matrices tend to be more complex than aqueous or gaseous (aerosols). Thus stringent sample manipulations are required, with the risk of errors. Due to the extreme low level of species in most samples, the instrument must be highly sensitive and a preconcentration step may be unavoidable.

Latest trends in application of FI in the analysis of solid matrices has seen the development of techniques for the direct insertion of solid sample into flowing systems coupled with pre-treatment like microwave digestion, ultrasonic assisted extraction as discussed under Dynamic leaching (see section 2.2.4)

Conclusion

The present day status of elemental speciation involving small mass bio-inorganic compounds is largely based on the use of a combination of various types of chromatography with inductively coupled plasma mass spectrometry and routine methodology exist.

A new field of interest is the identification and quantification of metals associated with large bio-molecules like proteins and DNA, so called metallomics. The analytical approach of metallomics i.e. the identification and quantification of metalloproteins and other metallomacromolecules present in life systems consists of (i) a separation technique (selectivity component) (ii) an element-high sensitivity detector (sensitivity component) and (iii) a molecule-specific detector, usually mass spectrometry (structural component) [108].

Since the determination of a particular element species is limited to those compounds that have been clearly identified and are sufficiently stable to be extracted from the solid material without risk of conversion, fractionation is more widely employed in environmental studies. A detailed overview of this method is given in the next subchapter.

2.2 Fractionation

Fractionation is usually carried out by extraction or leaching of the analyte group from the solid sample matrix. This takes the form of single or multistage extractions using carefully chosen reagents. Multistage extractions can be in the parallel or sequential mode, the difference being that in the former a sample aliquot per reagent is extracted while in the latter, the same sample is treated with various reagents one after the other.

The idea of fractionation evolved from the need to characterize sediments and soils according to metal burden for purposes of aquatic environmental impact but has since found applications in questions of mobility and bioavailability of trace metal in human toxicology, remediation of land, groundwater pollution, risk associated with landfill leachates etc. What makes fractionation versatile is that a wise choice of reagents in a definite order (so called extraction scheme) allows simulation of natural processes (e.g. acid rain) and therefore short and long term risk assessment. More complex simulations involve e.g. effects of fatty acids, humic and amino acids on mobility of such pollutants, assessment of human toxicological potential of sedimentary dust using epithelial fluid and availability to plants using root exudates.

In order to evaluate risk due to mobilisation of pollutants in the digestive tract, synthetic digestive fluids have been employed under close pH monitoring [109].

2.2.1 Effects of sample handling, storage and sample preparation on fractionation

The basic steps in sample handling can be divided into washing, drying and grinding. While these steps may not be detrimental in total content determinations, speciation analysis and chemical

fractionation can be badly affected by the procedures used in sample pre-treatment. A treatise on sampling and sample pre-treatment of plant material is provided by Mackert [110].

Poor sample storage is known to affect the total content of trace metals in biological samples. In order to stem microbiological activity (degradation), the samples are usually frozen till required or the moisture content reduced by drying. Thawing however may alter the speciation of metals. Addition of preservatives, coagulants and even column resin or eluent used in pre-concentration may cause changes in speciation if these substances are complexing agents or their presence changes the pH, ionic strength, redox potential and dielectric constant of the medium.

A wealth of information exists on the effect of sample preparation on fractionation results. For instance, the effect of drying of soil and sediment [111, 112, 113, 114], freeze drying of sediment [115], sieving and grinding (particle size) of soils [[116] on the speciation and distribution of metals in pre-defined fractions have been investigated. In summary, the literature shows that none of the studied pre-treatments appeared to preserve the original metal distribution. Further, not all metals are equally affected by the pre-treatment. Davidson et al. [114] investigated the effect of sample pre-treatment on the extraction of Cd and Pb using the Bureau Community of Reference (BCR) protocol on sediment prepared and stored in different ways. Cadmium speciation was particularly affected by freezing, whilst lead was more affected by oven-drying.

The conclusion can be drawn that the use of wet, fresh sediment samples is recommended for samples that are to be analysed for metal distribution.

In the dilute acid extraction of metals from biological matrices e.g. plant matter; it has been observed that few metals like Al and Pb behave differently under the extraction conditions, aluminium requiring more stringent conditions for quantitative recovery than say Zn and Cu.

It is therefore of interest to see how the sample pre-treatment affects the extractability of these metals in complex biomatrices like plants.

In this work, the effects of air drying vs oven drying; homogenised, milled samples vs coarsely cut up samples, on extraction yield, extraction profiles and total content were investigated.

2.2.2 Extraction theory

An understanding of the processes occurring and the mechanisms involved in extraction is necessary for the optimisation of extraction and estimation of analyte (bio)availability. Industrial processing based on extraction also requires the information about mechanisms to be cost effective. In environmental context, it leads to better assessment and control of pollution, since the interactions at the molecular level can be predicted.

The fractionation of native indigenous metal species is known to differ from anthropogenic or laboratory spiked forms indicating that the mechanisms of sorption and subsequent desorption are different.

There is a wealth of information available in the literature on the mathematical modelling of sorption and extraction processes, but this approach is outside the scope of this work. The following describe the probable mechanisms occurring in batch and continuous flow extraction of solid environmental samples as relevant to the present thesis.

Solid particle model [117]

Assume we have a heterogeneous porous solid matrix made of different phases- organic, silicate carbonate etc, which is insoluble or at most sparingly soluble in the extraction reagent.

Analytes are incorporated by adsorption, ion-exchange, complexation or occluded within the inorganic matrix /backbone.

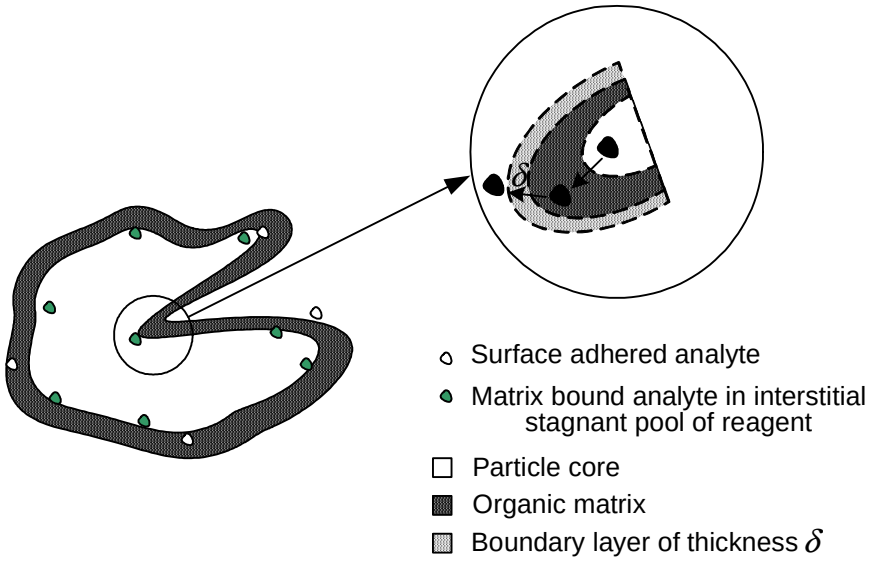


Fig. 2-1 Schematic illustration of heterogeneous solid matrix

Suppose that the particle is not totally soluble in the reagent, then in order to be extracted, the analyte must first be desorbed from the sample, diffusion must take place through the insoluble surface layer

(assuming it is organic in nature, marked grey in the above diagram, Fig. 2.1) into the boundary layer or matrix/fluid interface. Here, the now solvated analyte ion must diffuse through the stagnant fluid in the interstitial pore into the bulk of solution.

This implies that the ease with which the analyte is extracted depends on properties of sample matrix viz: porosity, specific area, sample morphology (meaning here grain size, heterogeneity and accessibility of solvent to analyte site or tortuosity) in addition to those of the reagent. Reagent properties like the affinity of analyte/matrix for the solvent (e.g. hydrophilicity or hydrophobicity) and solvation affect the extraction rate.

Water content due to sample preparation also plays a role, since dry samples have to be wetted before the extraction process can commence. Swelling characteristics of sample is also an important factor for consideration especially in flowing systems.

By extension of the above postulation, the fast leaching of metals may be a result of the analyte species being soluble in the reagent or as a result of easy accessibility or reachability i.e. site of adsorption is amenable for fast wash out.

Slow leaching on the other hand may be caused by hinderance of solvent penetration due to an enveloping (in)organic coating or layer or due to tortuosity i.e. large distance from site of analyte to bulk of solution or even a combination of both.

Mechanisms

Extraction of soluble species from solid matrices takes place through four major mechanisms depending on the site at which the analyte is held on to the solid matrix and the type of bonding/interaction.

1) In instant dissolution processes, there are no interactions between the analyte and the solid phase. The concentration of analyte in solid phase is zero. The analyte is contained in the volume of liquid phase around the solid matrix.

2) In desorption process, there are interactions between the solid and the analyte. The equilibrium is determined by the adsorption isotherm of the analyte on the solid in presence of the solvent.

3) A third mechanism is swelling of the solid phase by the solvent accompanied by extraction of the entrapped analyte through the first two mechanisms.

4) In reactive extraction, the insoluble analyte reacts with the solvent and the reaction products are soluble and hence rendered extractable.

Batch static extraction

In classical batch extraction, the mass transport of extracted analyte into the bulk extractant occurs by diffusion. This can be explained by the boundary layer model.

It is assumed that irrespective of the level of agitation, fluid in contact with the solid surface is always stationary, and as the distance from the solid surface increases, fluid movement gradually increases until it corresponds to bulk flow of fluid extractant.

In batch extraction, the solid is in contact with the extractant for long enough period of time that equilibrium conditions are reached. The transfer rate (flux of analyte, i) from the solid matrix could be described by Fick's Laws.

$$j = \frac{J}{A} = -cD\frac{\partial x}{\partial r} = -D\frac{\partial c}{\partial r}$$

Where j is the flux in moles per unit time and unit area, x is the mole, c the molar concentration (moles/volume), r the direction of flow.

Assuming that the concentration changes with time however in leaching processes, the diffusion equation comes into play.

$$\frac{dM}{dt} = \frac{KA(c_s - c)}{\delta}$$

where M is the mass of solute transferred in time t , A is the area of solid-liquid interface, c_s the solute concentration of fluid in equilibrium with the solid, c is the concentration of solute in the bulk solution, δ is the thickness of the liquid film surrounding the particle and K is the diffusion coefficient.

An increase in particle surface area would reduce the resistance and enhance mass transport.

In concentration terms, C=m/V

For batch extraction, where the total volume of solution is taken to be constant,

dM=Vdc, thus

$$\frac{dc}{dt} = \frac{KA(c_s - c)}{\delta V}$$

Using mass transfer coefficient K_m, assuming that δ and A do not change,

$$\frac{dc}{dt} = \frac{K_m(c_s - c)}{V}$$

The thickness of the boundary layer (δ) is determined by the rate of convection (agitation) in the fluid while the transport is affected by the diffusion coefficient of the analyte. Thus, in the same extraction process, the rate of extraction is different for the different analytes in the same sample, under the same extraction and hydrodynamic conditions. Swelling of the matrix and increase in temperature enhance the transfer across the boundary layer. In cases where the transport of analytes in the pores of the

matrix is rate limiting, finely grinding the sample and the use of ultrasonic or microwave energy, which induce convection even in the small dimensions of the pore can enhance the extraction. Also finely grinding the sample exposes areas of the sample matrix which would otherwise be hard to reach, increasing the specific (available, reactive) surface area. It can also be imagined that finely grinding may conversely lead to analyte loss by vaporisation or volatilisation than coarse grinding.

Batch vs. dynamic extraction

In batch system, the solid is in contact with a volume V of extraction solution. Metal release from the solid interspaces into the solution occurs as long as there is a driving force to maintain the distribution equilibrium. An increase in the liquid/solid ratio enhances the extraction efficiency but the overall solution is more dilute. In parallel, constant removal of the metal released in flowing system keeps the concentration in bulk solution low, such that more release is favoured.

In extraction, all processes of dissolution, exchange, release but also re-adsorption and redistribution take place concurrently. The attainment of equilibrium is achieved once in batch extraction after which the extracted substances are separated from the residue. In all, how much of analyte is recovered on equilibrium depends on the affinity of the analyte for the reagent/solubility product.

In flow through extraction on the other hand, the analyte is being removed as it is released, causing a permanent shift in equilibrium. This means that the recovered amount is not limited by the partition coefficient and as such, the solubility product does not play a limiting role. Furthermore, since the contact time is only a fraction of that of batch extractions, re-adsorption should be minimal if not absent.

Flow through extraction

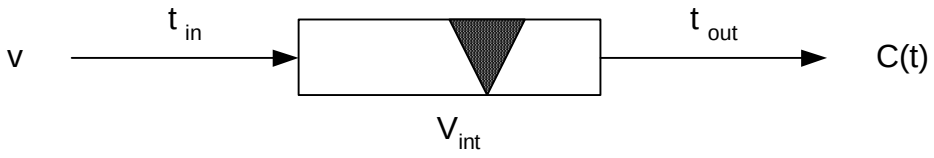


Fig. 2-2: Schematic illustration of flow-through extraction

In the flow system used in this work, with flow rate v (ml/min) time t in sec, the extractant has an initial concentration of C_{in} at time of entering the cartridge t_{in}. An aliquot of the extractant (volume fraction) enters the cartridge and interacts with the solid sample for a period τ. At time $t_{out} = t_{in} + \tau$, the aliquot exits the cartridge and contains the released analyte in concentration $C_{out.}$

According to Ficks law, the rate of extraction is defined as:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c_1}{\partial r^2}$$

In the flowing system, convection in addition to diffusion come into play, thereby increasing the concentration C (t). In this case, the rate of mass transfer depends on the flow rates of extractant and extract (and by extension, time of contact τ) as

$$\frac{dM}{dt} = \frac{dcV}{dt} = v_{in}\ (c,t) - v_{out}\ (c,t)$$

The amount released C after time t also depends on (i) particle size-sample morphology, (ii) diffusion route or accessibility (turtuosity) and (iii) solvent penetration.
These micro-structural effects of the sample matrix result in discrepancies between experimental and theoretical values of the diffusion coefficient.

2.2.3 Extraction methods for metal fractionation- A review

In 1962, Viets [118] proposed a concept of chemical pools in soil to account for the leaching behaviour of the elements studied. Since elements were extracted to different extents under different reagent and procedural conditions, he postulated that these are bound differently. Thus a water soluble pool, ion exchangeable pool, a strongly bound pool extractable by chelating agents, a secondary mineral pool and a primary mineral pool were proposed. This classification was to be broadened by the work of Tessier et al. [119] to describe metal fractions in sediment as discussed later under Sequential Extraction Schemes (SES).

Extraction methods have since been used as a means of assessing metal availability in solid environmental matrices especially for soils and sediments. The first part of this subchapter discusses the development of various extraction and elution protocols that have been used to date. The success of the older single extraction protocols for the assessment of metal fractions available to plants (estimation of related phytotoxic effects and/or nutritional properties) have made it possible to incorporate them as the first step in SESs. Aspects of validation, standardization (e.g. EU standard BCR three stage extraction method [120]) and the limitations of these schemes as applied to environmental analysis are also discussed. The second part focuses on flow through methods and their applicability to on-line extraction and leaching procedures. The prospects and development of a hyphenated (automated) microanalytical system for studying the leaching kinetics of heavy metals in solid samples of environmental importance is finally treated.

2.2.3.1 Single extractions

Single extraction methods were originally developed to provide agriculturalists with a relative empirical method for assessing fertilisation requirements. They were then adapted to estimate the nutrient status of ecosystems, focussing on the availability of micronutrients in soil. For instance, single extractions with unbuffered salt solutions have been widely used for the estimation of trace metals available to plants for root uptake [121].The DIN method (DIN 38406-29) for the analysis of soils recommends the use of $CaCl_2$ (0.0125M) for the determination of exchangeable Mg (plant available Mg and other essential elements) in soil [122,123] (see also DIN 38406-7method for single elements in soil). Since then they have found use in the assessment of soils, sludge and other solid environmental compartments for heavy metal (bio)availability. The evaluation (i.e. study of the mechanisms, factors affecting and absolute quantification) of the availability of heavy metals to plants through root uptake remains a viable research topic.

Metals that are weakly adsorbed onto the soil surface are thought to be particularly mobile and available for plant uptake because they can easily be exchanged by the major cations in the surrounding soil solution. Therefore, single extractants containing Ca or Ba salts are popular

candidates in assessment studies. The performance of single extractants in soil analysis has been the topic of research by many workers and several reviews have been published [53,124,125]. To date, there is no generally accepted method of estimating the bio-availability of heavy metals in soils. A plethora of methods exist depending on the type of matrix to be analysed, selectivity/specificity of reaction with the analyte, end analytical instrumentation and analyst preference to name a few. However, three methods are undergoing standardisation or are standardised in Europe: 0.01 M $CaCl_2$ (The Netherlands), 0.1 M $NaNO_3$ (Switzerland) and 1 M NH_4NO_3 (Germany). In tests using various single extractants, good correlation was observed between $CaCl_2$ extractable metals like Cd, Pb and uptake by plants [126,121]. This is explained by the fact that the divalent cation reportedly causes good coagulation in the suspension [127] and the reagent mimics the natural content of Ca^{2+} ions in soils, compared with exogenous substances like DPTA and EDTA. Synthetic complexing agents such as EDTA and DTPA as their sodium or ammonium salts are widely used as extractants because of their ability to form stable water soluble and well defined complexes with many polyvalent cations. They are used as single reagents in the assessment of plant available metals. EDTA is assumed to extract both carbonate-bound and organically bound fractions of metals in soils with low carbonate content (i.e. non-silicate bound fractions), whereas DTPA buffered with triethanolamine is considered suitable for calcareous soils. In general, higher percentages are extracted with EDTA in comparison with DTPA [128].

Ammonium salts of strong acids, e.g. NH_4NO_3 can lower the pH and promote the hydrolysis of clays, thereby overestimating the exchangeable fraction. This is not so for the unbuffered solutions of inorganic salts like $CaCl_2$, [129] where the pH of the extractant is mainly controlled by the pH of the soil. $NaNO_3$ was thought to be most suitable for predicting the plant (lettuce and rye grass) availability of Cd in soil, although the extraction efficiency was lowest of the reagents studied [130]. The analytical use of $NaNO_3$ as extractant is therefore determined by the detection capability of the instrument.

The suitability of 1 M NH_4OAc at pH 7 as a standard method candidate has been tested [120]. In comparison with EDTA and acetic acid solutions however, the correlation with plant uptake and the inter laboratory reproducibility was poor, indicating that further work is needed to validate the method. NH_4OAc is less suitable for calcareous soils and analytical problems due to the low metal content of extracts and large reagent blanks may arise [131].

Standard elution/extraction methods are available for the assessment of mobility and remobilisation of inorganic pollutants in waste depots, contaminated soils, sediments and other solid matrices of environmental importance. They have also found use in regulatory tests on the weathering stability for building materials, safety of children toys and extraction of essences/natural compounds. The DIN methods S4 [132] and S7 [50], pH_{stat} elution [133] and supercritical CO_2 continuous extraction (Switzerland TVA) are a few established examples.

The DIN S4 method [132] stipulates the overnight shaking of solid sample with water and analysis of eluate for the estimation/simulation of contact water seeping through to the ground water level from sediment and sludge. The use of water as extractant could however be problematic due to its low buffering capacity (the formation of hydroxides and other colloids) and the problem of re-adsorption in which ions released can be held on sites made vacant by the extraction itself leading to an underestimation of the mobile fraction. Also, the usually very low extract concentrations are often below the limits of determination of common atomic spectrometric instruments (like FAAS). Furthermore, in the natural environment, aquatic water is characterised by varying pH, organic matter (e.g. humic, fulvic acids) and salt content, etc. In this sense, the pH-stat method, involving extraction with acidified water at pH 4 would offer more realistic information as to immediate and potential risk. Still, the S4 method provides an initial estimate of the mobile fraction of metals in the solid waste sample.

Limitations of single extractions

Single extraction methods provide a relative empirical assessment of the amounts of heavy metal contamination that may be mobilised at the time of the measurement, or over a longer period of time. However, actual uptake by plants is determined by other factors such as the number of competing ions in soil solution and root uptake processes which vary between plant species. Thus the interpretation of results of a particular study as regards bioavailability is mostly based on statistical (correlation coefficients) relationships which cannot be extended to other soil-plant systems.

Since metal ions in soil and sediments are partitioned between the different phases present i.e. organic matter, oxyhydroxides of iron, aluminium and manganese, silicate minerals carbonates and sulphides, information of the different binding forms can be obtained using a series of reagents aimed at selectively attacking each particular phase.

Furthermore, the uptake of nutrients or pollutants is not only governed by the form in which the element occurs. External influences such as pH change, temperature, redox potential, organic matter decomposition, leaching, ion-exchange processes and microbial activity may change the availability of metal pollutants in the solid matrix. Such changes are more effectively simulated and studied using carefully designed sequential extraction schemes. They offer the advantages of information as to the physico-chemical properties of the sample, give a broader insight into the metal-solid interactions and allow long term risk assessment of the particular contaminant. However more research into the mechanism of root-soil interactions as well as of elements at interfaces is needed.

2.2.3.2 Sequential Extraction Schemes (SES)

The pioneering work of Viets [118] in which soil was classified as being composed of five major fractions or compartments which affect the availability of metals sparked off the idea of sequential

extraction schemes. Starting with the studies of Chester and Hughes in 1967 [134], many fractionation procedures have been proposed since then, most based on the work of Tessier et al. [119] and modifications thereof (e.g. Hall [135] and Förstner groups [136]) and culminating in the one proposed by the Bureau Community of Reference (BCR) now Standards Measurements and Testing programme (SM&T) [120, 137, 138].

SES in principle involves the shaking of a solid with suitable reagent (liquid/solid ratio) for sufficiently long time (typically 3 to 24 hours) to allow the establishment of equilibrium conditions. The liquid phase is thereafter separated from the solid residue by a chosen separation method, usually centrifuge and analysed for total metal content. The solid is washed and either kept for further extractions or analysed for residual content. By thus sequentially breaking down the matrix components, elements associated with each fraction can be separately determined. This gives a picture of the availability of the various metals and their potential toxicity since the reagents are used in the order of increasing aggressiveness. In sequential schemes, the reagents are offered one after the other, to the same sample aliquot, taking care to separate the liquid extract from solid residue after each extraction step. An extra washing step with water or a mild ion-exchange solution may be incorporated to enhance separation and avoid re-adsorption. Repeated extractions have also been used to ensure maximum efficiency, but of course the results are then dependent on the protocol used.

A look through the relevant literature shows that although SES differ in the strength of reagents and other experimental operational parameters like duration, liquid/solid ratio, particle size, operational pH, the same principal features are evident- namely

- the reagents are used in order of increasing aggressiveness so that the metal fractions are in order of decreasing mobility,
- the fractions targeted are usually similar for the same matrix e.g. for soils and sediments, viz. weakly bound ion-exchange, acid soluble fractions, reducible and oxidisable bound and silicate or matrix bound residual fractions.
- The length of a scheme is matrix and analyte dependent, though usually in the order of 3-8 stages. The trend of design however favours simple short schemes that allow routine analysis of large numbers of samples to be accomplished in short time.

An alternative approach to sequential schemes has been the introduction of parallel schemes whereby the same reagents and extraction conditions as the original SES are used but in this case each reagent is used with a fresh sample aliquot. This approach should be easier to implement, taking shorter times (since the extractions can be carried out simultaneously), and reduces the effects of re-adsorption or re-distribution. Therefore emphasis has been placed on obtaining comparable results with the original SES. The drawbacks are that more sample is needed and that sample heterogeneity may introduce errors. Tack and Verloo [139] in their work found good correlation between the two approaches (i.e. parallel and sequential) using the Tessier scheme. Estimates of the acid extractable, reducible and residual fractions from single extractions generally agreed with those determined by sequential

extraction. However, low recovery of organic fraction indicated non-accessibility and thus they recommended that the oxidisable fraction be determined after a prior extraction of the reducible fraction with hydroxylamine hydrochloride. Filgueiras et al. [140], in their comparison of parallel extractions with the conventional BCR scheme, found good agreement with each step except for the oxidisable and residual fractions. This is in agreement with the general recommendation that the reducible fraction be extracted prior to the oxidisable and residual fractions.

2.2.3.2.1 General Design of SES

Optimal design of a typical extraction scheme for soil necessitates consideration of the following factors:

- Sample pre-treatment employed like grinding, drying etc.
- Information on sample matrix like major composition, pH, particle size
- Pre-defined fractions/phases of metal interaction
- Suitability and stability of reagents considering chemical and physical interferences in extraction as well as end determination
- Experimental conditions like sample to liquid ratio, duration, means of mechanical agitation-shaking and way of phase separation.

Fractions and reagents

A typical sequential extraction scheme in soil would target the five basic chemical pools agreed to be present i.e. adsorbable, exchangeable, carbonate, reducible, oxidisable (organic, sulphides) and residual fraction (see Table 2.1). Reagents used are usually unbuffered salts, weak acid, reducing agents, oxidising agent and strong acid/peroxide, respectively.

Table 2-1: Typical fractions and reagents used in literature

Fraction	Reagent	Remarks/ref
Water soluble	water	Low buffer capacity
adsorbable, exchangeable	$CaCl_2$	McLaren and Crawford
	$MgCl_2$	Tessier
	NH_4OAc	
	$BaCl_2$	
carbonate	HOAc	[a,] Ure et al., BCR
	NaOAc/HOAc	Tessier
easily reducible (Mn oxides)	$NH_2OH.HCl$	Ure at al, BCR
Moderately reducible(arm.Fe oxides	NH_4Ox/HOx	
Moderately reducible(arm.Fe oxides	$NH_2OH.HCl$ /HOAc	BCR, Tessier
Poorly reducible(xtalline Fe oxides)	Dithionate Citrate Buffer	Gupta &Chen
Poorly reducible(xtalline Fe oxides)	NH_4Ox/Ascorbic acid	
Easily oxidisable (humic, fulvic acids)	$K_4P_2O_7$; NaOCl	
Oxidisable (oxides,sulphides)	H_2O_2	Readsorption problems
Oxidisable (oxides,sulphides)	H_2O_2/NH_4OAc	BCR scheme
Residual	$HF+HNO_3$	
	Aqua regia	BCR

[a] Not suitable for calcareous soils

Usually the first reagent is directed at the adsorbable, exchangeable fraction.

This fraction is regarded as the most bioavailable fraction present for plant uptake. It comprises metals weakly adsorbed on the sample surface, complexed to soluble organic matter, metals released during ion-exchange processes and those that can be co-precipitated with carbonates present in the sample. Almost all possible combinations of the major cations with chloride, nitrate and acetate anions have been used in the literature at neutral pH.

Such reagents include natural salt solutions like $NaNO_3$, $CaCl_2$ and NH_4NO_3 in millimolar concentrations (from 0.05 to 1 M) as they occur naturally in soil pores.

McLaren and Crawford [141] favoured 0.05 M $CaCl_2$ as the first extraction stage of their sequential extraction series because it had less effect on the natural soil pH and extracted fewer ions associated with soil organic matter.

However, the use of Ba and Ca salts reportedly interfered with the determination of Pb and other trace metals using AAS. This could only be circumvented by dilution of sample extracts by 1:10 resulting in correspondingly higher detection limit and the use of easily volatilisable salts like Mg $(NO_3)_2$ and NH_4NO_3 instead. The Tessier scheme stipulates 8ml 1M $MgCl_2$ (pH 7, 1hr) for the extraction of exchangeable fraction using 1g sample [119]. However, it has been found to overestimate the exchangeable fraction, particularly in the case of Cd [142]. The Cd complex formed with chloride ions has some stability at high chloride concentration (log K values lie between 1.98 and 2.4). The extraction efficiency of the anions at equal concentration is in the order nitrate < chloride < acetate [143]

The carbonate fraction is addressed next using reagents like dilute mineral acids, HCl and acetic acid (pH 3-3.5), acetate buffer solution (pH 5) or buffered Na_2-EDTA at pH 4.6. Metals bound to carbonates can be affected by the production or consumption of protons and represent together with the exchangeable fraction a readily available fraction. Release can be caused, for example, by anthropogenic acid deposition, natural acid production or oxidation. The Tessier scheme recommends the use of Na acetate/acetic acid buffer pH 5 (8 ml, 1 M buffer, 5 hr.) step. The operating conditions defined in Tessier's scheme are acceptable for a low carbonate content of soils or sediments. However, an incomplete dissolution of this phase was observed in the case of sediments with high carbonate contents (16% $CaCO_3$). Therefore, carbonate dissolution continued during the following step of the scheme, leading to an overestimation of the third (reducible) fraction [144].

While early schemes distinguish the carbonate from the exchangeable fractions, the BCR however proposes the use of dilute acetic acid (0.11 M, 16 hrs) to target the metals associated with the adsorbable, exchangeable and carbonate fraction together.

The reducible fraction consists of metals that are attached to Fe/Mn oxyhydroxides which can be released by suitable reducing agent. The association of metals with this fraction can be changed under anoxic conditions.

Most schemes use hydroxyl ammonium hydrochloride. The performance of this reagent depends on pH, concentration, extraction time and temperature, which means that a discrimination of Mn oxides from Fe oxides could be achieved, depending on the extraction conditions.

Chao [145] showed that the Mn oxide fraction can be extracted in 0.01M nitric acid medium, 30 min while the Fe oxide fraction requires more stringent condition (e.g. at high temperature). Simultaneous extraction of Mn-Fe oxides has otherwise been commonly achieved by employing hydroxylamine solution in sufficiently acidic medium (pH around 2).

The BCR recommends the adjustment to pH 2 using HCl. Other schemes have used acetic acid [146] and HNO_3 [147] successfully. Use of the reagent at pH below 1.5 causes the organic and silicate fraction to be partly extracted, leading to an overestimation of the reducible fraction [119].

A number of authors have established that the dissolution of iron oxides was incomplete during the reductive step of Tessier's scheme, leading to an overestimation of the residual fraction [148, 149, 150].

The addition of a supplementary iron-specific step is advised in high Fe content materials [149].

Some schemes have distinguished further between the Mn oxyhydroxide fraction and amorphous Fe complexed fraction. For this latter group, oxalates have been proposed as extractant. Tamm's reagent 0.2 M oxalic acid-0.2 M ammonium oxalate at pH 3, room temperature and in the dark [e.g. 136, 151] has been used after Mn oxide fraction removal for the extraction of Fe oxides due to its high Fe complexing capacity (log K for Fe^{3+}: 4.35-18.49; log K for Fe^{2+}: 3.20-5.15) and its low reducing properties (E°=-0.38V).

Tamm's reagent and ascorbic acid in addition to this reagent [143] has also been used for the extraction of amorphous Fe-oxides. However, due to the greater complexing power, the organic fraction will be extracted to a higher degree than with hydroxylamine. To circumvent this, the organic fraction should be extracted prior to this stage. Another disadvantage of Tamm's reagent is the formation of very stable complexes with aluminium, such that element association with Fe oxide fraction cannot be distinguished from that of Al oxide.

Some workers [152, 153, 154] have proposed the use of Na citrate/Na dithionite buffer, but this reagent suffers from metal impurities and lack of selectivity since the silicate fraction is attacked easily.

The oxidisable fraction concerns metals associated with organic matter and sulphides. These can be released under oxidising conditions caused for example, by dredging of sediments or re-suspension due to storm. Under oxidative conditions, sulphide will be oxidised to sulphate, releasing protons and metals. The released metals may in turn quickly be scavenged by solid phases or form complexes.

Oxidising reagents such peroxides H_2O_2 (E°=1.77V) or hypochlorites NaOCl (E°=0.90V) have been used to attack this fraction. For instance, the former at elevated temperature is used in the BCR scheme. However to avoid the re-adsorption of released metal on freshly exposed residual matrix sites, ammonium acetate extraction (pH 2) is usually carried out immediately after the peroxide treatment. $Mg(NO_3)_2/H_2O_2$ has also been used to extract Cd ions from organic matter [142].

Oxidation with peroxide is reported incomplete for materials with high organic matter like resistant organic and sulphide minerals [119]. Other workers incorporate an easily soluble organic fraction (humic and fulvic acids) using sodium pyrophosphate which seems selective for this phase but low extraction efficiency was observed [155]. Campanella et al. [156] in their 5 stage scheme, distinguished between organic fraction (mainly humic substances) and suphides using HCl, NaOH and HNO_3 in sequence after the extraction of the ammonium acetate and hydroxylamine soluble fractions.

The residual fraction is found by difference of the total (sum of extractables) or is attacked by aqua regia (as in the BCR scheme), by digestion with hydrofluoric acid or other digestion protocols and is attributed to silicate bound/crystal lattice incorporated metal, (lattice of primary and secondary minerals). Changes in natural environmental conditions are said to have no effect on the release of metals in this fraction in the time-scale of a decade.

Other matrices are subjected to stepwise degradation as described above, but with modifications based on sample characteristics or where particular elements are targeted, through careful choice of (element/analyte specific) reagents.

Thus, various schemes depending on the carbonate content, pH and other geochemical factors have evolved through the years. The EU Standards, Measurements and Testing (SM&T formally BCR) set out to harmonise the major schemes available and was able to produce a three-stage BCR sequential extraction standard protocol [121] presented in the following section.

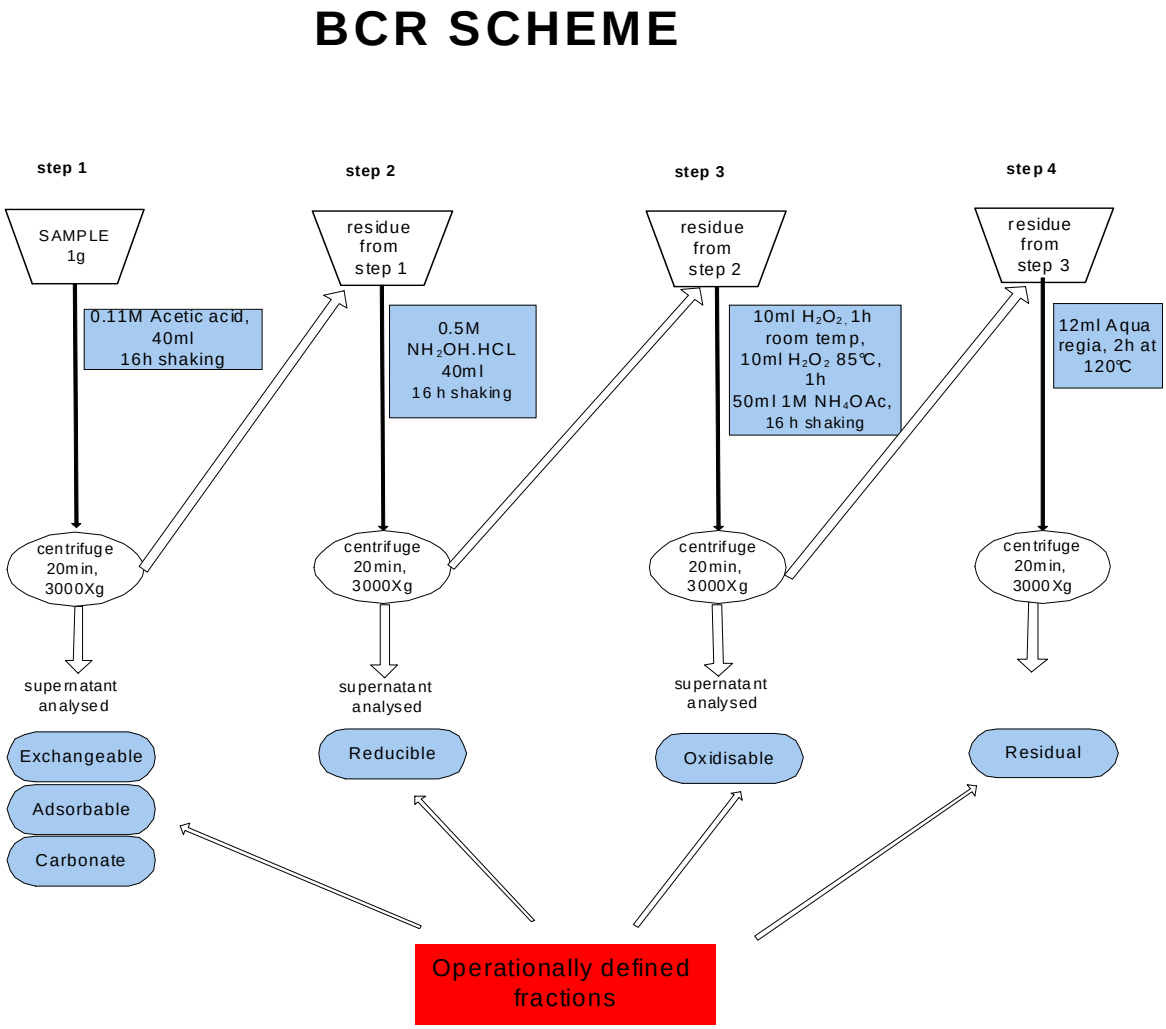


Fig. 2-3: Schematic representation of the BCR 3-stage extraction scheme

The BCR Scheme

The need for harmonisation of the various methods for interlaboratory studies and standardisation led the European Commission through the BCR Programme and its successor (Standards, Measurements and Testing Programme) to launch a collaborative project which aimed to

- design a three-step sequential extraction scheme,
- test the selected scheme in interlaboratory studies involving expert European laboratories,
- certify the extractable trace element contents of a sediment reference material.

The European Commission proposed a standardized three-step sequential extraction procedure through the BCR programme based on acetic acid extraction to liberate exchangeable/acid-extractable metals (step 1), hydroxylammonium chloride extraction for metals associated with the reducible phases (step 2) and then metals released by oxidation with hydrogen peroxide extracted with ammonium acetate (step 3). This scheme was tested in a first inter-laboratory trial on Cd, Cr, Cu, Ni, Pb, and Zn in soils and sediments and was reported by Quevauviller et al. [157]. The three-step sequential extraction procedure was then used for the certification of the trace metal extractable contents in a sediment reference material (CRM 601) [158]

To assess sources of uncertainty in the developed BCR three-stage sequential extraction procedure, different factors, such as extractant pH, type of acid used in pH adjustment, temperature, duration of extraction, performing extractions in an inert atmosphere, technique used to separate liquid and solid phases after extraction and reagent type and concentration, were studied [159]. pH was found to be a serious criterion. Recommendations were made to amend the second step, by addition of a fixed amount of nitric acid at pH 1.5 in the preparation of extracting reagent and increasing the concentration of the reagent to 0.5 M. Also, centrifugation at 3000 *g* for 20 min to separate the liquid from the solid phase was proposed. Finally, the residual fraction was obtained by pseudo digestion with aqua regia as suggested by Rauret et al. [160]. The modified BCR scheme (see Fig. 2.3) has been used for the certification of new sediment, soil and sewage sludge amended soil reference materials [161].

The use of alternative reagents was also considered. Davidson et al. [162] investigated the use of 0.2 M ammonium oxalate (pH 3) as a replacement for 0.5 M hydroxyl ammonium chloride (pH 1.5) in step 2, the reducible fraction step of the modified, four-step BCR sequential extraction for soil or sediment. Their proposed method is suitable for substrates with high Fe oxide content, but problems arise when analytes, such as lead or calcium which readily form insoluble oxalate precipitates, are to be determined.

Ho and Evans [163] in their work used a smaller sample mass (0.5 g) than in the original BCR procedure, but the ratio of soil mass to extractant volume was maintained. 0.1 M hydroxylamine sulfate was used in Step 2, rather than 0.1 M hydroxylamine hydrochloride. Step 4 is not standardized in the original BCR procedure, but has typically been an aqua regia digestion. However, Ho and Evans

applied a total digestion involving a hot plate digestion of 0.5 g of material, using an HF-HNO_3–$HClO_4$ mixture in a Teflon dish.

The effect of ultrasonic agitation on the release of copper, iron, manganese and zinc from soil and sediment using the BCR three-stage sequential extraction method was reported. Although lower recoveries were obtained than in the conventional method, the time saving aspect made it attractive [164]. Filgueiras et al. [140] developed a small-scale extraction method with ETAAS determinations (i.e. 25 mg mass in 1 mL extractant), with considerable time saving by using ultrasonic probes. Extraction yields were comparable to those of the conventional BCR protocol and the method could be useful in situations in which rapid assessment of metal mobility is required, rather than high accuracy.

Validation

Optimisation and validation of SES may involve testing whether repeated extraction increases the fraction extracted but the operationally defined nature of the fractionation scheme method usually means that interpretation of results is influenced by the actual scheme used. Corroboration using other methods has also been used to validate fractionation results. Some researchers have combined the SES with direct solid analysis of samples [165, 166, 167] or extraction residues after each step [168] to study the extraction efficiency as well as selectivity of the reagent chosen. La Force et al. [149] combined fractionation with spectroscopic studies to monitor the extraction of crystalline materials but the method presumes the presence of crystalline material.

Fractionation analysis is also enhanced by measuring major elements like Fe, Al, and Ca, which influence dissolution/precipitation and adsorption/desorption equilibria [169].

The use of multiple parallel extractions (i.e. one reagent per sample) as opposed to sequential regimes has been proposed by various workers [170,171]. Comparison of single parallel extractions with SES [113] showed that generally useful estimates of the various fractions in the samples could be obtained.

Applications

Solid samples of environmental interest for which the BCR SES has found application include soil [172, 173, 174,175], sediment [176, 177 178, 179], sewage sludge [180,], air particulate matter [181, 182] and fly ash [183].

Table 2-2: Table showing the main SES methods available since the 1980s

Scheme	Fraction/ reagent				
	exchangeable	Acid soluble	Reducible	organic	residual
Tessier, 1g sample	8ml of $MgCl_2$, 1M, pH 7, 1hr at room temp.	25 ml of NaOAc 1M /to pH 5 with HOAc, , 5hr at room temp.	20ml of $NH_2OH.HCL$, 0.04M in HOAc 25% (v/v) solution, pH~2, 6hr at 96°C	3ml HNO_3, 0.02M + 5ml H_2O_2, 30% , 2hr at 85°C 3ml H_2O_2, 30% + 5ml NH_4OAc 3.2M, 30 min at room temp. (H_2O_2 to pH 2 with HNO_3 solution)	HF + $HClO_4$ mixture
Zein and Brummer	deionised water (S4, water soluble fraction, 1M NH_4NO_3 exchangeable fraction,	1M NH_4OAc easily soluble fraction	$NH_2OH.HCL$, 0.1M, 1M NH_4OAc		
Shuman 1993	$Mg(NO_3)_2$	#NaOCl	$NH_2OH.HCL$, easily reducible fraction NH_4Ox/HOx,moderatel y reducible		
McLaren	0.05 m $CaCl_2$: (weakly bound, soluble)	0.43 M HOAc (extractable, mainly inorganic)	0.1 M $(NH_4)_2\ C_2O_4 \cdot H_2O$ occluded by free oxides *	1 M $K_4P_2O_7 \cdot H_2O$ later changed to 0.1m, organic	HF (residual).
Emmerich *et al.*	H_2O (adsorbed fraction0.5 M KNO_3 exchangeable fraction	0.05 M Na_2 EDTA (carbonate fraction)		0.5 M NaOH (organic fraction	4 M HNO_3 (sulfide/residual fraction)
BCR	-together with acid soluble-	40ml of HOAc, 0.11M, 16 hr at room temp.	40ml of $NH_2OH.HCL$, 0.1M, (to pH 2 with HNO_3 solution), 16hr at room temp.		

* order changed: 4,then 3; # order changed 2 after 4

2.2.3.2.2 Limitations

SES are usually criticized for the *lack of selectivity* of the chemical reagents, such that a reagent may attack several phases at the same time. For instance, acetic acid meant for the acid soluble fraction is reported to attack also silicates [129]. Also, the *critical influence of the extraction conditions* like pH, solid/liquid ratio, small operational variations in factors such as speed of centrifugation, shaking mode, and solid–liquid phase separation procedures on fractionation have made it difficult to compare results that have been obtained using different protocols on the same sample [184, 185,186]. Sutherland et al. [175] compared the extraction patterns of Cu, Pb and Zn metals in sediment samples using the Tessier, BCR (original and optimised procedures) and Geological Survey of Canada (GSC) schemes [135]. Significant differences were noted between the four schemes, with the GSC scheme producing the least comparable results. Different distribution of metals in the various fractions analysed were reported, greatest for Pb, moderate for Cu, and least for Zn. The extraction efficiencies of the 2nd step for Pb for instance increased in the order BCR original < Tessier < BCR optimised < GSC scheme. A careful look at the extraction protocols could explain the differences. The Tessier scheme uses more stringent extraction conditions in the 2nd step, than the original BCR procedure, while in the optimised BCR procedure higher concentration of reagent is used. The GSC conditions are furthest apart from the other schemes considered. As such it was not surprising to find the results least comparable with the other schemes. The GSC scheme employs a multi-extraction approach, repeating extractions twice using first 20 ml of 0.25 M NH_2 OH.HCl in 0.05 M HCl (60 °C) for 2 h, repeat while heating for 0.5 h. Then 30 ml of 1 M NH_2 OH.HCl in 25% HOAc is used at 90 °C for 3 h, repeat under heating for 1.5hr.

Indeed the Tessier and BCR scheme in original and modified forms have been compared by other workers [171,187,188]. Similar differences in extraction efficiency were observed, i.e. the 2nd step of the BCR yielding less Pb than that of Tessier, but with higher organic fraction than the latter. These have been explained as readsorption of metal from the exchangeable and reducible fractions onto an oxidizable phase, such as organic matter, during extraction. Alternatively, the high temperature Tessier reducing step may be extracting Pb from organic matter as well, enhancing the apparent concentration in the reducible fraction.

The effect of extractant concentration has also been studied (160,189) and found to affect extractability in both the exchangeable and acid-soluble metal extraction steps. Varying the S/E ratio from 1:5 to 1:50 has been found to affect extractability of some metals but not others (190, 159).

The effect of extraction time on metal extractability is two-fold. On the one hand, the long times usually involved in SES allows equilibrium yields to be obtained. On the other hand however, prolonged contact time may increase the risk of dissolution of non-target phases, re-adsorption and redistribution. Furthermore long procedures limit sample throughput which is of interest in commercial routine laboratories. Maiz et al. [189] proposed a rapid sequential extraction procedure by reducing the extraction time in their metal bioavailability study of contaminated soils. Perez-Cid et al.

[191] used an ultrasonic probe to reduce the extraction time from 16 h to 7 min. Other researchers have recommended the use of a minimal extraction time to allow a steady state to be established between sample and extractant, but not so long that dissolution of other phases can occur [125].

The manner of *sample handling*, sieving, grinding, treatment like drying at high temperature and manner of storage all can affect the distribution of metals in solid matrices as mentioned in Chapter 2 section 2.2.1.

The problem of *standardisation* was successfully taken up by the BCR and a certified reference material (CRM) with certified extractable fractions for 6 metals has been produced. However due to the static nature of the sequential extractions, sufficient time still has to be allowed for the attainment of equilibrium between liquid and solid matrix. This leads to enormous time costs, which have characterised these schemes. Worse still the chances of re-adsorption and re-distribution of metal forms are increased by prolonged extraction or even incomplete dissolution of some phases as well as changes in pH.

Studies on extraction efficiency and re-adsorption have been done by metal spiking and the use of CRMs. Early tests were carried out by spiking the extractants with metals and comparing solution metal concentrations before and after extraction [192] or by using synthetic models [193], but there has been a discussion on the suitability of such material. It is argued that the distribution of metal spiked onto a soil sample does not represent the natural distribution in natural samples (this means that the extractability of metals cannot be compared). Thus Tessier and Campbell [194] questioned the validity of an experimental procedure using artificial sediments to test the performance of the SES and stated that the only important problem was the lack of selectivity of reagents to attack a particular phase. Bermond [195] underlined the difficulty of preparing synthetic models without avoiding metal transfer from one phase to another. Ribeiro and Mexia [196] admitted that an artificial contamination (spike) did not reproduce the geochemistry encountered in polluted sites and so could not perfectly represent a real case. However, McLaren and Clucas [197] showed that sufficient incubation of about six months produced metal spiked material in which the metal distribution was similar to that in non-spiked native sewage sludge.

Other approaches to study readsorption and redistribution phenomena have included the use of deionised water or ion exchangeable washes between extractions or determining correction factors using trace element spikes [198]. However there is no consensus on whether this improves data interpretation.

Missing kinetic information

In general, extraction procedures are designed to allow the establishment of thermodynamic equilibrium between phases through sufficient time for mixing and interaction. With this approach, problems of readsorption, redistribution and non selectivity occur as discussed in the last subchapter. Thus they at best deliver empirical information on the extractable content. However, natural leaching

occurs on a larger timescale and just like sorption, shifts in equilibrium are experienced due to ongoing environmental processes. Indeed enhanced information on the strength of pollutant binding, stability, mobility (lability) and potential of long term release of pollutants can be better obtained using kinetic methods [53,184]. Single extraction procedures proposed as alternatives include the use of chelating agents with or without external energy source (microwave, ultrasonic waves) to assist the extraction and acid leaching. Since these methods are equilibrium based, the kinetic information on the leaching of metals, which could lead to enhanced information about the inherent environmental risks, is lost. Kinetic investigation of the leaching process as it occurs could provide valuable additional information about the sample, such as speciation or complete characterization of the natural distribution of analytes in the respective system under investigation. Bermond et al. [199] demonstrated the feasibility of fractionation of trace metals in soil using an otherwise non-specific reagent, EDTA. Two metal fractions could be identified according to the extraction data into labile and non-labile. Gismera et al. [200] in their own work distinguished between a labile fraction (corresponding to exchangeable fraction) with the use of acetic acid and a less mobile fraction (corresponding to the other sediment fractions) with the use of EDTA. Although these studies emphasised the need for kinetic studies, very fast leaching (i.e. instantaneous) could not be followed due to the long sampling interval.

Hyphenated techniques based on the combination of chromatographic separation with a powerful multi-element detector like ICP-OES or ICP-MS have become a routine tool for heavy metal speciation in environmental samples and biological tissues. For species determination of a single element, flame, hydride or graphite furnace AAS is often employed. Corresponding automated methods for fractionation of heavy metals in solid samples are rare.

Furthermore, in-line kinetic (non-equilibrium) methods are faster and more convenient to perform, without extra separation steps, and should not suffer from re-distribution and re-adsorption problems. Also, because natural processes are dynamic, the in-line method would better simulate these conditions.

Flow through techniques (or more elegant a hyphenated (in-line) system allowing the real time monitoring of leaching kinetics) would yield better information and as such this approach was followed in this work. In the following subchapter, a synopsis of dynamic leaching and its relevance in environmental analysis is presented.

2.2.4 Dynamic leaching

Primarily the goal of leaching is to attain completeness of extraction with a given solvent. *Batch* extraction involves the suspension of solid material in a volume of extraction agent, over a specific time period. Optimisation of static batch extraction methods typically includes varying the

conditions like temperature, pH, solid/ratio, particle size etc. The release of analyte into the extracting liquid occurs so long as the driving force of concentration difference between the interstitial solid phase and the liquid exists. Thus the maximum concentration of analyte is obtained at equilibrium and this could be achieved by sufficiently long contact time but with the attendant problems of re-adsorption as discussed before. More important however is the fact that equilibrium is achieved only once. The equilibrium concentration of analyte is determined by the rate of transport, the effect of other substances in the sample matrix like organics, as well as pH and redox conditions.

Dynamic leaching on the other hand involves the continuous or intermittent renewal of leaching agent, thereby forcing a shift in equilibrium, and typically leads to higher extraction yield than the static counterpart.

According to partition theory, extraction efficiency is enhanced with repeated extractions using fresh solvent as opposed to a single extraction. Improvements in sequential extraction schemes have thus included the repeated extraction with fresh solvent [135]. This entails the shaking of a solid sample with the extraction liquid for a specified time, removal of liquid extract from solid residue, washing in between extractions etc. which could be tedious to perform in serial batch mode. Also the inherent risk of loss of analytes at each stage cannot be overlooked.

Flow through methods using column [201] and lysimeter [202] have been employed to mimic the natural leaching processes in the environment. Column experiments differ from lysimeter in that the former deals with the release of analyte from the solid sample while lysimeter deals with the release and transport phenomena under natural real conditions like sorption, facilitated routes, microbiological degradation etc. In all, the solvent is allowed to percolate through the soil body and analytes in the effluent are determined. At most only a stepwise renewal of solvent through the sample body is achieved. Although information is gained on the potential mobilisation of pollutants (from soil for instance), these experiments usually involve kg quantities of sample and take weeks or months to perform. Also channelling, clogging of the system by fine particulates and biological growth in the system could be problematic [53].

Continuous extraction methods on the other hand would involve the continuous renewal of solvent and better still the simultaneous removal of extracted analytes from the system. Here a constant shift in equilibrium is achieved with much higher extraction efficiency than the static method.

Continuous extraction has been realised in the literature in various ways. In most available methods, quantitative extraction of analytes from the sample matrix for total metal determination is the aim. Therefore the use of temperature, pressure and external energy sources, (mainly microwave and ultrasound), to enhance the extraction have been described.

The groups of Valcarcel and Luque de Castro in Spain were among the first who exploited the in-line leaching and extraction of heavy metals (and also other compounds) using a flow-through approach with samples held in cartridges. However, their studies were equilibrium based, with the use of extraneous energy sources to enhance the leaching process aimed at complete recovery of the metals

in order to obtain comparable results to conventional batch leaching or digestion protocols. For instance, a dynamic ultrasound-assisted extraction step was proposed for the quantitative extraction of Cd and Pb from plant leaves prior to determination by electrothermal atomic absorption spectrometry (ETAAS) [203]. Quantitative leaching of metals and also organic pollutants from various matrices has been reported by circulating the same reagent for a set period of time (usually 5-10 minutes under ultrasonic assistance) through the sample, after which the extract is collected and analysed, using continuous extraction systems. Thus, Yebra et al. [38] extracted iron from solid mussel samples by a simple continuous ultrasound-assisted extraction system (CUES) connected to a flow injection manifold, followed by on-line flame atomic absorption spectrometric determination. Here again, although the extraction system was independent of the detector stream, on-line monitoring of the leaching process was not exploited. Instead extraction was carried out for a fixed time after which an aliquot was injected into the detector stream. Usually, the system is optimised for particle size, flow rate, extraction time etc. and after the set time, the extract is transferred into a collection vessel for offline determination or as recently reported [204] coupled to a suitable detector for in-line determination after completion of the leaching procedure. A method for the routine analysis of essential metal elements in animal feeds using a dynamic and automated ultrasound-assisted extractor was also recently proposed [205].
The advantages of the method include a drastic reduction in extraction time (18 min versus 4.5 h) and lower volume of extractant required.

Extraction techniques like supercritical fluid extraction (SFE) and pressurised liquid extraction (PLE) [206] involve the flow of solvent through the sample held in a cartridge and transport of the extracted analytes into a collection vessel. Static and dynamic modes of extraction can be combined but possibilities of real time monitoring of the leaching process have not been considered.
In all these methods, the aim was to achieve quantitative extraction of the analytes from sample matrix and thus the leaching kinetics was only studied for optimisation reasons.

It is increasingly recognised that enhanced information useful for the long time risk assessment is obtained through studies of the leaching behaviour of different metals in a sample matrix over time. This is an addition to information about the mobile fraction useful for bioavailability assessment and fraction mobilisable under specific conditions such as gastric digestion.
Fangueiro et al. [207] studied the kinetics of the leaching of metals from sediments with EDTA. They observed that the protocol used for extract collection influenced the results of EDTA extraction. Two protocols were employed. In the first protocol, a single sample aliquot was mixed with the reagent and extract aliquots collected over time. In the second, several sample and extractant mixtures were stirred, each for a specific time. The latter protocol gave better results. However due to the long time intervals of 30 min, fast leaching kinetic information could not be captured.

Flow systems offer a practical solution for gaining kinetic information about the extraction of pollutants in solid samples of environmental importance with high time resolution. In general, this would involve the pumping of solvent(s) through a solid sample held between filters in a suitable container. Sample extracts would then be collected at set time intervals or in portions e.g. at the fraction collector for off-line determination of extracted analytes. The results obtained not only give an indication of the mobility of the metals under the studied conditions, but also provide an estimate of the long time risk potential. For metal fractionation involving sequential extraction schemes, the use of such continuous flow extraction systems have been increasingly reported in the past five years.

Shiowatana et al. [208, 209] proposed a continuous-flow sequential extraction system for metal fractionation in soil materials. The sample (250 mg) was held between two filters in a stirred cell, while the respective reagents were pumped through. The effluent was collected in about 20-30 mL portions for AAS analysis. Advantages of the method over the batch system include speed of extraction, ease of manipulations like change of solvent with no need for extra separation step, less risk of contamination and less dependency on extraction conditions. Furthermore, noteworthy reduction of re-adsorption was achieved using this system.

The innovative use of rotating coiled columns (rcc) as sample holder in a continuous flow extraction system has been reported [210]. In these experiments, slurried sample (0.5 g), was kept in the column by gravitational forces due to constant rotation while extractants were pumped continuously for 3 hours to effect the extraction of metals in sediment sample. Here, extracts were also collected at intervals and analysed offline, but using ICP-AES in this case, simultaneous detection of several elements was possible. Apart from less risk of contamination, a closed system i.e. on-line analysis certainly has advantages over open vessel fraction collection as discussed before.

Several SES have been applied for arsenic [211] and phosphorus and heavy metal fractionation [212] in soil using the method. This system offers the advantages of constant renewal of solvent, potential of automation and ease of obtaining kinetic information.

Speciation of nickel in airborne particulate matter by means of sequential extraction in a micro flow system and determination by graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry has also been described [213]. Here extractant was pumped through the aerosol sample held in specially made filter holder and analysis of metals in effluent stream carried out offline.

Another approach of growing interest is the coupling of the continuous flow extraction system to the detector such that the analytes in the effluent stream can be in-line detected. The advantages of this approach over the off-line procedure include analysis in closed system, with less risk of contamination from laboratory or loss of analyte and most importantly almost real time monitoring of leaching processes or at least highly time resolved kinetic information is achieved.

Shiowatana et al. [214] had devised a flow system in which samples in the order of 0.25 g were held between micro filters in a stirred vessel and pumping continuously the extractant at high flow rates (10 ml/min). Extraction time was 4-6 hours after which extracts were determined with FAAS. Using direct coupling of extract stream to the detector, they reported gradual blockage of the filter by fine solid particles, resulting in decrease in flow rate of extractant to the FAAS with time. As a result, peak area of the extraction profile could not be directly related to the amount of metal extracted and finally off-line detection was resorted to.

There are only few reports which describe in-line flow methods to assess the mobility and fractionation of elements by kinetically following the leaching process. Scokart et al. [215] proposed a fully online method for the study of leaching processes. In their work, a flow system comprising continuous flow extraction of metals from samples held in a vessel, with direct online determination of the metals in the effluent stream was developed. Extractograms were obtained showing the leaching of metals from the sample with time. However, problems were encountered with the detection due to matrix effects.

Recently, Beauchemin et al. [216] using a similar flow system, was able to monitor the leaching of heavy metals from various geological samples with the advantages of ICP-MS detection.

Concentration profiles were obtained during sequential leaching of elements from soils, sediments and geological samples using various concentrations of nitric acid thereby affording more information on the possible phases involved (since surface adhering elements are released more readily than those bound to the matrix). Here also, the results were hampered by interferences in the ICP-MS detection due to the different concentrations of reagents used. Table 2.3 shows the development of flow methods for continuous extraction of metals from solid environmental matrices (both offline and in-line detection) to date.

Due to the identified need for kinetic information and problems hitherto encountered by various workers, the development of an online flow through method for studying the kinetics of leaching processes was therefore embarked on in this work. The instrumental configurations developed and method optimisation are described in the next chapter.

Table 2-3: Chronology of continuous (solid sample) sequential extraction methods in the literature

Date of publication	Worker/Group	Extraction Method	Detection mode	Remarks/Ref
1987	Scokart et al.	1g sample in minicolumn-CF of extractant	In-line direct flow of extract into ICP-AES detector	Poor detection, extractograms
2001	Schiowatana et al.	1g sample in stirred flow cell-	Off-line fraction collection FAAS	214
August 2002	Kurosaki et al.	5g sample disc shaped cell, CF of extractant	Off-line fraction collection	217
2002	Beauchemin et al.	minicolumn-ICP-MS	In-line direct flow of extract into detector	216
2003	Fedetov et al.	1g sample in rotating coiled column	Off line fraction collection	210
2003	Jimoh et al.	5- 25mg sample, mnicolumn, FIA	On-line flow injection analysis, FAAS, ICP-AES and ICP-MS	228
2005	Chomchoei et al.	25mg sample in micro column- SIA	Off-line, FAAS	218

CF continuous flow, FIA flow injection analysis, SIA sequential injection analysis

2.2.5 Bottom line: Interpretation of fractionation data obtained in extraction schemes

Environmental trace metal analysis serves to gain information about the levels of contaminants for effective control and long term monitoring programmes. Human risk can be assessed through monitoring of known pathways like food, water, air (respiratory), and skin contact. For this, information may be required as to total amount of contaminant present, chemical form or structure, stability and distribution of contaminant in the sample as well as the availability (bioavailable, bio accessible).

As mentioned earlier (Chapter 1), in order to recognise pollution, the 'normal' or native levels of the metals in various environmental compartments must be known. Thus total content determination provides background information as well as the potential risk (at worst case scenario) on the environment. Speciation analysis approach on the other hand enables the identification and quantification of known species present in the sample. This provides an assessment of the immediate risk. The question of distribution, stability (lability) and bioavailability can be effectively answered using fractionation schemes. Information on the fraction available for uptake, be it at the grass root – soil pore level (plant available fraction), or fraction available on ingestion and ready for assimilation (absorption) in the organs can be obtained using the appropriate schemes. Fractionation schemes can be designed to incorporate the changes in pH, Eh and such chemical factors which are known to govern the release of metals from solid matrices. Thus fractionation schemes offer a tool for the assessment of immediate and potential long term risk.

Speciation analysis could also be carried out in the various fractions/residue after extraction in order to gain more information about the identity and behaviour of metal species in the sample.

Finally, a unified approach to speciation as a whole is still missing. To this end, correlation should be sought between the results of chemical extraction schemes, speciation analysis and eco-toxicological (in vivo) approaches.

3 Aims and objectives

Two approaches (microcolumn and a membrane based approach) were followed in this work with the aim of developing microanalytical flow-through methods or at least improvement of existing methodologies for the fractionation of metals in solid environmental matrices.

In the microcolumn approach, an in-line (flow-through) microanalytical system suitable for the study of the leaching kinetics of heavy metals in solid environmental samples was developed. The method is based on the continuous extraction of metals from the solid substrate and in-line analyte determination using hyphenated atomic spectrometers.

The next chapter deals with the development and optimisation of the flow method. The challenges of the method development included steps to

- accommodate microgram quantities of solid samples in a flowing system
- allow the study of slow as well as fast leaching kinetics in almost real time
- design an interface to optimally introduce the sample extract to the detectors

Furthermore, optimisation of the flow system was aimed at the generation of good reproducible transient signals which could be accurately detected (above detection limits) even at the otherwise continuous flow (non-transient) measuring instruments like the ICP-AES.

The system was to be rugged yet flexible permitting changes according to the analytical problem at hand.

Validation and real applications of the developed system are the focus of Chapter five. To this end, assessment of method performance through the use of certified reference materials (CRMs) and the development of laboratory internal reference materials, comparison with batch methods, independent methods and literature sources were carried out.

Finally, the system was to be applied for the simulation of various scenarios in environmental risk assessment studies. Application of the micro-analytical system to the speciation of metals in selected plants and soil material are discussed fully in the next chapters.

The second approach considered in the thesis involved the application of microdialysis to metal fractionation. A microdialysis probe suitable for the continuous in-situ sampling of metal ions from solid samples was constructed. The probe was then coupled with an ETAAS detector to facilitate the analysis of dialysates.

4 Method development

In this chapter, the experimental considerations made in the development of the micro-column extraction system used in this work for the kinetic study of leaching processes in solid matrices are described. Although it was possible to lead the extract directly to the detector for analysis as had been done in literature, this approach was not followed in this work. Instead the flow injection (FI) variant, which is well established for sample introduction and fluid manipulation, was employed in this work. To facilitate the description, the flow manifold is treated in two parts, the extraction line separate from the detection one. The design of the interface between the two parts is then handled.

4.1 Experimental design

The design of flow system depends on the detector and the chemical aspects of the determination. In this work, the analysis of samples was carried out using atomic spectrometric detectors exclusively.
Instrumental considerations on the microanalytical extraction system thus consist of three subsections:
Method of extract analysis- detectors, flow configuration, instrumental details
Description of the sample extraction system employed in this work
Sample interface joining the two systems, comparison with literature, pros and cons
In the following section, the detectors used are described and considerations that needed to be taken detailed.

4.1.1 Detectors

Detector requirements for integration into FIA manifolds include small volume need, low noise level fast and linear response over a wide concentration range and high sensitivity. Furthermore the ability to recognise transient signals (i.e. register the appearance and disappearance of the analyte introduced into the detector stream), ease of coupling to the extraction system and compatibility of extractant flow rates and extractant composition with the instrument are added advantages of the candidate detector.
The usual operational requirement of various atomic spectrometers is compatible with flow systems. Normally, about 1-3 ml of sample is required per analysis by continuous aspiration, which can be reduced to 100-200 µl in FIA. Since atomic spectrometers do not require a flow cell to hold the sample, no significant dead volumes are encountered. The manifold design can be quite simple, requiring only pumping of the carrier into the nebuliser for detection. The use of FAAS, ICP-AES and ICP-MS in flow systems had already been treated in Chapter two. The micro analytical flow through extraction system was optimised for FAAS, ICP-AES and ICP-MS detection.

4.1.1.1 FAAS detection

The FAAS in general works by the continuous pneumatic aspiration of carrier at about 4 ml/min into the nebuliser. Nebuliser efficiency is put about 5%, probably the cause of the poor detection limits compared to the other AS detectors.

The extraction system was coupled with a tubing to deliver the injection loop contents into the carrier stream. Since the detector measures continuously, the concentration of analyte in the detector stream could be monitored over time as peak signal. Matrix interference was minimal with the sample extracts injected and background spectral interference correction was by deuterium lamp. The FAAS response is fast but the LOD is not low enough for the concentrations encountered in plant samples. Also the method allows the determination of a single element per sample run only.

4.1.1.2 GFAAS-detection

GFAAS at first seems not to be suitable because it is discrete, requiring 10-50µL sample in the graphite tube.

The facts that the various steps of drying, ashing prior to atomisation must be accomplished sequentially, that matrix effects need to be countered with modifier or modification of temperature program, all make it difficult to couple GFAAS directly to a flowing system. In the past decade, two means of marrying FI to GFAAS have emerged: fraction collection in vials and subsequent off-line determination or using an injection loop as holding vessel for sampling the sample stream at intervals for introduction into the furnace. The feasibility of such coupling with the extraction system developed in this work was considered. The idea was to design a suitable flow-through vessel, to temporarily hold the effluent stream while aliquots of the extract are introduced into the furnace by auto sampler arm. The flow injection could be implemented in stop and go mode. However, the relatively longer stopped time of two to three minutes during which extraction proceeds had to be considered. The time constraints outweighed the advantage of LOD over the FAAS method. Furthermore, only a single element can be monitored per sample, making the multi elemental ICP-AES more attractive and this was pursued as described below.

4.1.1.3 ICP-AES detection

The operation of the instrument involves the aspiration/pumping of the liquid sample into the nebuliser, aerosol formation, drainage of large droplets in the spray chamber and propulsion under gas flow (Ar) of suitable sized droplets to the plasma. Here the sample is desolvated, atomised and ionised. Excitation at the high temperatures in the plasma cause light emissions which are led into the spectrometer where the light is diffracted and sent to the detector for quantification. The instrument used was equipped with a solid state detector of charge coupled type (CCD).

ICP-AES has several advantages over FAAS apart from lower detection limit, like the multi elemental character, scanning over wavelengths such that matrix interference can be eliminated by choosing a line that is not disturbed or by multi component spectral fitting (MSF). However, since measurements over the time range are averaged, according to the instrument software (Spectro CCD v. 3.2) transient signals could only be followed by extra software programming. This was achieved using Origin software (www.originlab.com) platform.

Problems with AES detection

The ICP-AES instrumental software used was not capable of detecting transient signals. The usual operation involved continuous nebulisation of sample solution for a period of time to allow stability of plasma conditions and enhance reproducibility of readings, i.e. continuum, non-gradient, therefore extra software was necessary to collect data at chosen intervals and display these in graphical form for easy monitoring of the leaching processes.

Data collection:

Several reading modes on the CCD detector were tested by varying measurement phase times in order to optimise the data collection at the AES. Still the following problems persisted:

1. Digital resolution: In contrast to the MS version, the AES detection was only able to describe a peak with some 7 points, by sampling rate of 3 to 6 sec and typical half width of 30s. At least 10 points was considered adequate for a chromatographic peak, considering that the baseline may slope [219].
2. Signal to noise ratio: the sensitivity of the method was reduced for interesting metals like Pb and Cd which occurred at near detection limit in the plant matrices analysed.
3. Instrumental response was slow, partially because the transport of sample to plasma alone took ca 30 seconds (shortening of the length of tubing within the instrument was not feasible) and also the slow detector response.

Problems were encountered with the ICP-AES detection of leached metals from plant samples due to the insufficient LOD (Cd and Pb were worst affected) and plasma instability from the high organic content especially for parsley and spinach samples.

Interferences

The method suffers from spectral and matrix interferences. Spectral interference can be alleviated in batch process by choice of alternative lines, inter element correction or multi component spectral fitting. The first approach was used in the work.

For the flow injection mode, the interference could not be manually corrected, such that a predefined instrumental correction was relied on.

Matrix interference could be avoided by using matrix matched reagents and standards. In the on-line mode, high organic content of some plant material led to plasma quenching during extraction.

4.1.1.4 ICP-MS detection

In this method, the flow of ions from the plasma is led through a sampling cone, skimmer to the ion lens and into the quadrupole where separation according to mass to charge ratio (m/z) occurs. The ion of interest then impinges on the detector surface where it is counted. Once the detector measures the ions, the computerized data system is used to convert the measured signal intensities into concentrations of each element and generate a report of the results. Basic components of the instrument are described below:

Sample introduction system

The sample introduction system generally consists of the peristaltic pump, nebuliser, and spray chamber and provides the means of getting samples into the instrument.

Traditionally, ICP-MS is designed to accept liquid (aqueous) sample solution which is converted into an aerosol by means of a pneumatic nebuliser. Concentric, cross-flow and grooved variants may be employed, depending on the viscosity, solid (salt) content, quantity of the sample etc. The performance of the nebuliser determines to a large extent the detection capabilities of the instrument. The most common Meinhard nebulisers (concentric) have an efficiency of 1-2%. Improvement in the efficiency of sample introduction into the ICP saw the development of alternative nebulisers like the ultrasonic nebulisers with 10-fold increase in efficiency and the direct insertion technique with 100% efficiency.

The spray chamber removes the excess large droplets formed at the nebuliser. Thus the efficiency of the atomisation and ionisation depends on the performance of the spray chamber. A comparison of several spray chambers (standard Scott double-pass, cyclonic, and two low-volume cyclonic type), which was carried out in combination with a MCN in emission spectrometry, should be applicable to ICPMS [9]. The study indeed showed that at very low liquid flow rates (10-160 µL/min), a conventional cyclonic spray chamber provided higher analyte and solvent transport rates and, as a result, higher sensitivity and lower limits of detection than the others.

For hyphenated systems however, the geometry of the spray chamber (internal volume) reportedly affects the peak characteristics, a double pass giving rise to worse precision than the single. Other factors investigated were the length of tubing between nebuliser and extraction unit, the longer the distance, the more the peak broadening.

Mass filter

ICPMS instruments vary in the type of mass spectrometer that is attached to the ICP ion source. Quadrupole mass spectrometers only allow ions of a specific m/z value within a narrow mass window to reach a detector, ejecting all other ions. The multi-element capability (varying the m/z values) is ensured by changing the voltage and radio frequencies applied to the quadrupole rods thus selecting the position of the mass window. Two modes are possible, the peak jumping or hopping mode, where the instrument dwells on particular pre selected m/z value for a given time (ms) or the scan mode in which the mass spectrum can be scanned in seconds. The quadrupole on the ELAN Series ICP-MS from Perkin Elmer SCIEX used in the work can scan from m/z = 1 to m/z = 240 in less than 0.1 seconds, the so called scan speed of the quadrupole. Where necessary the measurements were carried out repeatedly in 100 or 500 ms.

Sector instruments on the other hand, use magnetic and electrical fields to focus a beam composed of spatially dispersed ions. Focusing permits ions of a particular m/z to reach the detector through a slit. In TOF instruments, ions travel through a tube at different velocities, depending on their m/z. All ions are detected, though lighter ions reach the end of the tube before heavier ions.

Detector

Most instruments use an electron multiplier in which the ion is counted individually and corrected for background. Photons are excluded using a shadow stop. The active surface of the detector, known as a dynode, releases an electron each time an ion strikes it. The electrons released from the first dynode strike a second dynode where more electrons are released. This cascading of electrons continues until a measurable pulse is created. By counting the pulses generated by the detector, the system counts the ions that hit the first dynode. This is for low concentrations, i.e. the pulse created by the individual ions is measured. At higher count rates, the electron multiplier switches to analogue mode and an output signal whose intensity is proportional to the intensity of the ion beam striking the detector is measured instead. Thus, a wide range of linearity is maintained.

Table 4-1: Common interferences

Metal	Isotope	Relative abundance %	Interferent
Al	^{27}Al	100	$Be^{16}O$, $B^{16}O$, CN
Cr	^{52}Cr	83.7	$^{40}Ar^{12}C$, $^{34}S^{18}O$, $^{36}Ar^{16}O$, $^{36}S^{16}O$, $^{35}Cl^{16}OH$
	^{53}Cr	9.51	$^{36}Ar^{17}O$, $^{36}Ar^{16}OH$
Ni	^{60}Ni	26.2	$^{44}Ca^{16}O$, $^{43}Ca^{16}OH$
	^{61}Ni	1.14	$^{44}Ca^{16}OH$
Cu	^{63}Cu	69.2	$^{46}Ca^{16}OH$, $^{40}ArNa$
	^{65}Cu	30.8	$^{48}Ca^{16}OH$, $^{33}S^{16}O_2$, $^{33}S^{32}S$
Zn	^{66}Zn	27.9	$^{34}S^{16}O_2$, TiO, $V^{16}O$, Ba^{2+}
	^{68}Zn	18.8	$^{40}Ar^{14}N^{14}N$, VO, ClO_2, $^{36}S^{16}O_2$, $Ti^{16}O$, ArS, Ba^{2+}, Ce^{2+}
Cd	^{111}Cd	12.8	
	^{114}Cd	28.7	
Pb	^{206}Pb	24.1	-
	^{208}Pb	52.4	-

Interferences

Table 4.1 shows the common interferences that could be encountered for the determination of metals in environmental matrices using ICPMS.

In this work, the elements (isotopes) analysed were Al (27), Cr (52, 53), Ni (60, 61) Cu (63, 65), Zn (66, 68), Cd (111,114) and Pb (206,208).

Polyatomic interferences are a major problem for the analysis of some elements. For example, $^{40}Ar^{16}O^+$ is a classic interfering species when detecting ^{56}Fe.

The use of cool plasma conditions (low RF, higher nebuliser flow rate) leads to a substantial reduction in the intensity of Ar^+ and Ar based ions. However the cool conditions adversely affect analysis of elements with high ionisation potential, the intensity of other molecular ions e.g. oxide ions increase and matrix effects become more pronounced [220].

To address this issue, researchers have used either a multipole collision cell or a dynamic reaction cell. In collision mode, interfering polyatomic ions collide with an inert gas in a cell containing 6 or 8 rods to which an RF only voltage is applied. In reaction mode, interferents or the analyte ions undergo

chemical reactions with a gas [221]. Here the quadrupole is maintained which serves additionally as a mass filter. Both technologies help prevent interfering species from spectrally overlapping with the target isotopes.

The use of sector field mass spectrometer gives the required resolution such that the detection of ions differing in fraction of a mass unit could be achieved [222].

ICPMS as on-line detector for FIA Systems

Flow injection (FI) is often preferred over continuous nebulisation of sample solution because of its numerous features (reduced sample consumption, higher sample throughput, minimized solid deposition problems on the sampler, lower matrix effects, less contamination etc).

The multi-element detection capabilities of ICP-MS and the range of linearity over several orders, make the instrument attractive for metal speciation analysis.

Moreover, the capability of transient signal detection is a reason why it has been successfully used in on-line determinations coupled to chromatographic separation.

The instrumental software used in this work already incorporated a transient signal detection mode making on-line hyphenation/coupling easier.

The lowest detection limits were achieved, allowing detection of ng level of metal species especially Pb, Cd which were poorly detected by the AES. Further quality control of data could be achieved with the added isotope information. In this case, the ratios of the isotopes measured were compared with theoretical ratios based on the relative abundance, to exclude artefacts. Due to interferences from $^{40}Ar^{16}O$, Fe (which luckily occurred in high enough concentration in the samples analysed), was measured with ICP-AES or FAAS.

Signal Processing

Due to the injection of sample extract into the carrier /detector stream, transient signals in the form of peaks were obtained as detailed later (section 4.2 Basic concepts). A typical peak as shown in Fig. 4.1 was almost Gaussian in shape and characterised by width at half height, peak position and area. Latter was obtained by simple integration using Origin software (www.originlab.com). In cases where noise was prevalent, smoothing had to be done using moving point average prior to integration.

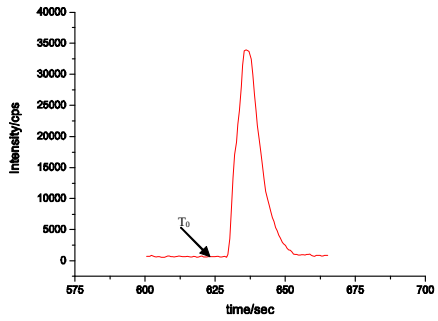


Fig. 4-1: Typical transient peak shape obtained in flow injection mode (time of injection t_0)

4.1.2 Extraction system

Fig 4.2 depicts the constructed micro analytical components i.e. sample cartridge (microcolumn, mini-column) and adjustable holder. The sample is placed between two frits in the microcolumn (Fig 4.2 b) and this is inserted into the adjustable cartridge holder (Fig. 4.2a). The necessary tubing is screwed on at both ends of the holder to facilitate reagent delivery. The design of these components is described in the following. Criteria for sample mass and particle size are also discussed.

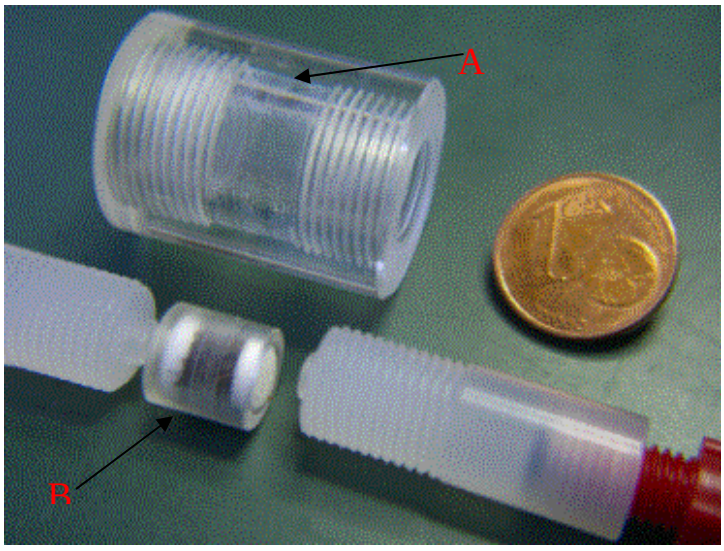


Fig. 4-2: Photograph showing adjustable cartridge holder (marked A) and sample cartridge (B) used in the work

4.1.2.1 Sample cartridge

Sample containers that have been proposed for flow through extractions include the stirred flow cell, but mostly cylinders have found resonance by workers since the flow conditions of mixing and dispersion are optimal. Thus, in this work, for optimal hydrodynamics, the cylindrical shape was chosen. Cartridges were made of perspex material in three sizes- 3, 7 and 12 mm height respectively, all of 5mm diameter, to accommodate the different sample sizes (5-80 mg as required). The

dimensions chosen ensured that sidewall effects were avoided, since the length of column was at least 10 times the particle size of samples analysed. Further, the diameter of column was compatible with the tube diameter, such that flow was not impeded. The inlet and outlet frits served to distribute the extractant fluid and collate the extract respectively in addition to keeping the sample from escaping from the sample cartridge. Sample was loosely packed in the column held between two frits of polyethylene (20 µm, Varian) as compact packing had been observed to increase back pressure.
Finally the packed cartridge was inserted in a special adjustable cylindrical holder fitted at both ends with screwable adapters through which the various cartridge sizes could be conveniently held. Also, the connections from lines to the cartridge were effected through the adaptors (see Fig 4.2a).

Sample mass

The weight of sample that was transferred into the cartridge was dependent on the kind of sample material, the particle size, its swelling characteristics under the influence of the solvent and the expected metal concentration. For elements present at higher levels and those having low limits of determination using the applied detectors, a sample weight of ca 5 mg was generally sufficient. Smaller amounts were not used to avoid problems due to insufficient homogeneity. The upper limit was 20-30 mg since larger samples tend to create problems with back pressure when the leaching solvent was pumped through the cartridge at elevated flow rates.

4.1.2.2 Flow manifolds

In order to study fast leaching as well as slow leaching kinetics, a continuous (foward propulsion) and a re-circulating manifold were developed.
The basic flow systems used are depicted in Figs. 4.3 and 4.4. In both configurations shown, solutions are propelled by multi-channel variable speed peristaltic pumps (Ismatec IPS-8, Zurich). Injection and switching valves (4 and 6-port all PTFE rotary type, Latek) were installed at different positions in the manifolds, facilitating the flexible manipulation of the various liquids. Sample injection loop was left constant for all experiments at a volume of 210µl. All interconnections were made from PTFE tubing (0.5 mm i.d.).

4.1.2.3 Procedures

Continuous (forward) flow mode

At the beginning of the experiment, the flow lines of the leaching system were air filled. Then the sample cartridge was placed in position and the desired leaching solvent was aspirated at a preset flow rate (usually 0.7 ml/min). The leaching solvent was directed through the cartridge and further through the injection valve of the FIAS system. Immediately after the loop of the injection valve was filled, the

first injection was made giving a transient detector signal that represented the concentration of metals in the initial volume of leaching solvent in contact with the sample. When the sample had been flushed out from the loop, the injection valve was returned to the loading position and was refilled by a fresh sample extract. Then the next aliquot was analysed. The procedure was generally continued until no further elements under investigation were leached by the respective solvent. The next stage of operation was the introduction of another leaching agent and repetition of the entire procedure.

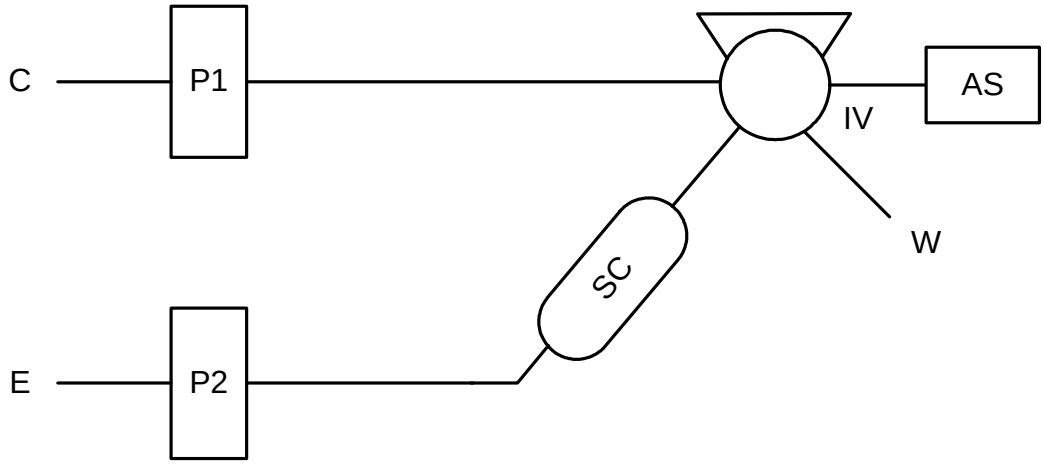


Fig. 4-3: Diagram showing manifold for the study of fast leaching processes

P1 und P2 = Pumps, IV = Injection valve, SC = Sample column, C = Carrier, E = Eluent, W = Waste, AS = Atomic spectroscopic detector

Stopped flow mode

The stopped flow mode is a slight variation of the continuous flow leaching procedure in that the flow of the leaching solution was temporarily stopped whenever the injection valve of the detection line had been just filled with fresh extract. Only after the return of the valve was the leaching agent propelled further to refill the loop. Proper timing can be achieved when flow rates of the leaching agent and the loop volume of the injection valve are known. The minimum stopped flow time is dependent on the time required for detection by the respective atomic spectroscopic technique and that for refilling the loop. In this way of operation, the complete extract could be brought to analysis hence permitting quantitative information by integration of the sum of all signals of the absolute amount of metals leached with a particular solvent. This is particularly interesting because comparison with manual leaching procedures could be made on a quantitative base.

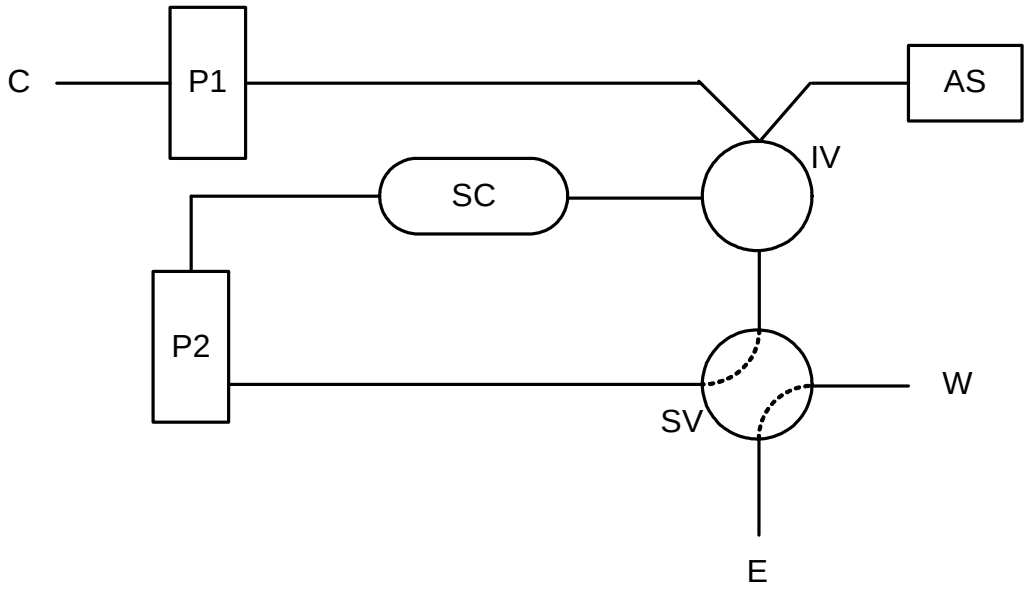


Fig. 4-4: Re-circulating manifold for the study of slow leaching processes

P1 und P2 = Pumps, SV = Switching valve, IV = Injection valve, SC = Sample column, C = Carrier, E = Eluent, W = Waste, AS = Atomic spectroscopic detector

Re-circulating mode

In the re-circulating mode the leaching agent on reaching the selection valve SV was redirected to the sample cartridge and continued to circulate for a preset time or as long as desired (e.g. until no further increase in analyte concentration occurred). Thus the method offers the flexibility of extraction time. The concentration increased with each cycle till equilibrium was reached. The change in concentration with repetitive cycling was followed by successive injection (via the injection valve, IV) of a small volume of the re-circulating liquid into the detection line. This time dependent concentration change is a measure of leaching kinetic and can in principle be followed until equilibrium is attained under the given conditions. A new portion of leaching agent was simply introduced by switching the valves SV and IV until the recirculation loop was refilled.

4.1.3 Sample interface

In the direct introduction of sample effluent into ICP-MS, degradation of measurement precision and cone orifice clogging may be expected. When FI sample introduction is used, the attack of harsh matrices on the sampler and skimmer cones and instrument drift when analysing difficult samples are reduced and there is essentially no degradation of the ICP-MS detection power [223]. Combined with standard additions and internal standardization, FI is an excellent tool to decrease matrix effects with complex samples.

A major difference from the earlier procedures is that the extraction line was separated from the detection line i.e. the injection valve served as sample interface. Thus the two parts could be optimised independently and flexibly while avoiding the matrix effects of reagents used at the detector.

Interfacing of leaching flow system to the detectors

The carrier stream was continuously pumped into the nebuliser of the AS detector while sample eluates were introduced by loop injection, in given time sequences, into the carrier. The leaching flow is thus separated from the detection line using a six-port injection valve as the interface between the two streams. Both flow systems were individually optimised.

This is in contrast to the methods described by Scokart et al. [215] and Beauchemin et al. [216] employing a single line stream where the extractant was continuously pumped through the sample column and the resulting extract or eluate directed into the instrument.

There are several advantages connected with decoupling the streams. Firstly the optimum flow rate for nebulisation can be set irrespective of that for the leaching process. This also permits the application of the stopped-flow procedure without introducing fluctuations in the AS-response. Secondly, the leaching agent is only in short contact (depending on the injection volume used) with the AS instrument. Thus corrosive agents can be used without detrimental effects. Thirdly re-calibration of the AS instrument can be conveniently performed by injection of standards without interrupting the leaching flow stream. Fourthly, the composition of the carrier stream can be made to reduce potential transport interferences or chemical interferences in AS determination e.g. matrix matched. For instance dramatic signal suppression occurred in the method by Beauchemin et al. [216] when concentrated nitric acid was used as the leaching agent and submitted directly to the ICP-MS nebuliser. Calibration was hence necessary for each individual reagent matrix. This problem was not encountered in the course of the experiments, possibly because the small volume of leaching agent was partially diluted in the carrier stream and the nitric acid carrier served as a kind of matrix adjustment solution. Also as mentioned earlier, Shiowatana et al. in their work reported problems of blockage of detector lines with microfine sample particles when sample extracts were directly and continuously pumped to the detector. Indeed the fluctuations of flow rate made a reliable quantification of peak areas impossible.

Sampling interval and stop time

Injection time was optimised for each instrument since the timing depended on the response of the instrument, number and concentration level of elements and the time required for complete washout to avoid memory effects and ensure good resolution/separation of extraction peaks. Thus minimum injection interval (between peaks) was found to be 6 s for FAAS (single element), 18 s for the determination of 12 isotopes by ICP-MS and 30 s for ICP-AES. In order to bring the whole extract to analysis, the extractions were carried out in the stopped flow mode. Generally, stop time was set at

20s, but for some analyses at the ICP-AES, longer times were necessary to accommodate the slow washout at high analyte concentrations. As expected, the amount of metal extracted (mg/l) varied with the stop interval, longer times allowing more contact between phases thereby enhancing the extraction. This is comparable with the static time in PLE techniques. It was thus necessary to keep the time constant for reproducibility. Indeed method comparability between measurements at the ICP-AES and those at the ICP-MS was hampered by this factor (see subsection 5.3.1.4 Collaborative studies). Typical total extraction time (number of injections, ca 10-15) per reagent was between 2-5 min depending on the analyte concentration, sample size and physico-chemical properties.

4.2 Basic concepts

4.2.1 Analysis of Extract

Several options are available for presenting the effluent of the extraction vessel for measurement to the detector. These could be summarised as collection of extract in fractions, prior to off-line measurement, direct leading of the effluent to the detector (termed in-line detection) and injection of the sample extract into a carrier stream with on-line detection. In the following, the merits and shortcomings of each option are discussed. A theoretical sketch of the recirculating mode is also presented. Further, a description of the detector signal as the extraction proceeds is made. The section is rounded up with a discussion of methods of quantification from each option.

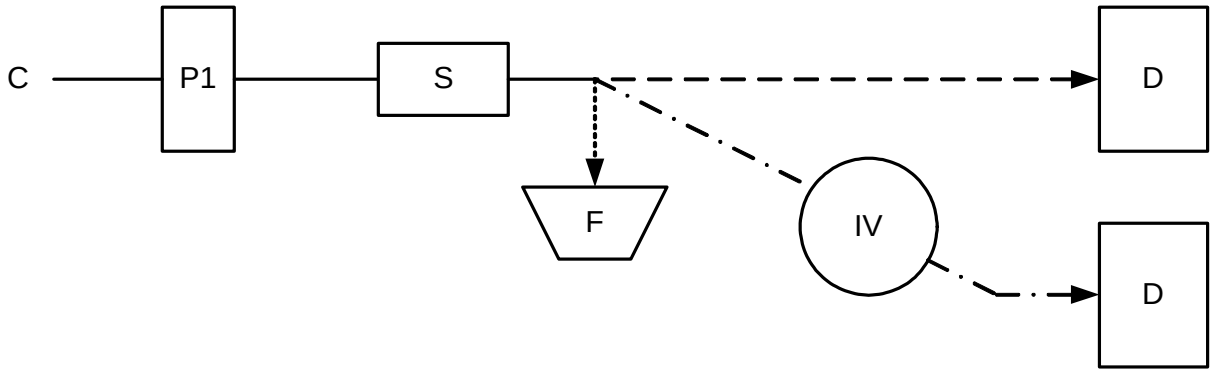


Fig. 4-5: Scheme of online sample extraction and detection options

P, peristaltic pump; S, sample cartridge; F, fraction collector, V, injection valve; D, detector

4.2.1.1 In-line detection

This involves the continuous flow of extractant through the micro column and direct introduction of column effluent to the detector. The signals obtained indicate the time resolved continuous release of metals, without dilution and dispersion (i.e. steady state). The whole extract is brought to measurement at the detector. The time resolution, i.e. interval between measurements, depends on the distance to detector and instrument delay (instrument response time). As already mentioned, this option is mostly followed in the literature to date, being fast but with serious consequences. Disadvantages include the increased matrix effects, potential damage to reagent sensitive instrument parts and the technical difficulty of calibration and matrix matching as had been mentioned before.

4.2.1.2 Off-line detection

This involves manual or automatic collection of the extract at time or volume intervals for subsequent analysis, thus the sample is presented in aliquot “slices” to the detector. The sampling interval for fraction collection is determined by the volume required for a set of analyses. The advantage of this option is that sample aliquots can be stored for future analysis, whereas in in-line determinations, the sample extract is consumed. Where necessary, difficult analytes can be determined separately using individually optimised instrumental settings. Needless to say, the number of fractions collected determines the quality of kinetic information obtained, the fewer the fractions, the less the information. Therefore, labour and time resolution constraints make this approach less attractive. For instance, analysis was carried out every 0.6 s using ICP-MS, 3 s using ICP-AES and 6 s with FAAS, a comparable time resolved information cannot be achieved with this approach.

4.2.1.3 On-line detection

4.2.1.3.1 FIA with stopped flow (Stopped flow injection)

The stopped flow mode aims to achieve the quantification of the whole extract, section for section. Here, the whole sample extract is brought to analysis by injecting “slices “of the extract at intervals into the detector stream. As an analogy, the injection loop serves as the collection vial in the off-line option. Since the flow is stopped, the rest of the extract is prevented from going to waste. However, the stop time must be regulated and kept constant for the whole experiment, since extraction or metal release still takes place. This is analogous to the static time of extraction in PLE. Also to be considered is that the whole interstitial volume of the extraction cell is injected, and exchanged for fresh extraction agent in each injection cycle. The time interval between injections is determined by the time required to fill the loop in an injection cycle (i.e. emptying of loop, return of valve and refill of loop), the instrument delay and time required for washing the sample out of the detector system. The optimum injection volume was empirically assessed by monitoring the transport of air bubbles introduced into the micro column. Since interstitial volume of the column at the dimensions mostly used (5mg sample, 70 µm particle size, cell dimensions 0,5 cm x 0,5 cm) was in the order of 15 µl, due to dispersion, a 10 to 15 times larger loop volume would ensure that all the extract is actually measured. Also for FIA the usual sample size at near steady state conditions is about 200 µl. Advantage is that all the extract is brought to analysis but the problem of carryover (zone overlap due to adjacent ‘slices’) occurs. This means that since there is axial dispersion in the system, the concentration of the analytes measured in the extract is a net result of the adjacent incoming and outgoing flows. The procedure is also time consuming and tedious compared to in-line detection, except where fully automated.

However, it allowed quick integration of the area under the peak for quantification purposes with enough sensitivity and precision and as such was the preferred method chosen in this work.

4.2.1.3.2 FIA with continuous pump

In continuous flow injection, the flow of extractant through the column is continuous, i.e. uninterrupted while the effluent is injected into the detector stream. Here, the whole sample extract cannot be measured since there is loss of extract to waste during each injection step. Rather, each n^{th} slice (corresponding to the grey areas under the curve e, Fig. 4.7) is measured, giving an integral over the interval sampled. The time interval between sampling is also dependent on instrument and loop configuration. When injections are carried out at regular intervals and the total extraction time is known, the missing information can be corrected for by interpolation.

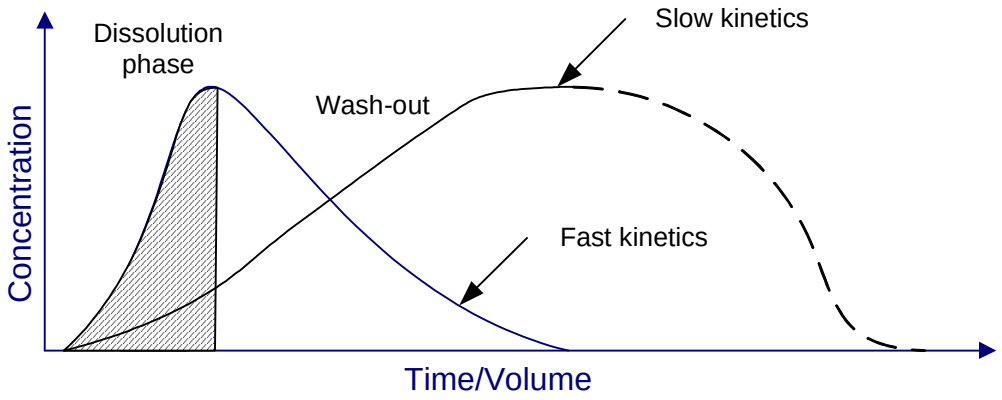


Fig. 4-6: Schematic diagram of the typical signal shape for continuous extraction showing fast and slow kinetic profile

4.2.2 Recirculating mode

The uni-directional continuous flow mode is suitable for studying the kinetics of reasonably fast leaching processes. In some samples it was observed that a slow but steady leaching occurred over a longer time period. This indicates the presence of a slowly available pool of metals for which a recirculating manifold was devised.

In the recirculating mode, sufficient leaching time is gained by circulating the same reagent through the mini-column several times in a closed loop, allowing the attainment of equilibrium. At the end of each cycle, the circulating extract becomes richer in analyte concentration i.e. accumulation until no more analyte is released, signalling equilibrium. Thus the recirculating mode can be regarded as a dynamic form of the batch process. It is comparable to the back and forth propulsion of extractant in some flow-through systems [224].

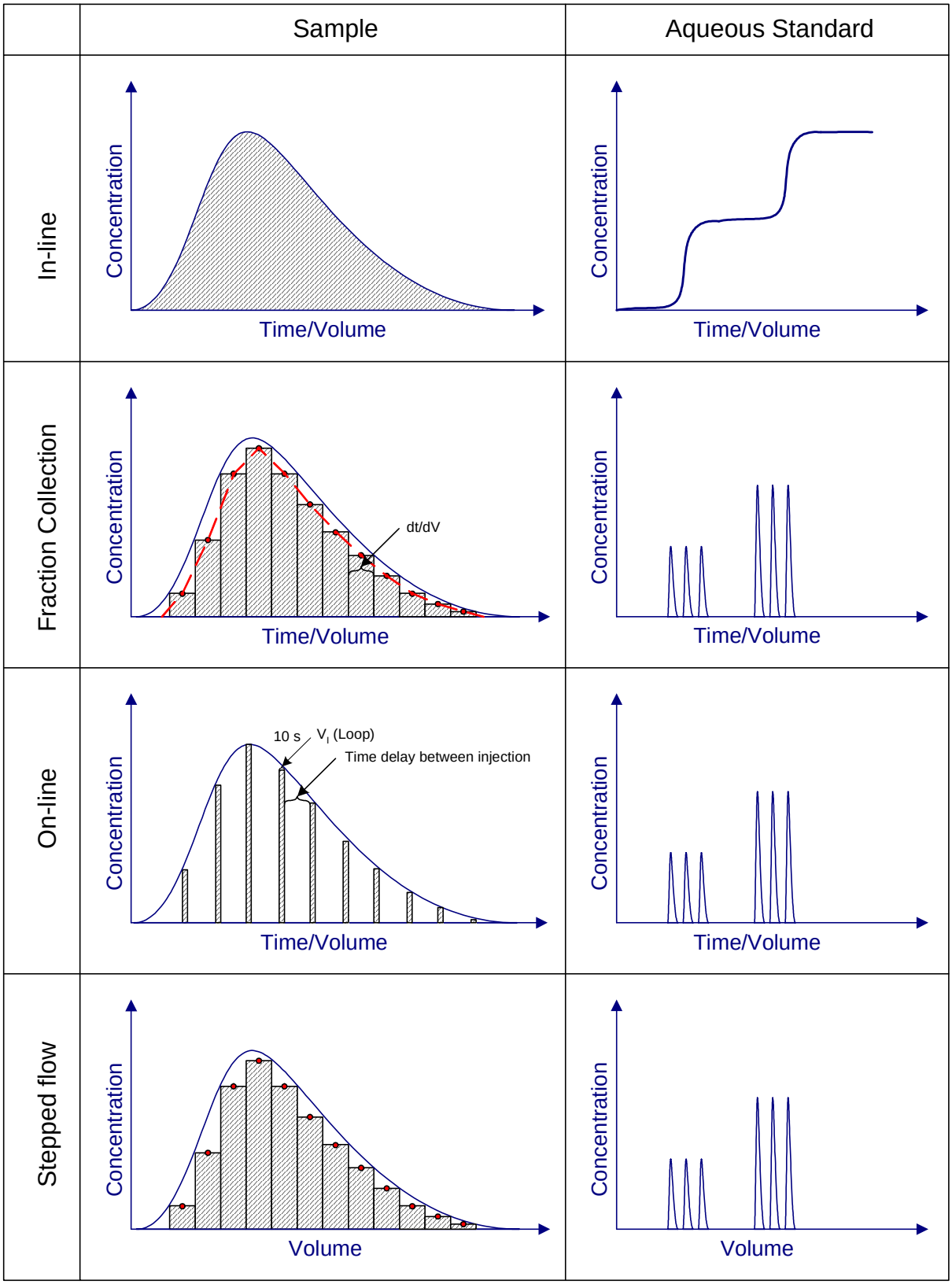


Fig. 4-7: Schematic representation of hypothetical extraction profile and detector signals from time dependent concentration change in a)in-line, b) off-line, c) on-line mode, i) continuous injection and ii) stopped flow injection options

Aliquots of the sample extract are analysed by injection into the detector stream at chosen time intervals. Assuming that the injected volume of extract is much smaller than the total recirculating closed loop volume, the accompanying dilution effect of the injection can be neglected. Thus, monitoring of the kinetics of extraction as well as information on the equilibrium (total) concentration is made possible. This concept is illustrated below with an example profile.

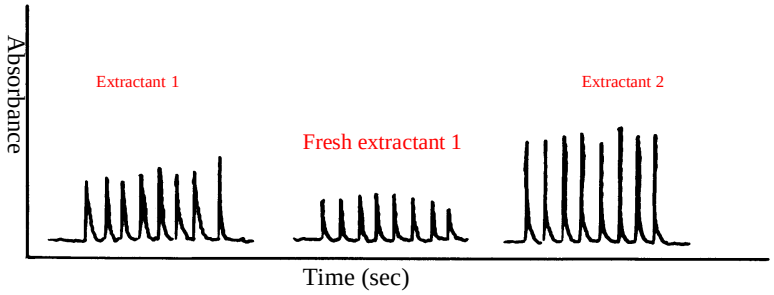


Fig. 4-8: Extraction profiles obtained for Fe in BAM2b FAAS detection

4.2.3 Signal generation and quantification

Signal profile

The shape of signal obtained at the detector is the same for all options chosen. It is characterised by a first part (region a) depicting an increase in concentration of analyte on changing to a suitable releasing agent (by dissolution or other extraction process) and a second part corresponding to the fast (region b) or slow (region c) decline of analyte release and washing out of the released analyte from the detector system. The slope is indicative of the rate at which the analyte is leached. The signal obtained at the detector in the flow through method signifies an integral response to the total amount extractable under given conditions. This is in contrast to the batch extraction where only one value is obtained representing the total extractable content.

Quantification

Quantification of the so obtained signal is dependent on the option chosen. The extract concentration is given by the sum of fractions.

In the flow injection options, the area under the curve is taken to be the sum of concentration of the extract slices. In stopped flow, the entire extract is measured while in continuous flow injection, the correct curve has to be reconstructed from the data points available.

4.3 Preliminary investigations

Preliminary experiments- Flow system characterisation

The analytical characterisation of the flow system occurred in three parts. Firstly, the transport of a standard iron nitrate solution was studied. Since this was only dissolution and not a desorption process, injection of aliquots of the 'extract' into the detector stream resulted in transient signals due to dispersion.

Thereafter the selectivity, distinction of phases and accuracy of the leaching process was studied by sequential extraction of a mixture of model compounds. The third step in the characterisation involved the use of further commercial and lab made reference materials, as reported in Chapter five.

a) Dissolution of iron nitrate

In order to optimise the overall flow procedure and in particular the timing of the switching intervals in a first set of experiments small amounts of a water soluble salt were introduced into the micro-cartridge. The dissolution can be assumed to be almost instantaneous so that the concentration profile obtained in the eluate solely represents the dispersion caused by transportation from the cartridge on its way to the detector. For the flow system described in the last subchapter, loop size of 210 µl was adequate for multi-elemental detection (see Tables A-3-A6 Chapter 9 for typical calibration data). Since the interstitial volume of the cartridge was much less in all cases, complete injection of the extract at any given time was expected. However, switching of the injection valve causes loss to waste, which could be circumvented by stopping the pump just before the injection. The stop time affects the extraction efficiency as discussed above and the approach has the inherent problem of zone overlap (carry over).

The analytical features of the developed method were further investigated using a mixture of iron nitrate, $Fe(N0_3)_2$ and iron oxide, Fe_2O_3 as sample. Figure 4.9 shows a typical extraction profile obtained with a two-stage leaching of metals. The first leaching agent was pumped through the cartridge containing the sample and extracts injected into the detector stream at set intervals until the level of metal had reached baseline or a plateau. The next extractant (eluent) was then pumped through yielding a series of peaks corresponding to the concentration of analytes.

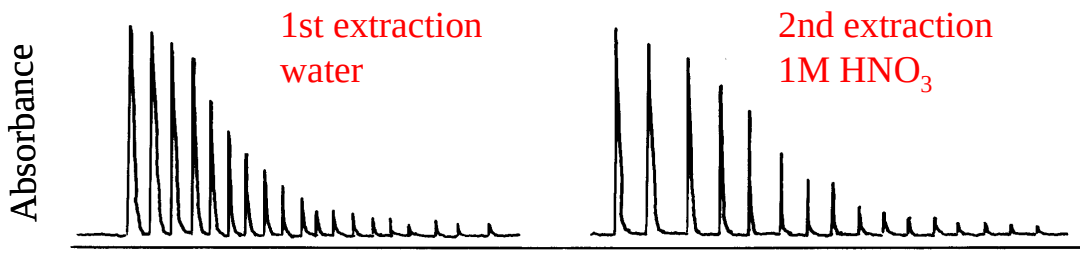


Fig. 4-9: Typical peak profile as obtained in a sequential extraction of metals (here FAAS detection of Fe).

b) Study of selectivity of reagents, distinction of phases (phase overlap) and accuracy

In order to check further the performance of the system as regards selectivity and accuracy of the quantitative response, a standard mixture containing solid PbO and $Pb(NO_3)_2$ in ratio 37.5% and 62.5% respectively (calculated as Pb content) was sequentially extracted with water, 1% and 10% nitric acids respectively (see Fig 4.10). It is expected that the nitrate is quantitatively recovered in the water soluble phase, while the oxide will be mainly recovered in the acid fraction. Results are given in Table 4.2 below.

Comparison of the amount leached by the different reagents (5 injections each) showed that 60% of extracted Pb was water soluble, 35% of the released Pb was found in the 1% acid fraction while about 5% were in the 10% acid soluble fraction, in good agreement with the expected values. The small discrepancy of 2.5% can be attributed to insufficient homogeneity of standard mixture prepared and carryover in the stopped flow mode used for detection as has been discussed under optimisation of flow system.

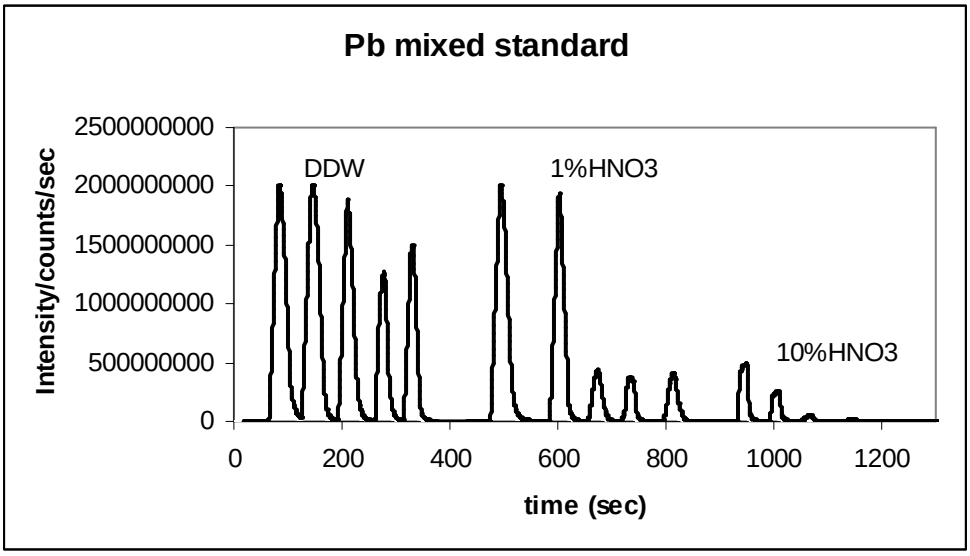


Fig. 4-10: Sequential extraction of lead nitrate and lead oxide standard mixture

Table 4-2: Results of the on-line sequential extraction of Pb from a standard mixture

Fraction	Concentration (ppm)	% fraction
Water	101	59.8
1% nitric acid	60	35.5
10% nitric acid	8	4.7

5 injections per extractant

5 Analytical Quality control aspects

Because it is dynamic in nature, the in-line method is not a quantitative extraction method but the aim is to extract sufficient amount of analyte in a repeatable fashion while ensuring that signals well discernable from baseline are obtained.

5.1 Calibration

Calibration strategy and quality control aspects to combat different spectral and non-spectral interferences are described. For all instruments, external calibration was used and usually samples were measured against matrix matched standards. Significant matrix effects are known to occur above 500 mg/l concentrations of major elements for ICP-AES [225, 226]. Plant sample digests contained much higher Ca, K and Mg levels. Digest of BCR 701 also contained about 1000 mg/l Al showing the necessity of matrix matching.

Optimisation of instrumental detection

To exclude spectral interferences, alternative isotopes and emission lines were monitored for ICP-MS and ICP-AES, respectively. However, Fe was determined at the FAAS and ICP-AES while Cd and Pb results were better using the ICP-MS.

5.1.1 ICP-AES Measurements

Calibration was effected for the ICP-AES measurements by employing matrix matched standards. Thus for plant samples, standards were modified to contain appropriate levels of K, Ca, Mg and Fe. Aqua regia digests were measured against standards prepared in acid. For the BCR digests, standards were also prepared in the BCR reagent solutions by addition of the appropriate volume of metal standard. As a quality control check on the measurements, a further standard solution was prepared containing the major matrix elements in the BCR 701 lake sediment reference material. Analysis of this solution at the ICP-AES was compared with that at the ICP-MS as given in Appendix Table C-1.

The argon line served as monitor line for all ICP-AES measurements. Background equivalent concentration (BEC) and other calibration /optimisation parameters were checked daily.

The measurements were mostly in semi-quantitative scan mode for the plant samples. Some soil and reference sample measurements were made in the normal quantitative mode. For the in-line measurements, several programs were tested for peak signal collection at the detector. The method

chosen involved a compromise between time, number of points per peak, peak shape and resolution (see Chapter 9 Table A-6).

5.1.2 ICP-MS Measurements

Several methods exist for the avoidance of interferences at the ICP-MS. Here, matrix matched standards were chosen to compensate for any matrix/reagent interferences.
Potential spectral interference was checked by comparison of intensities measured for the different isotopes of the elements.
In order to avoid bias, several single standards were mixed in varying proportions of the elements. The resulting solutions (stdmix I, II, III and IV; composition given in Appendix Table A-1) were measured and intensities compared.
For the in-line experiments, water, reagent blank and standards were measured, a stable baseline established and thereafter samples were analysed. A check on standard was made at the end of each measurement series. Instrument sensitivity and other calibration parameters were tested daily.

5.2 Figures of merit

5.2.1 LOD and LOQ

The limit of detection (LOD) concerns the limit below which the analyte signal is indistinguishable from the instrument background. Blank levels were therefore kept to a minimum by using Millipore grade water, ultrapure reagents and disposable containers at the ICP-MS. Furthermore, all glassware were soaked in acid (10% nitric acid solution) overnight or treated with acid in an ultrasonic bath, thereafter rinsed thoroughly with water before use. Flow lines were flushed between analyses with nitric acid solution and then water. Instrumental settings were adjusted for optimum signal detection at the instruments used according to manufacturer recommendations. LOD values were determined by measuring blank solution at least 10 times and calculating the average and standard deviation, σ. LOD was taken as 3 σ of the blank. Limit of determination or quantification (LOQ) on the other hand is the limit above which the quantity measured is considered statistically significant. LOQ is calculated as 10 σ. Since several elements were measured simultaneously and the levels at which they occur were unknown before the extractions, it was only possible to compromise instrumental settings for suitable detection. Thus, in the determination of elements like Cd and Pb at the ICP-AES, settings to suit the detection could not be individually made since this would affect instrument sensitivity to other elements.

FI peaks are characterised by high reproducibility. Replicate injections of standard solution into the detector stream gave satisfactory precision (<6% rsd)

Reagent blanks, a range of multi-element matrix matched standard solutions (100 µg/l, 1 mg/l and 10 mg/l) and solutions of mixture of standards in various concentration ranges, i.e. stdmix were injected into the detector stream using the continuous flow manifold as described in Chapter 4. Regression curves were linear for the metals in the studied range (Fig. 5.1, Table 5.1). Other results obtained for instrument detection limit, limit of quantification and other figures of merit for the assessment of method performance are given in appendix Tables A-2 and A-3.

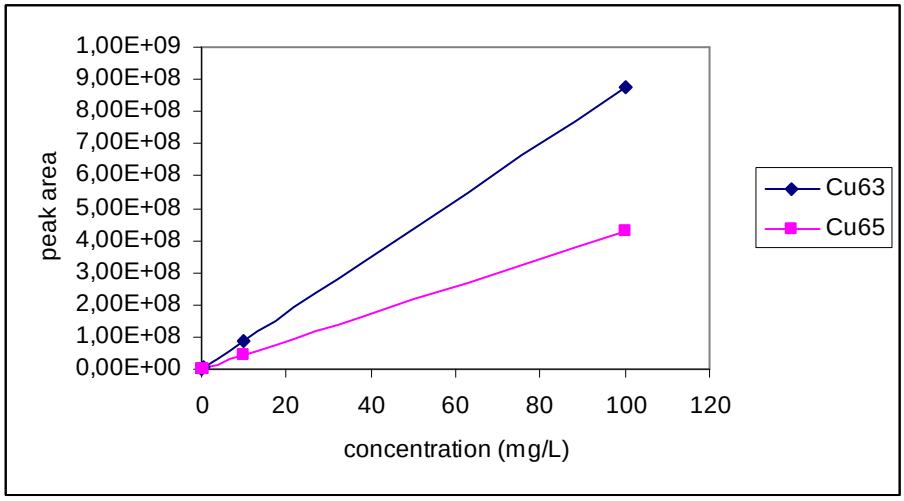


Fig. 5.1: Calibration curve for Cu (ICP-MS detection)

Detection limit was calculated from the blank data obtained by measuring 15 injections of reagent bank using the equations [227] as follows:

$$x_{DL} = \frac{y_{DL} - c}{m}$$

where xDL is the detection limit, yb is the mean peak area of the blank, sd the standard deviation of the blank, c is the intercept and m the slope of the calibration curve.

$$y_{DL} = y_b + 3\,sd$$

Thus, for Cu in the example above, the detection limit is calculated to be 0.15 mg/L (see Table 5.1. below). For the other elements studied, calculated values of detection limit and limit of quantification are given in Appendix Table A-4.

Table 5-1: Calibration data for Cu

conc mg/L	Cu63	Cu65
0,1	7,94E+05	3,86E+05
0,5	3,84E+06	1,88E+06
10	8,45E+07	4,26E+07
100	8,77E+08	4,29E+08
slope	8784621	4290424
intercept	-1246476	-206126
3sd	31080	19205
yb	448444	198367
yDL	479524	217572
xDL (mg/L)	0,196	0,099

5.2.2 Reproducibility of the extraction procedure

Reproducibility of the in-line extraction system was tested by running separate aliquots of dandelion samples through the acid sequential scheme. Results in Table 5.2 show an average precision of 30% rsd. This modest precision is acceptable considering that only mg quantities of real samples (with unknown heterogeneity) were involved. Best values were obtained for Cu which was at higher concentration.

Table 5-2: Reproducibility test of the extraction system (dandelion samples, n=4)

Dandelion	1	2	3	4		
isotopes	ng/mg	ng/mg	ng/mg	ng/mg	sd	%rsd
Ni60	7	6	4	4	1.5	27
Ni61	13	13	9	8	2.7	25
Cu63	7	8	7	7	0.4	6
Cu65	8	8	7	7	0.5	6
Zn66	106	106	66	62	24.5	29
Zn68	108	111	69	65	24.7	28
Cd111	2	1	1	3	1.0	65
Cd114	2	1	1	3	1.1	73
Pb206	13	13	5	10	3.7	36
Pb208	14	13	5	11	3.8	35
Al27	21	20	21	18	1.7	8
Pb208_1ppm	3	3	1	2	0.8	35

5.3 Measurement uncertainties

Instrumental precision and repeatability was assured through calibration and fine tuning according to manufacturer recommendation before sample measurements.

Method precision was assessed by using various reference materials instead of samples. Standard deviation values were calculated for the various total determinations.

Also, it must be borne in mind that the method that was developed in this work was meant for the semi-quantitative, quick screening of large sample contingents. For real samples, poor reproducibility was observed especially at concentrations near the limits of determination. This was also aggravated by the heterogeneity of the samples at the low mg level employed. Furthermore, most experiments were carried out in duplicate or triplicate. In all, the results were meant to serve more as proof of concept rather than standardisation of an established method. Nonetheless, the reproducibilities obtained are considered adequate to obtain enhanced information on the leaching (and fractionation) of metals in solid environmental matrices.

For the in-line methods, comparison of sum of fractions with maximum extractable amount obtained by aqua regia extraction served as further proof of correctness of the methods.

5.3.1 Method validation (accuracy)

Testing the accuracy of the method gives confidence that the results obtained are analytically sound according to good laboratory practice. Instrumental precision and calibration had been carried out as reported in the last subsection. Here, the steps taken towards validation of the extraction process and subsequent detection are described. These ranged from the recovery of spiked analytes, to use of reference materials, both commercial and laboratory tailored.

In order to study the applicability of the developed in-line microanalytical system for studying the kinetics and monitoring of leaching and other processes in solid environmental samples, and to assess the accuracy of the method, several reference materials including the BCR 701 lake sediment reference material were analysed. However, the BCR701 sample was the only material at hand certified for extractable contents (6 elements- Cr, Cu, Cd, Pb and Zn in four fractions). Therefore quality assurance was achieved by several means. Where appropriate the analysis was compared with those available in the literature. Otherwise comparison of results using different AS detectors (to eliminate instrument bias), recovery of spikes, as well as introducing variation in the mode of extraction were also means of testing the robustness of the method. The design of laboratory made reference material was a further attempt to ensure the quality of data in the presented work.

5.3.1.1 Recovery test

The completeness of the extraction was tested by recovery of spiked analytes in a matrix similar to that of the real samples. Earlier the preliminary assessment of the flowing system had been done using mixtures of standard Fe and standard Pb compounds, but this was without the sample matrix and therefore could not be taken as representative of the extraction of real samples. Recovery tests were therefore also carried out using the certified lake sediment reference material BCR 701 and the laboratory prepared reference material (section 5.3.1.3 below).

5.3.1.2 Certified reference materials

Laboratory quality control was further achieved with the analysis of certified materials, where comparison of the declared total content, experimental total content after digestion and sum of extracted fractions were made. The use of BCR 701 lake sediment sample, the only reference on the market with declared extractable metal concentrations in the predefined fractions (BCR sequential extraction scheme) enhanced the quality assurance efforts.

5.3.1.3 Laboratory made reference material

Due to the deficiency of the certified standards in dynamic systems, the suitability of mixtures containing known amounts of metal species as lab internal standards was tested. In a first attempt to make available a solid sample that has a defined chemical composition and represents different fractions (e.g. water and acid soluble) known amounts of $Fe_2(NO_3)_3$, Fe_2O_3 and iron filings have simply been mixed in various ratios. A small amount of the homogenised mixture was placed between the two frits of the micro cartridge. The leaching of the various species was monitored using FAAS. Results of this material were basically in agreement with expectations. The iron nitrate is instantaneously dissolved with water whereas the iron oxide and the metallic iron are only soluble in acid. However, the kinetics of dissolution of the iron oxide is much faster compared to the iron filings, which is certainly due to the smaller particle size of the former.

Though useful for basic studies this material is far away from optimum as far as fractionation of metal species is concerned. The absolute amount is much higher than that expected in real environmental samples (and smaller amounts cannot be accurately weighed), the accessibility of the elements is certainly different and matrix effects are absent.

Another approach tested was spiking of an inert material with small amounts of various compounds. Using solid materials problems were encountered with proper homogenisation so that the procedure was abandoned. The use of dissolved compounds or slurries of insoluble materials for spiking the inert matrix appeared more promising. In order to simulate the plant matrices which were of major concern in the investigations, cellulose fibres were used as the inert matrix. After spiking with appropriate

volumes of the liquids and/or slurries, the cellulose was treated with a household blender for homogenisation.

For qualitative studies (ratios of element leaching), small portions of the wet material were directly filled into the micro cartridge. The elution pattern was again in agreement with the expectations in that the lead nitrate is dissolved readily in water and lead dioxide and lead sulphide are only soluble in acid, the rate of dissolution of the two latter compounds being similar, yet considerably slower than for lead nitrate.

5.3.1.4 Collaborative studies

Corroboration of results can be done by carrying out comparative studies using an independent method.

Comparative measurements were carried out on BCR 701 samples using the simBCR scheme at different detectors i.e. ICP-AES and ICP-MS. Preliminary results showed extracted metal was generally higher at the AES than at the MS. A close look at the peak analysis data shows that the peaks were much broader and number of points considerably smaller at the AES. The time elapse between injections and thus contact time during which extraction took place was higher than for the MS experiments due to peculiarities of the instrument which could not be changed.

Better comparison was obtained especially for aluminium and copper on adjusting the stop time during extractions with ICP-MS as end detector (Table 5.3)

In order to check the validity of the AES measurements, ‘cross validation’ was done by employing different modes of extract measurement (as outlined in Chapter 4) at the ICP-AES detector. Results using the recirculating manifold are compared with batch, online and semi-online in Table 5.4 below.

Table 5-3: Comparative study of on-line extraction of metals in BCR 701 reference material using MS and AES detection

		BATCH		ON-LINE	
Element/ total content		reference	experimental	online- MS	online- AES
Ni	step 1	15	14	10	32
	step 2	27	28	15	72
103	sum	42	42	25	104
	ratio step1/step 2	0.6	0.5	0.7	0.4
Zn	step 1	205	194	149	247
	step 2	114	122	70	66
454	sum	319	316	219	313
	ratio step1/step 2	1.8	1.6	2.1	3.7
Cr	step 1	2	2	4	30
	step 2	46	47	28	17
272	sum	48	49	32	47
	ratio step1/step 2	0.04	0.04	0.14	1.76
Pb	step 1	3	2	4	198
	step 2	126	130	138	137
143	sum	129	132	142	335
	ratio step1/step 2	0.02	0.02	0.03	1.45
Cd	step 1	7	7	6	5
	step 2	4	5	3	7
12	sum	11	12	9	12
	ratio step1/step 2	1.8	1.4	2	0.7
Cu	step 1	49	48	34	28
	step 2	124	125	111	94
275	sum	173	173	145	122
	ratio step1/step 2	0.4	0.4	0.3	0.3

Table 5-4: Extraction of metals from BCR 701 reference material under different manifold configurations (ICP-AES detection)

(Metal concentrations in mg/kg)

SEMI-ONLINE MODE		FI-CONTINUOUS FLOW			FI-STOPPED FLOW			RECIRCULATING MODE			BATCH MODE			
	10ml soln A	solnA	solnB	sum	solnA	solnB	sum	solnA	solnB	sum	batchA	batchB	batchsum	total content
Al167.078	508	22555	550	23105	458	18	475	506	539	1044	181	1187	1368	27061
Cd228.802	13	20	38	58	5	7	12	16	10	27	7	5	12	12
Cr267.716	10	55	118	173	11	17	28	7	16	23	2	47	50	272
Cu324.754	158	174	45	219	24	14	39	93	42	135	48	125	173	275
Fe259.940	234	2502	3620	6122	604	216	820	67	1054	1121	78	5158	5237	24815
Ni232.003	140	43	70	114	15	26	41	119	297	417	14	28	42	103
Pb220.351	59	167	318	485	42	83	125	28	15	43	2	130	132	143
Zn213.856	422	360	41	401	51	16	66	194	31	225	194	122	315	454

Manifold configurations are described in detail in Chapter 4, sections 4.1 and 4.2

6 Applications of the microanalytical extraction system

Synopsis

The applications of an in-line microanalytical system for the rapid screening of solid environmental samples for heavy metals in risk assessment studies fully described in the previous chapters and elsewhere [228 229] is discussed here. Having established the analytical merits of the method as discussed under Instrumental configuration, two standard batch schemes viz.–the BCR three-stage sequential extraction scheme [137] and the DIN 19738 method, an in vitro digestion model for the assessment of bioaccessible fraction [230], were rendered on-line.

Thus, using a two-stage sequential scheme employing the BCR steps 1 and 2 reagents (termed simBCR in the following), the extractable fraction of heavy metals in BCR 701, SRM 1648 and a garden soil were investigated. Comparison was done with the revised BCR protocol, and the nitric acid scheme developed in this work and which had also been described before [231].

It is of importance to emphasise that the developed method is more of a semi-quantitative screening approach with the attraction of speed, ease of operation and automation potential.

The system offers the advantage of

- Obtaining the kinetic information required in long term risk assessment,
- Less sample (ca 5 mg) and reagent consumption needed in comparison with other methods,
- Enabling the monitoring of the processes as they occur in almost real time (instrument delay ca. 10 s)
- Reducing the total analysis time to less than 1 hour and
- Minimal sample preparation requirement. No extra separation or dilution steps are required before the in-line detection, thus affording greater convenience and more importantly, less contamination risks.

However, the method validation and standardisation has proved to be a challenge. In all operationally defined speciation methods, the amounts extracted depend on the extraction conditions used. The interpretation of data using the BCR reference material with certified values for the extractable contents is limited in on-line (dynamic) processes, because the operational conditions are significantly different. The development of laboratory internal standards built to simulate the expected fractions in real sample matrices was therefore embarked on.

For the first time, correlation was sought between the bioavailable fraction obtained using sequential schemes and a totally different physiological approach, i.e. an in vitro digestion model for assessing bioaccessibility (see section 6.2 below).

6.1 Assessment of bioavailability

The bioavailable fraction is thought to comprise the water soluble, ion-exchangeable and dilute acid accessible metals. This corresponds to the double distilled water (DDW), 1% nitric acid and perhaps 10% acid fractions for the nitric acid scheme. The first fraction of the BCR scheme is regarded as readily bioavailable, while the second fraction is potentially available. Thus, assessment of bioavailability involved the sequential extraction of metals from a diverse range of environmental samples using the BCR scheme and nitric acid scheme reagents.

Four types of samples were available for this work viz:

Category 1: Laboratory prepared reference materials i.e. standard mixtures with known compound ratios. This offers an inexpensive, tailored standard for evaluation of the developed system and for providing information and proof of concept studies.

Category 2: Standard reference materials with certified total metal content. This has the added advantage of homogeneity, being professionally prepared.

Category 3: BCR 701 with extractable metal content for four pre-defined fractions

This was the main quality assurance sample to evaluate the recovery of in-line fractions in comparison with the batch method.

Category 4: Plant material from the open market and real soil sample, prepared as required in the laboratory

This offers real conditions with high heterogeneity (lower precision), but also allows spatial and temporal distribution patterns to be studied. The total metal content was determined by several methods

Efforts on quality assurance using the samples of categories 1 and 2 have been described in the previous chapter (subchapter 5.3)

In the first section (6.1.1), the results of experiments carried out using BCR 701 under batch reference, simBCR and nitric acid extraction scheme conditions are discussed. The batch experiments provided valuable extraction information on elements not available in the certificate or literature. Thereafter in section 6.1.2, results of the analysis of SRM 1648 also using the various schemes at hand are presented and discussed. Finally, the rational behind the on-line acid extraction scheme and a comparison with batch experiments mainly carried out on plant sample material of category 4 are given in section 6.1.3.

6.1.1 Analysis of BCR 701, lake sediment reference material

The BCR 701 material is the only available on the market which provides the extractable content of four-predefined fractions of the improved BCR sequential scheme. The shortcomings of the use of the

material are two-fold. Firstly, the material is certified for the contents of only six elements. Secondly, the distribution of metals under batch static conditions is not expected to be the same under dynamic conditions (see discussion of extraction theory in chapter 2) posing a limitation of the reference material for verifying the on-line procedure developed in this thesis.

6.1.1.1 Total content vs. extractable content

Analytical information on the BCR701, SRM 1648 and a real soil sample (garden soil from a private property in Brandenburg region) used in the BCR experiments are given in Appendix Tables B1-B3. The BCR 701 lake sediment sample, which had been certified for extractable metal content in four defined fractions according to the BCR scheme, was used as sample for the batch as well as the online translation of the scheme- coined simBCR experiments.

Sequential extraction of 13 elements (Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) from BCR 701 according to the batch BCR procedure was carried out in replicate, using ICP-AES detection. Results show good agreement between values certified (Table 6.1) and those obtained experimentally for the different fractions (Table 6.2) and the total content which was determined separately on a 3g sample for the metals under study (Table 6.3) except aluminium (181, 1187, 1507, 27078 mg/kg for steps 1, 2, 3 and total contents respectively). With Al good agreement with literature values [232] was obtained for the extractable fractions, while the residual fraction and aqua regia extractable content with the current approach seemed to be heavily underestimated. This could be due to the incomplete release of Al from the silicate matrix under the current experimental conditions. Thus the mass balance was not achieved for this element. Still, the fractionation results obtained could be used in relation to each other. Residue analysis for the in-line methods was not possible due to the small quantity of material available and the attendant problem of contamination during digestion and analysis.

Since only six elements were certified by the BCR, results obtained for the other elements in this work could serve as indicative values for all further work.

Table 6-1: Extractable trace elements in sediment certified reference material BCR 701 following the BCR sequential extraction procedure

Element	BCR values Extractable fraction (mg/kg)			residue	Total
	Step 1	Step 2	Step 3	Indicative values only	
Cd	7.34 ±0.35	3.77 ±0.28	0.27 ±0.06	0.13	11.7
Cr	2.26 ±0.16	45.7 ±2.0	143 ±7	62.5	272
Cu	49.3 ±1.7	124 ±3	55.2 ±4.0	38.5	275
Ni	15.4 ±0.9	26.6 ±1.3	15.3 ±0.9	41.4	103
Pb	3.18 ±0.21	126 ±3	9.3 ±2.0	11.0	143
Zn	205 ± 6	114 ±5	45.7 ±4.0	95	454

Table 6-2: Experimental results

Element	Experimental Extractable fraction (mg/kg)			residue	Total
	Step 1	Step 2	Step 3	(aqua regia extraction)	
Cd	8	5	0.4	2.4	16
Cr	2	48	135	56	275
Cu	46	125	49	27	323
Ni	15	28	15	31	103
Pb	3	130	8	19	153
Zn	223	122	42	74	504

In implementing the scheme, the following observations were made which were deemed to affect the quality of results obtained.

Time span: Each extraction step requires a minimum of 18 hrs, the whole process taking a week of laboratory work.

Separation of phases after extraction: Problems arose even after centrifuging, as the solid residue easily disintegrated, more time was lost by use of Pasteur pipette to carefully remove supernatant. Also loss of solid could not be totally prevented.

Re-adsorption /re-distribution: Lower concentration of extracted metal was observed where the separation could not be done immediately after the extraction period had elapsed. This could be attributed to readsorption process.

In the BCR protocol, rinse liquids are discarded after each stage, without analysis. However, radiotracer measurements of the rinse phases reportedly confirmed that small amounts of metal were being lost, primarily all after the first extraction [233]. In the in-line version of the BCR extraction, no extra washing was required reducing the probability of analyte loss.

Table 6-3: Characterisation of BCR 701 reference sample (aqua regia extractable content mg/kg)

BCR 701	1)[1]	2)[2]	3)[3]	*given[4]
Al %	8.3	7.6	2.7	n.a[5]
Ca %	1.9	1.7	1.1	n.a
K %	2.1	2.2	0.3	n.a
Fe %	4.5	3.9	2.2	n.a
Mg %	1.9	1.3	1.1	n.a
Cr mg/kg	304	254	268	272
Cu mg/kg	234	273	310	275
Mn mg/kg	675	n.a	611	n.a
Ni mg/kg	111	97.3	99	103
Pb mg/kg	152	280	149	288
Zn mg/kg	463	451	499	454

Table 6-4: Time sampled extraction compared with batch BCR protocol

solution A	Time sampled extraction		End time	Batch extractions (16 h)	
mg/kg metal	10 min	60 min	960 min	experimental	certified
Cd	7	6	8	7	7.34
Cr	3	2	4	2	2.26
Cu	39	34	82	48	49.3
Ni	13	12	20	14	15.4
Pb	7	3	8	2	3.18
Zn	170	149	206	194	205
Al	253	170	467	181	n.a
Ca	5663	4893	5662	5669	n.a
Fe	169	126	346	78	n.a
K	107	110	152	139	n.a
Mg	454	557	738	759	n.a
Mn	160	140	184	182	n.a
Na	75	63	73	76	n.a

[1] A. Sahuquillo,G. Rauret,M. Bianchi,A. Rehnert and H. Muntau·Anal Bioanal Chem (2003) 375 : 578–583

[2] Guevara-Riba et al.. Sci total Environ (2004) 321: 241-255

[3] Experimental results, ICP-AES detection

[4] given from BCR indicative values

[5] n.a: not available

6.1.1.2 Kinetics of metal release

According to chapter 2, kinetic information about the leaching process is available.
Two concepts were followed in the study of leaching kinetics using BCR protocol. In the first, batch extractions were carried out with sampling of extract at three time intervals for metal determination (time series). Results in Tab 6.4 show that the different metals were extracted at different rates. For example 80% of acetic acid extractable Zn was obtained in the first 10 minutes, while for Na practically no change was seen over the time examined (i.e. instantaneous extraction). However discrepancies occurred in the amounts of Al, Fe and Cu extracted using the time series and conventional batch methods. The cause could not be clarified within the timeframe of this thesis, but is probably due to the peculiarities of these metals.
Results for the extraction steps 2-3 using reagent solutions B and D of the BCR scheme as well as residue analysis are given in Appendix Table A-4.

The second approach was using the online adaptation of the BCR scheme, i.e. simBCR as described in Chapter 8 under Methods. In order to test the feasibility, reproducibility and accuracy of the method, the BCR 701 lake sediment reference material was taken through simBCR in replicate.
The simBCR consists of sequential extraction with the first two reagents of the original BCR protocol i.e. 0.11 M HOAc, and 0.5 M $NH_2OH.HCl$. These two reagents are designed to release metals from the bioavailable and potentially available fractions of the sample matrix and as such were of particular interest in these studies. The third step of the original BCR scheme involving peroxide digestion at elevated temperature could not be accurately realised on-line and was left out. The metal isotopes Cd (111, 114), Cr (52,53); Cu (63, 65); Ni (60,61); Pb (206,208) and Zn (66,68) were detected in-line using quadrupole ICP-MS as detector.
Replicate analyses on BCR 701 gave precisions ranging from 2% to 30% rsd for the different metals, the poorest reproducibility found for metals at concentration levels near the detection limit. This moderate reproducibility is probably due to insufficient homogeneity, the presence of so-called hot particles, since the usual sample weight was only 5 mg. Sample amount had been chosen considering flow characteristics and the linear range of determination for the detectors used. Nonetheless, the results are regarded more than adequate for rapid metal screening.

A typical extraction profile, here for copper in BCR 701, is shown in Fig. 6.1. All other metal profiles are given in Appendix-Figures, Fig. F-2b.

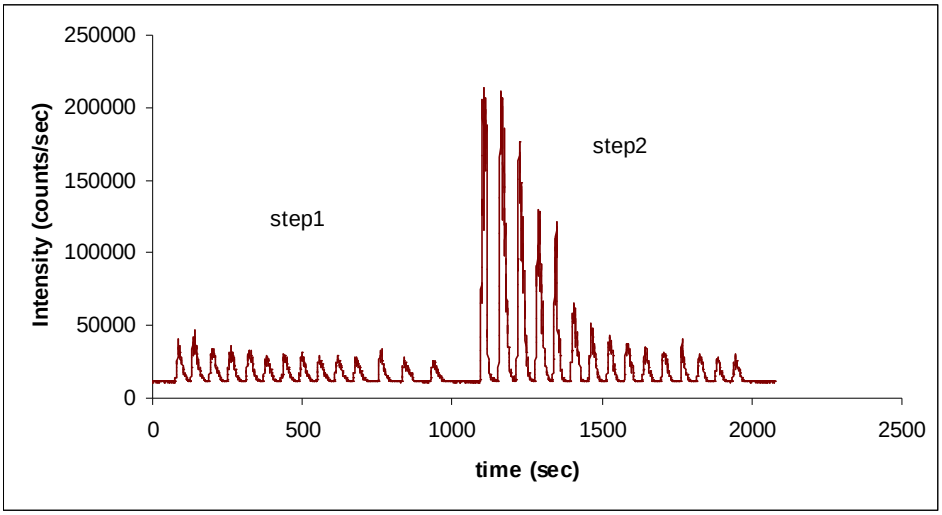


Fig. 6-1 Extraction of Cu using simBCR on-line procedure

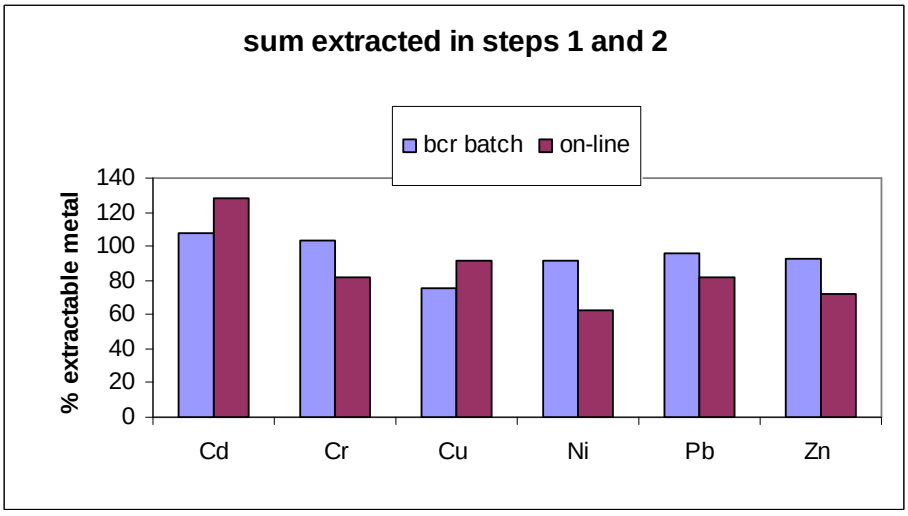


Fig. 6-2: Comparison of batch BCR procedure and on-line simBCR for the extraction of metals in BCR 701 reference material

Table 6-5: Extractability of metals in BCR 701 using on-line simBCR procedure in comparison to the BCR standard protocol

	bcr batch		simBCR		certified	
	Soln A	**Soln B**	**Soln A**	**Soln B**	A	B
Cd	7	5	10	4	7	4
Cr	2	48	6	34	2	46
Cu	48	83	41	119	49	124
Ni	14	25	12	14	15	27
Pb	2	121	4	102	3	126
Zn	194	102	163	68	205	114

Method comparability

Table 6.5 compares the concentrations of metals extracted in BCR 701 using the batch method and the simBCR with the certified values for each step. The extractability of copper under different extraction schemes is presented graphically in Fig 6.4 below.

In comparing results between studies that have applied different sequential extraction procedures, allowance must be given for variations due to the operational conditions, type of sample and indeed the characteristics of the sample material under investigation. In this work, the effective leaching time was about 5 min, as opposed to 16 hours equilibrium conditions in the batch protocol.

It was therefore surprising to see a reasonably good agreement of recoveries for step 1 in batch BCR and on-line simBCR methods. For step 2, less recovery was observed especially for Zn and Pb with ≈ 55% and 60% recoveries, respectively. It is worth noting again that the aim of this work was neither to find best conditions, under which the BCR certified result may be obtained, nor to replace the BCR protocol, but rather to develop a simple and rapid method useful for screening purposes.

6.1.1.3 Online Acid extraction scheme

Acid or acid mixtures have been widely used for the digestion of plant samples prior to determination of metal content.

From a practical point of view, the non-specific extraction method has a number of analytical advantages. The simple nitric acid leaching solution does not cause analytical matrix problems. Further, it is readily available in trace metal laboratories and affordable.

Comparisons of the results of extraction of metals in BCR 701 using the in-line 2-stage scheme with those of the revised BCR protocol and a nitric acid scheme are presented in Figures 6.3 and 6.4.

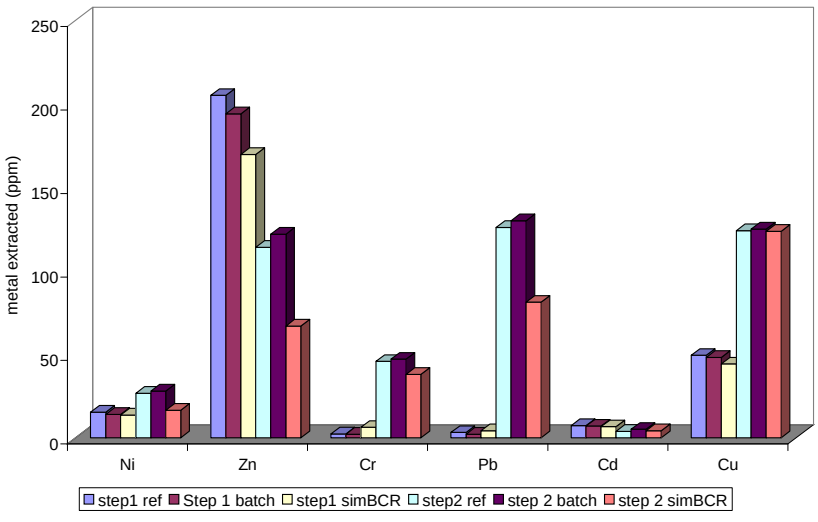


Fig. 6-3: Metals extracted from BCR 701 using BCR reagents in batch and online schemes

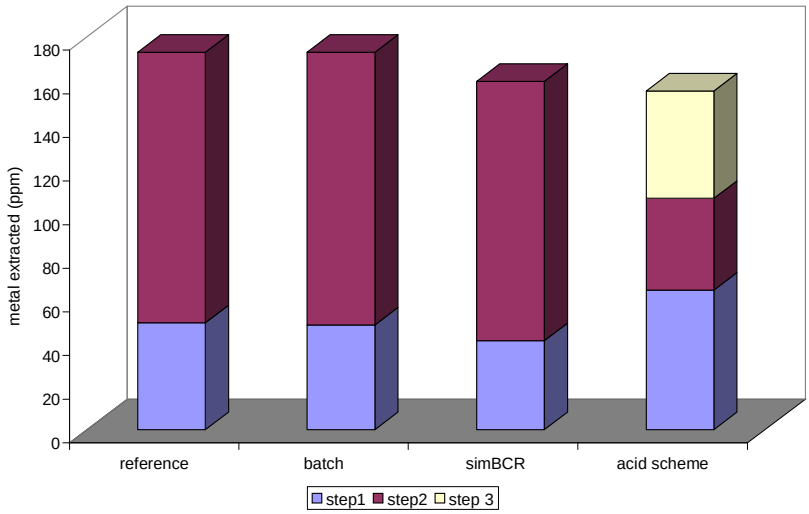


Fig. 6-4: Extractability of Cu in BCR 701 reference material using different protocols

*reference = BCR certified values, *batch = BCR found values, *simBCR = online extraction using BCR reagents, *acid scheme = on-line nitric acid extraction

Good agreement between the total amount extracted in steps 1and 2 of the simBCR and those obtained from steps 1-3 of the acid scheme is also evident for most metals extracted from BCR 701 (see Fig.6.4 for example). It must however be considered that the reasonably good agreement of results does not necessarily mean that this holds true in general, i.e. for other types of samples with variable chemical composition and different morphology. It might in turn only be an indication that the elements present in the particular BCR reference material investigated are quickly releasable.

The extraction profiles (extractograms) of metals leached from BCR 701 using the acid scheme have been compared with those of the simBCR (Appendix-Figures, Fig. F-2a and 2b).

6.1.2 Analysis of SRM 1648 using the on-line methods

As part of the validation exercise, SRM 1648 urban particulate matter reference material, for which literature data on extractable contents as well as some data on x-ray absorption fine structure spectrometry (XAFS) analysis exist, was chosen for comparison. Results of the simBCR and nitric acid extraction of metals in urban particulate reference material (SRM 1648) are given in Table 6.6. Recoveries of metals (except Al) in the 2 steps of the simBCR were between 70 and 100% of the total content, with Ni, Cu, Zn and Cd mainly in adsorbed, exchangeable and carbonate fraction defined by extraction with 0.1 M acetic acid.

Pb and Al on the other hand were distributed evenly between both fractions (steps 1 and 2).

Results for Cu and Zn are comparable with those obtained by Dabek-Zlotorzynska et al. [234] using ultrasonic assisted extraction. For Cd however, higher recovery ≈ 90% was obtained. Results of the acid scheme extraction of the same material indicate that Cd occurs mainly in the water soluble fraction. The nitric acid scheme distinguishes between water accessible (surface bound, adsorbed, inorganic salts) fraction and dilute acid accessible (carbonate, exchangeable, oxide) fraction. Thus ca 80% of total extracted Ni, Cd and Zn was in the water fraction, while Pb and Al were mainly found in the 1% acid fraction. Extraction profiles of the metals in SRM 1648 using the simBCR as well as acid scheme are available in Appendix-Figures, F-1a and b.

Table 6-6 : Results obtained with in-line extraction of metals in SRM 1648 using various schemes. Values are given in mg/kg

	BCR scheme				certified	Nitric acid scheme			
Metals	simBCR			Dabek et al.[234]	SRM 1648	step 1	step 2	step 3	step 4
	step 1	step 2	sum steps1-2	sum steps1-3	total content	DDW	1% HNO_3	10%HNO_3	30% HNO_3
Ni	54	6	60	n.a	82	52	5	3	4
Cu	344	75	419	425	609	323	181	67	26
Zn	4680	74	4754	3323	4760	4721	606	nd	nd
Pb	2205	2676	4881	n.a	6550	328	6699	158	260
Al	1068	1142	2210	n.a	34200	405	2314	845	416
Cd	66	1	67	42	75	43	10	2	nd

DDW deionised distilled water

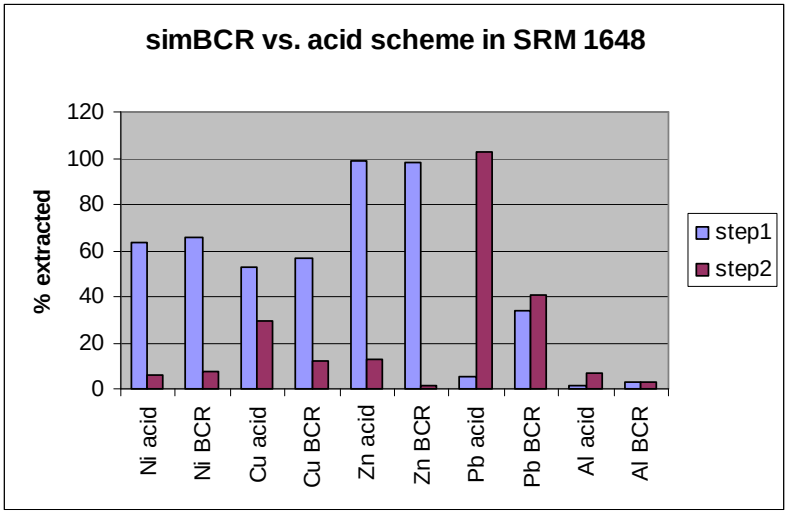


Fig. 6-5: Graphical representation of extractability of metals in SRM 1648 using two different on-line schemes

In comparing results of the acid extraction with those of the simBCR (see Fig. 6.5 above and also Appendix Fig. F-1), it was observed that for most metals investigated, the water soluble fraction of SRM 1648 corresponded to that of step 1 (0.1 M acetic acid) of the simBCR. This suggests that the metal species are mainly water soluble/loosely bound to the surface (probably sulphates) which is in agreement with the results of Huggins et al. [235] using X-ray absorption fine structure spectroscopy (XAFS). Pb required 1% acid to be quantitatively released, suggesting it's occurrence in the form of low water soluble sulphate or as oxide. Al was not quantitatively recovered in any of the schemes, probably being present as aluminosilicates or polynuclear hydroxo complexes.

6.1.3 Leaching kinetics of heavy metals in various environmental samples

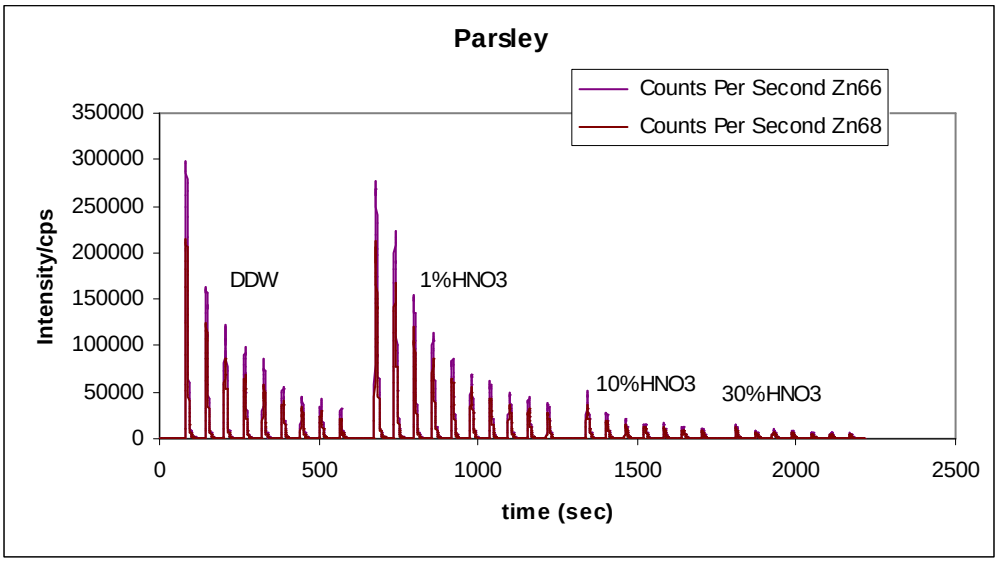
6.1.3.1 Extraction of metals in plants and soil

Several real samples were investigated using a four step extraction with water, 1%, 10% and 30% nitric acid as eluents. Some typical results (using multi element ICP-MS detection) representing basically different elution patterns are shown in Fig. 6.6a and Fig. 6.6b for a plant and soil sample, respectively. It must be noted that in having analysed many different samples and several of them twice and more, the data received allow for some general conclusions without implying that they can be taken as representative for the particular matrices.

6.1.3.1.1 Qualitative analysis

As illustrated in Fig.6.6a and 6.6b, the elution profiles of Zn in the plant sample differs from that in the soil sample significantly. In the plant sample, the water and 1% nitric acid leachable fractions are equally large and rapidly decaying. The subsequent treatment with more concentrated nitric acid does not lead to a significant enhancement of the leaching of the metal. This indicates that Zn is obviously easily accessible (surface bound) and present in the form of readily soluble salts.

a)



b)

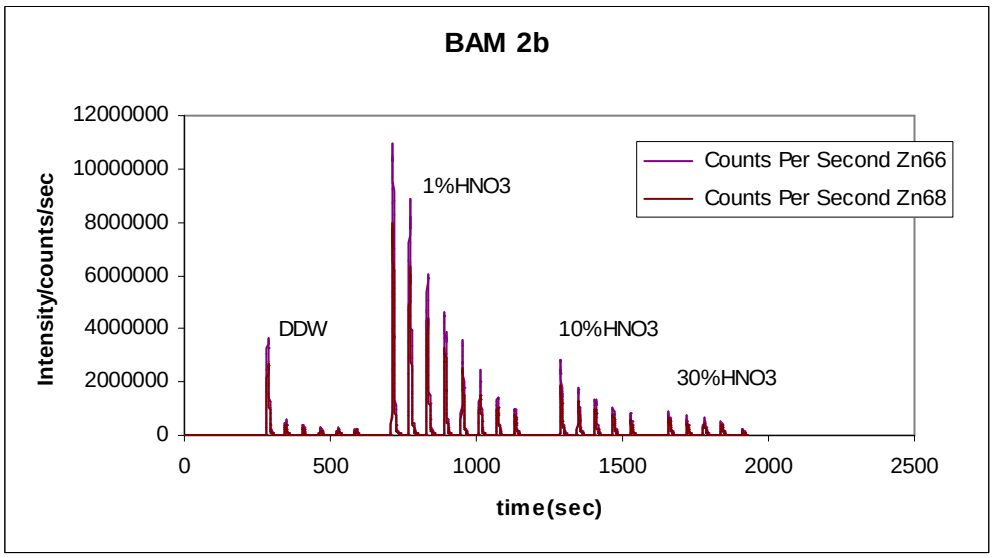


Fig. 6-6: Extraction profile of Zn from a) parsley sample (ICP-MS detection) and b) contaminated soil sample (BAM 2b)

The elution profile of Zn in the soil sample (Fig. 6.6b) is distinctively different in that a small water leachable fraction is followed by a significantly larger acid fraction. It is also evident that the decay of the leachate concentration is fast so that the element is probably also bound to the surface yet being less soluble in water.

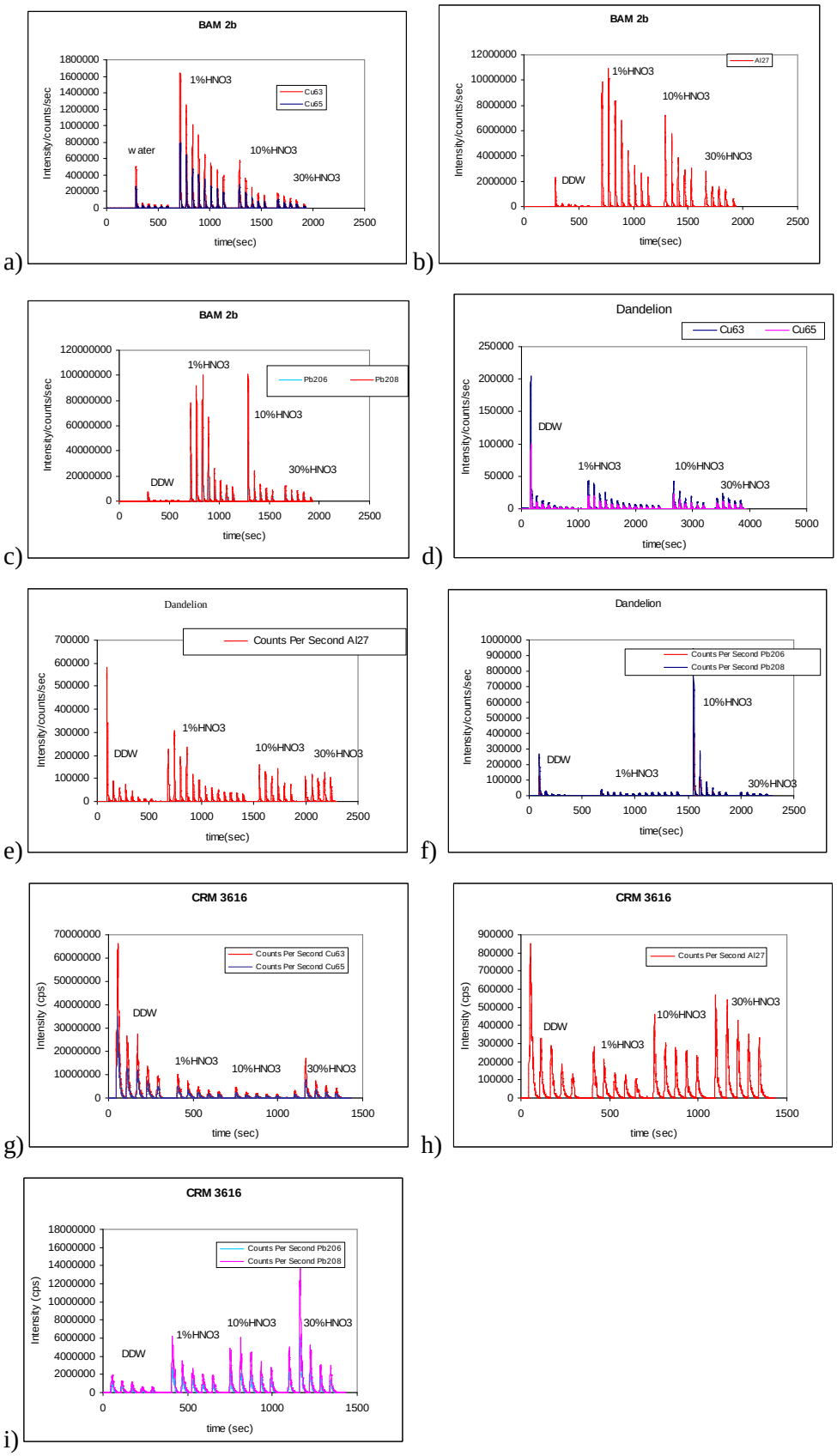


Fig. 6-7: Elution behaviour of 3 different metals Cu, Al and Pb in (a-c) BAM2b contaminated soil, (d-f) Dandelion and (g-i) CRM 3616 pyrrhotine ore samples.

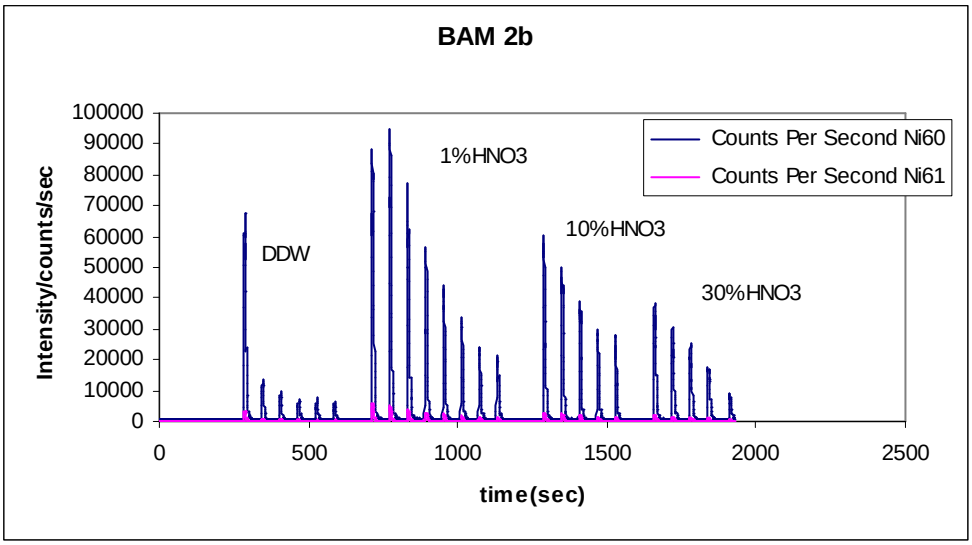


Fig. 6-8: Extraction profile of Ni in BAM2b, contaminated soil candidate sample

A comparison of the metals within a particular sample show similarities that indicate two metal groups viz. a mainly easily accessible/water soluble group and a mainly acid soluble group. The elution profile of Cu (Fig. 6.7 d), Zn (Fig. 6.6b) and Ni (Fig. 6.8) in BAM2b contaminated soil sample is characterised by a small water soluble fraction followed by a high concentration in the acid leachates. The decay rate of the metal ion concentrations in the water fraction is fast, while moderate in the three acid leachates. For lead only negligible amounts are found in the water fraction but high concentrations are observed in all three acid leachates. The decay rates are similar to those of the three other elements. Aluminium behaves like lead with the exception that the decay rate is considerably slower. In the plant matrices investigated, Cu again shows similar patterns to Zn and Ni. Here however, is a substantial water soluble, easily accessible fraction. A different behaviour is evident from the aluminium leaching profiles (see Figs. 6.7 b, e and h). Irrespective of sample matrix, the distribution is almost equal over all fractions and the decay, particularly in the two stronger acid leachates is slower. This indicates that aluminium is less well accessible compared to the other elements studied. Lead on the other hand occurs mostly in the 1% acid fraction, with a small water soluble portion.

In comparing the leaching pattern of the various elements in soil and plant materials (see Appendix Figs. F-1 to F-7), the following trends are apparent. The amount of water soluble metals in comparison to that leachable by acid is generally higher in the plant samples. A grouping of elements can be made in that Cu, Ni and Zn behave similarly within one matrix while Pb and Al though similar in some respects differ mainly with regard to the leaching kinetics.

6.1.3.1.2 Quantitative approach

As found before in the qualitative study, the fractions eluted differ significantly with respect to the element and the kind of sample material. The additional information that can be obtained is the extent of extraction in relation to the total amount present (i.e. after wet acid digestion). Results obtained for a selected number of samples are presented in Table 6.7.

For the urban particulate matter (SRM 1648) for instance, only 1% of Al but 99% of Zn of the total amount are eluted with water. The sum of the extractable fractions under given conditions is 11 % and 112% for Al and Zn, respectively. Taking the results of the plant sample (Spinach leaves) likewise considerably different amounts of elements in the water and the acid fraction are seen and the ratio of extractable elements to the total amount also differs. However, looking for a particular element and the same eluent it is observed that the percentage extracted relative to the total amount can be significantly different for soil and plant materials.

With few samples the decay (estimated from the time required to reduce the concentration to half of its initial value) was slow indicating a diffusion limited leaching rate. This can in principle be expected when the analyte in the bulk of the particulate sample is only slowly reached by the solvent and transport of the dissolved ionic form back to the interstitial volume must occur (see section 2.2.2 for discussion of extraction model).

Table 6-7: Online extraction of some environmental samples a) urban particulate matter (SRM 1648), b) spinach leaves (SRM 1570a)

Amount extracted as % total content								
SRM 1648	DDW	1%acid	10%acid	30% acid	SRM 1648	found mg/kg	mg/kg given	yield%
Ni	64	6	4	5	Ni	65	82	79
Cu	53	30	11	4	Cu	596	609	98
Zn	99	13	nd	nd	Zn	5327	4760	112
Pb	5	102	2	4	Pb	7445	6550	114
Al	1	7	2	1	Al	3981	34200	12

nd = not detected

Amount extracted as % total content								
Spinach	DDW	1% acid	10% acid	30% acid	Spinach	found mg/kg	mg/kg given	yield%
Cu	66	36	9	0,1	Cu	13	12	111
Zn	74	32	4	1	Zn	90	82	110
Cd	45	49	17	0,4	Cd	3,2	2,9	112
Pb	12	27	77	7	Pb	0,24	0,2	122
Al	1	2	1	1	Al	17	310	6

Effect of sample preparation on the extraction of heavy metals in biomatrices

Results (Fig. 6.9 below and Table 6.7) show that the sample preparation method affects the extraction behaviour of Al and Cd in vegetable samples from the same lot. Parsley and spinach were used as example matrices. For washed, blended and dried spinach samples (SWBT), Al and Cd had the highest concentration in the 10% accessible fraction, while the other methods unwashed (spdirty) and fresh scissored (spfresh) gave highest yields of these metals in the DDW and 1% fractions. A look at the intensities indicates that higher amounts of the metals were leached in the mildly prepared (spfresh) sample than in oven dried, blended and ground (swbt) sample. The highest intensity as expected was obtained in the unwashed (spdirty) sample again confirming the ability of the developed method to discriminate spatial distribution of metals. More details are available in the Appendix-Fig.F-6 Comparing the total metal contents of the samples given in Table 6.7, fresh, scissored plant material yielded highest Cd content indicating loss of analyte due to stringent conditions of sample preparation. Cu, Zn and Pb were relatively unaffected by sample preparation.

Although 100% extraction was not achieved with the online extraction procedure, yields for the various metals under investigation were reproducible, see Table 6.7, making a semi-quantitative assessment of heavy metal load possible. Under restrictive conditions of homogeneity and reproducibility, for the same sample type, an estimate of the total burden could be made by extrapolation of extraction yields. This means for example, Al was only extracted with 10-20% yield, therefore estimation of Al requires only multiplication by a factor considering the moisture content if and only if the above conditions are met.

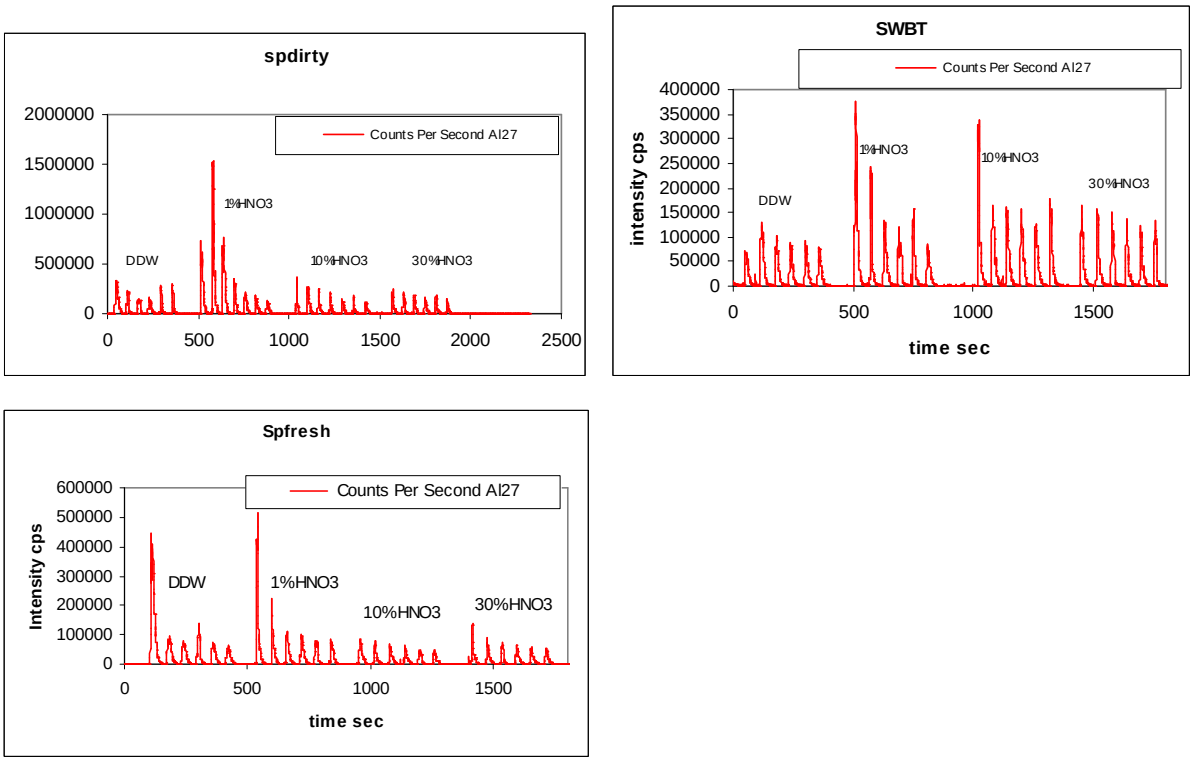


Fig. 6-9: Extraction profile of Al in spinach samples prepared differently

6.2 Assessment of bioaccessibility

DIN 19738 method for the assessment of bioaccessibility

The versatility of the microanalytical system was proven by adapting the DIN 19738 method for the assessment of bioaccessible fraction of metals. Here the BCR 701 reference material was carried through the extractions with synthetic digestive juices prepared as summarised in Appendix Table D-1. The procedure is outlined in Chapter 8 Materials and Methods, section 8.5. The online adaptation involved the sequential extraction of the sample with the same reagents as the batch method but using the general stopped flow procedure as had been described before in Chapter 4.
For the first time, correlation was sought between the bioavailable fraction obtained using sequential schemes and a totally different physiological approach, namely an in vitro digestion model for assessing bioaccessibility.

The online methodology offered the possibility of separate quantification of the metals leached by each extracting agent i.e. saliva, stomach and intestinal juices, unlike the total amount approach of the original DIN 19738 method, where the end exposure at the intestine is targeted.
The advantage is that the additional information on toxicity of the metals at the various organs can be assessed considering that absorption or damage to organs could occur at the different compartments on the way to the intestine. Thus it was interesting to observe that the amount extracted in the stomach exceeded that found in the intestine for most heavy metals (see Table 6.8). This is probably due to the fact that the acidic conditions in the stomach favour the extraction of metals which are then rendered fixed or inextractable in the intestine (e.g. as precipitates) under the highly basic conditions there.
In terms of bioavailability and bioaccessibility, studies based on stomach extract values are more stringent (less liberal) than those based on intestinal values. A comparison of the different schemes made by Oomen et al. [236] suggests that no consensus has been reached on which scheme gives the better assessment. At best, judgement can only be made according to the question at hand.

Table 6-8: DIN19738 extraction of metals in BCR 701 reference material

BCR 701	saliva	stomach	**intestine**	% bioavailable	residue	intestine+res	total content	stomach+res
Ni	3	35	**15**	15	85	100	103	120
Cu	26	173	**39**	14	100	139	275	273
Zn	34	203	**65**	14	209	274	454	412
Pb	nd	68	**23**	16	26	48	143	94
Al	690	2831	**651**	2	21800	22451	27061	24631

Interpretation of results

The feasibility of the microanalytical system for the rapid assessment of leaching behaviour of metals in various environmental samples has been proven. The method is an additional tool for risk assessment purposes. It has been possible to analyse some samples using notable international schemes i.e. BCR scheme, DIN 19738 method as well as a nitric acid scheme. Inter-comparison of data obtained is interesting because the BCR and DIN method are based on different principles, yet serve to assess the immediate and potential risk on exposure.

Fig 6.10 below shows a comparison between the values obtained for Al and Zn with the different schemes. There seems to be good agreement between the simBCR, acid and DIN methods. The view that the bioavailable fraction in the acid scheme is the sum of the DDW and 1% HNO_3 fractions was confirmed. For the DIN method, the ratio of metal concentration in intestinal fluid extract to the total metal content is defined as the bio-accessible fraction. The first step of the BCR scheme is shown to describe the most bioavailable fraction, the second step being a potentially available metal pool.

The results also indicate the suitability of the nitric acid scheme in predicting the immediate risk. The nitric acid scheme has the advantage of low cost, simplicity of preparation and non matrix interference at the ICP-AES in comparison with the DIN method and BCR reagents.

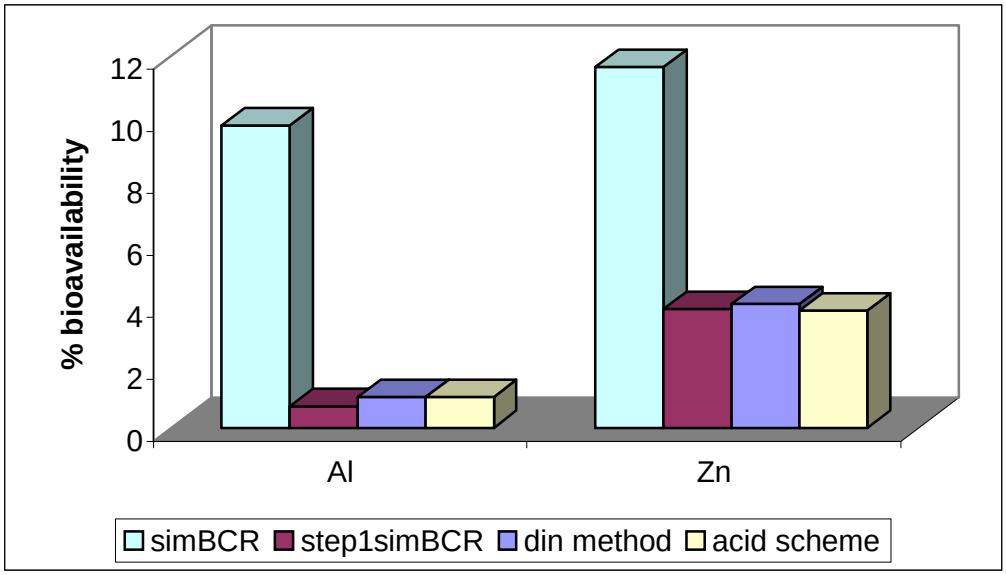


Fig. 6-10: Bioavailability estimates of Al and Zn in garden soil using three online schemes

7 Membrane-based method (Microdialysis-ETAAS system)

7.1 Dialysis concepts

Introduction

Dialysis was introduced in the 19th century by Graham [237]. It has been used for the separation of colloids from small molecules as in haemodialysis and much later found important technological applications like the industrial removal of alcohol from beer.

Dialysis is the process by which components of a fluid sample are separated according to diffusion rates across a semi-permeable, hydrophilic membrane into an accepting fluid. Depending on whether external energy has been applied or not, we distinguish between passive and active dialysis. The latter is not within the scope of the present work and shall not be discussed further.

7.1.1 Principle

In passive dialysis, the sole driving force of the separation is a concentration difference at opposite sides of the dialysis membrane. The membrane acts as a molecular sieve, allowing ions and small molecules below the molecular weight cut-off (MWCO) to pass through, while other molecules are held back. Mass transfer across the membrane is diffusion controlled. The rate of dialysis is dependent on the type and molecular properties of the membrane and the chemical milieu.

In classical equilibrium dialysis, the dialysis bag containing the sample is immersed in a vessel containing the acceptor solution (usually water or buffer). Since equilibrium dialysis is typically carried out under quiescent conditions, the donor and acceptor are kept stagnant. Here only 50% maximum recovery is achieved for equal donor and acceptor volumes. To improve recovery, the concentration gradient must be kept as high as possible e.g. by constantly removing the analyte from the acceptor channel i.e. constant or intermittent flow of acceptor. In principle quantitative recovery of analyte is possible (as in exhaustive dialysis). However, the transfer of ions of interest from sample into the accepting solution takes hours. Another disadvantage is the high volume of sample and *handling* requirement. Miniaturizing the fluid chambers reduces sample volumes required and dialysis time. In order to enhance dialysis, apparatus allowing the flow of acceptor were developed.

In dynamic dialysis, the two channels containing the sample and acceptor solutions are separated by a semi-permeable membrane. The most common dialysers incorporated in flowing systems are the sandwich type and the tubular hollow fibre type. The former consists of miniaturised sample and acceptor channels grooved on the surfaces of two halves of a glass or Perspex plate (planar) with the

membrane held between. In the tubular type, the acceptor channel is concentric with the donor channel, both separated by the hollow fibre.

7.1.2 Transfer efficiency

The transfer efficiency (or dialysis efficiency, relative recovery, recovery efficiency) can be described as a ratio of the concentration of given analyte in the acceptor solution to that originally in the sample solution. Usually, a dialysis efficiency of 3 - 30% is obtained with planar dialysers as applied in analytical flow through systems. The efficiency is a result of a combination of physical and chemical factors which can be manipulated to suit the particular analytical problem. The influence of physical factors like geometry of the dialyser-channel length, depth, radius, cross-sectional area and membrane characteristics (MWCO- pore size, thickness, chemical composition) on the dialysis efficiency have been well documented [238, 239]. The fundamental aspects of on-line dialysis, the manifolds and the interfaces for different detectors necessary for optimisation were described by Fang in 1993 [240].
Thus depending on the flow rate, ratio of donor to acceptor flow rates, the transfer efficiency can be manipulated. This flexibility opens possibilities not only for the optimisation of the dialysis efficiency but also allows individual analytical needs to be met.

7.1.3 Chemical effects

Properties of other ions present in the sample or acceptor stream affect the rate and efficiency of dialysis. The concentration gradient should favour the movement of analyte species across the membrane into the acceptor stream.
Ideally, the transfer efficiency should be independent of the concentration of analyte in the sample. However, due to membrane polarisation in the presence of ions, a lower efficiency is observed for low analyte concentration, and higher efficiency for the higher concentrations. This bias negatively affects the calibration. The overall ionic strength should therefore be balanced on both sides with an ionic strength adjustment buffer (ISAB). The buffer chosen should not react with the analyte of interest and not interfere with the instrumental detection. 0.1-0.5 M acid or salt solutions (e.g. potassium nitrate or sodium sulphate solutions) are usually selected as ionic strength adjustment buffers for in-line dialysis separations of inorganic analyte using sandwich-type units.
The mobility and type of counter ion also determines the dialysis rate. For instance, chloride ions diffused faster from an acidic (H^+) environment than a neutral or alkaline one [241]. This is because the hydrogen proton is faster, causing a ‘Donnan’ effect (pulling across a negatively charged ion to preserve electro-neutrality). Other chemical factors affecting dialysis are the presence of chemical agents like ligands. Complexing agents with high affinity for the analyte ions may inhibit dialysis by reaction with free analyte ions in the sample. On the other hand, addition of such agents to the

membrane as in permeation liquid membranes [242] or to the acceptor (perfusion liquid) enhance dialysis greatly.

7.2 Microdialysis (MD) today

Microdialysis is a form of dynamic dialysis involving the flow of perfusing acceptor solution through a small diameter tubing (probe) to which the dialysis membrane is attached. Microdialysis could be seen as a miniaturisation of continuous dialysis in which there is only one channel, the acceptor (perfusion) channel. The sample now occurs in an undefined (perhaps infinite) volume, while the acceptor volume is minimal. This means that the sampling does not deplete the concentration of analytes in the sample and the equilibria are not disturbed. This non-invasive property of microdialysis sampling and the fact that the chemical composition of sample is not usually modified makes it suitable for in-vivo studies. Indeed MD has found widest application in the neurosciences and pharmacology, for the sampling of various substances in brain extra-cellular fluid and in drug efficacy studies, respectively. The MD probe mimics the passive function of the capillary blood vessel, allowing compounds with molecular weight smaller than the MWCO to cross into or out of the slowly flowing perfusion liquid, while large molecules are held back.

The efficiency of microdialysis sampling is commonly reported as the relative recovery (RR), the ratio of analyte collected by the probe to that available in the sample. The relative recovery is influenced by factors such as perfusate flow rate, membrane properties, probe geometry (e.g. accessible volume) as well as analyte diffusion coefficient. Different probe designs are available commercially, in various configurations – hollow fibre, planar and concentric geometries and sizes depending on the tissue to be sampled. The most common commercial probes are concentric. The MD is implanted into the tissue to be in contact with extra-cellular or physiologically similar fluid. Membranes are made of biomaterials such as polycarbonates and cellulose acetate, while the tubing could be of Teflon material. Usually, MW cut-off of the membranes employed range from 10 to 100 Kda. The lower range is used to discriminate proteins and enzymes such that the dialysates do not need to be cleaned up prior to instrumental analysis (mainly by liquid chromatography).

The full potential of MD has yet to be exploited in the environmental analytical field. The group of Torto has published several articles on the sampling of metal ions from waste water and tomato fruit using commercial probes [243 244]. However, the determination has been off-line with the attendant risks of contamination and/or analyte loss since only microlitre quantities have been collected for analysis. The on-line in vivo determination of Mn in rat tissues has recently been described [245]. The issues of calibration and standardisation of such in vivo methods for quantitative purposes remain problematic.

The novel development of a microdialysis system hyphenated to ETAAS for the in situ sampling of metal ions from soil interstitial (pore) water is the main topic of this subchapter. The applications and importance in environmental field are discussed.

7.3 Modelistic view of microdialysis

During MD, analyte ions move from the interstitial fluid of the sample through the membrane into the perfusion liquid which may be collected at intervals for analysis or more conveniently analysed on-line. The prerequisite for microdialysis is that the sample be a liquid or at least a solid containing pore water or some other interstitial liquid.

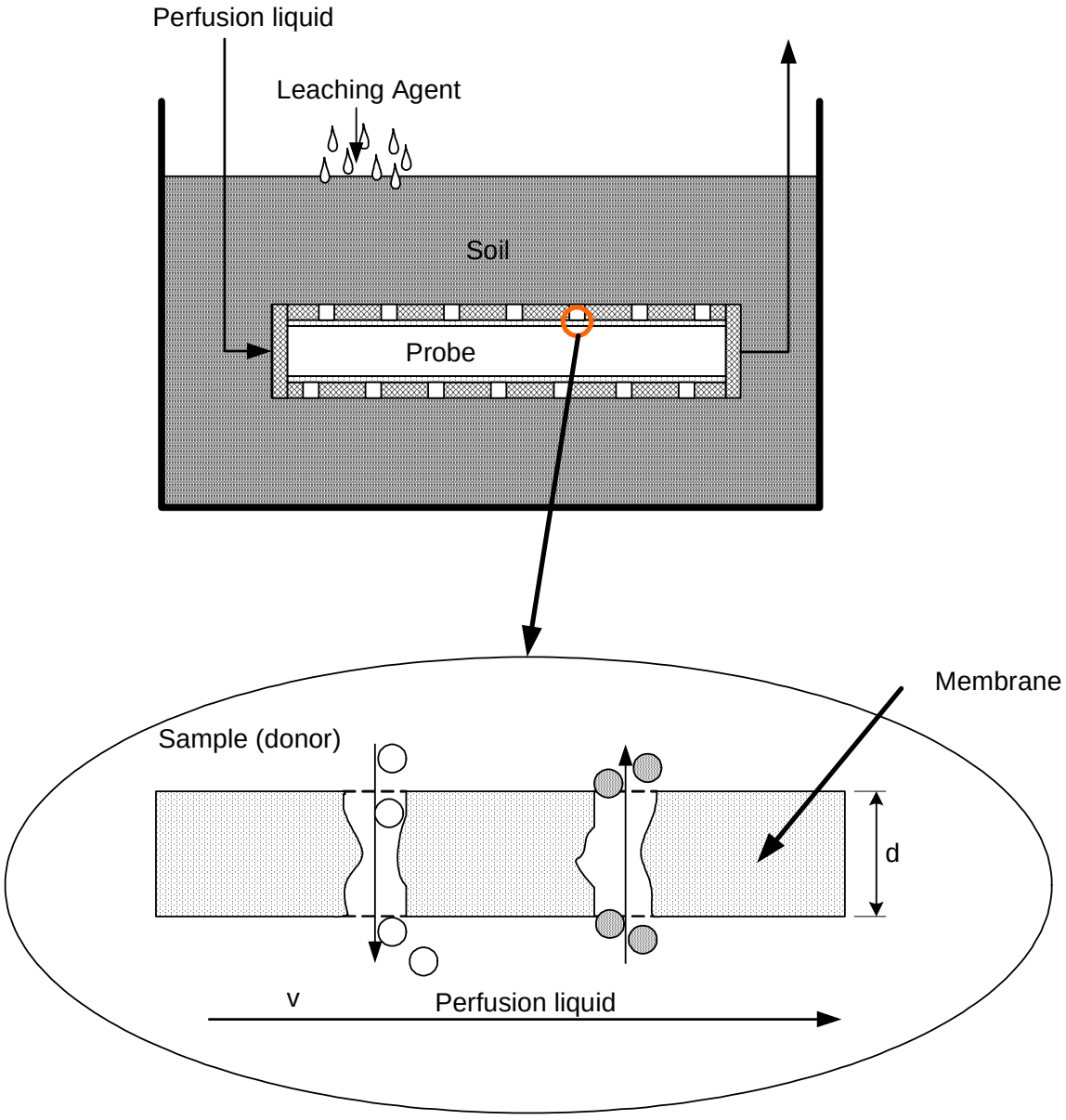


Fig. 7-1: Schematic representation of (micro) dialysis showing bi-directional function

v, flow rate of perfusion liquid; d, membrane thickness

7.3.1 Mass transfer model

When a suitable perfusion liquid is pumped through the microdialysis probe, the analytes traverse three different regions. Diffusion occurs from the bulk sample (or solid interstitial water) into the sample-membrane layer and then through the membrane into the membrane–perfusion liquid layer and are subsequently carried on by the bulk perfusion liquid to be detected in the effluent stream.
If the system is homogeneous, i.e. single phase for sample and acceptor, given that no chemical effects occur, the concentration of metal analytes in the perfusate gives a measure of the maximum dialysis efficiency (effective efficiency or relative recovery) of the probe under the almost equilibrium conditions (i.e. low flow rates) used. The perturbation of the sample environment is minimal at low flow rates [246].

7.3.2 Probe principle

Since the membrane is semi-permeable, the transfer of analytes is bi-directional (Fig. 7.1). This opens opportunities of manipulating the transfer efficiency and thus controlling dilution. Through choice of perfusion liquid, pH and addition of chemical agents, the chemical environment in the vicinity of the probe can be changed and the resulting relative recovery monitored.
Considering a model case in which a weak ion exchange buffer is made to perfuse a probe placed in a heterogeneous system as soil contained in a vessel, the metal content in the dialysate reflects the original dialyzable fraction (free metal ions, dissolved molecules) in the interstitial water in the ultimate vicinity of the probe. In this case, there is no convection or disturbance of solid-liquid equilibrium. The relative recovery (RR) is affected by the resistance of the sample medium (e.g. due to turtuosity)

However, if an external flow of an extractant solution is introduced onto the soil column via an auxiliary (secondary) flow (see Fig. 7.2 below), a dynamic (non-equilibrium) situation is produced, and the efficiency of dialysis would be additionally affected by the partitioning of analyte between interstitial fluid and the introduced extractant. As a result, “vertically integrated” information about metal release from the soil column above the probe is obtained.
The concept can be extended to using variable leaching partners of different strengths as in chemical sequential extraction schemes for the fractionation of metals in soil and sediments. The analogies with and difference from the microcolumn experiments of the first part of the thesis are discussed separately in the next sub-chapter. The fate and transport of metals released can easily be monitored over time and space using a probe array.

7.3.3 Retro-dialysis

Apart from in-situ sampling, micro dialysers could be used to change the ionic milieu directly at the vicinity of the probe by careful choice of perfusate. For instance, introduction of acid or organic ligands or other releasing agent to the soil through the dialysis probe would simulate the real scenarios of acid rain and localised accidental spill respectively. This is reminiscent of the principle of retrodialysis whereby the bi-directional nature of microdialysis sampling allows infusion of agents through the microdialysis probe to give a localized delivery of the infused substance. [247,248]. The formed product can then diffuse back into the probe. In this case the response of the probe reflects the change in analyte concentration in the microenvironment of the probe. This means that a particular pollutant can be applied to the micro-environment of the probe while at the same time, the behaviour (e.g. transport) and 'effects' can be monitored.

Novel applications of such stimulus-response technique were tested in the laboratory soil column for proof of concept and the findings reported [249 250] and discussed below.

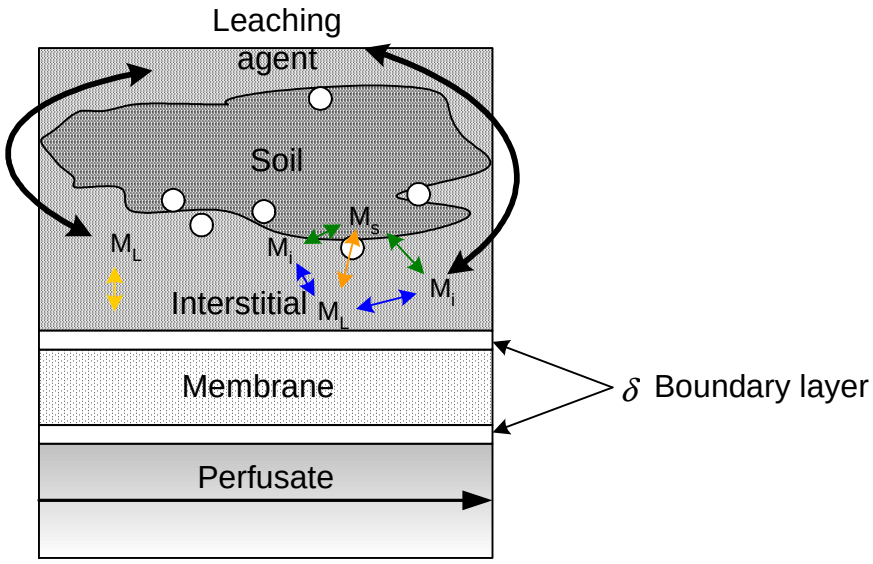


Fig. 7-2: Schematic representation of mass transfer in heterogenous system

M_s, M_i and M_L represent the metal "concentrations" in solid, interstitial fluid and bulk extractant liquid phases, respectively.

7.3.4 Probe geometry

The microdialysis probe acts as a barrier to molecules above the MWCO of the membrane. Assuming that no further interactions occur between the molecules and the membrane material, the flow of metal ions from the interstitial fluid surrounding the probe is controlled by the concentration gradient. Ions

in solution must pass the solid-liquid interface into the dialysis membrane and travel further to be dissolved in the perfusion liquid.

Bungay and co-workers [251] introduced mathematical expressions to describe mass transfer processes in microdialysis. The resistance to diffusion through the dialysate and membrane is governed by the effective membrane dialysis length (EDL), the inner and outer radius of the membrane, the outer radius of the inner cannula, the diffusion coefficient through the membrane and dialysate, and the void volume of the membrane. They concluded that the efficiency of dialysis is enhanced by short distance, thin membrane layer, high surface area and accessible volume.

7.4 Hyphenation of MD to AAS

7.4.1 On-line analysis of perfusates

For the sensitive determination of metal ions in dialysates, atomic spectrometric techniques, such as electrothermal atomic absorption spectrometry (ETAAS), have been commonly employed

These studies used off-line collection of the microdialysis sample and subsequent analysis by ETAAS. However, the sample volumes collected are only a few microlitres and evaporation and contamination may be problems with manipulation off-line. For high temporal resolution, the number of samples to be analysed makes the conventional off-line procedures unattractive being time-consuming and tedious. Also, the analysis of complex matrices is often hampered by spectral and non-spectral matrix interferences, which are not completely circumvented despite the development of efficient background correction techniques [252]. As a consequence of the intrinsic molecular-size discrimination of MD, the on-line analysis besides sampling also offers a unique sample clean up especially for complex highly organic environmental samples like sewage sludge and compost.

On-line microdialysis sampling coupled with ETAAS thus provides many advantages such as in situ sampling and measurement, simplified sample preparation, rapid analyses and dynamic monitoring of trace elements in living systems.

7.4.1.1 Procedure

The microdialysates were on-line delivered to the graphite furnace by pumping through a PTFE tubing attached to a constructed sampler arm (depicted in Fig. 7.3). The time-resolved signals were processed in the peak area mode. The operating parameters of the graphite furnace program for analysis of the clean microdialysates were those recommended by the manufacturer and are given in Appendix-Table MD-1. Working range limit for Pb, Fe and Cu were 50, 30 and 20 ppb respectively for 20µl sample. Typical calibration data for the GFAAS detection are also given in Appendix Table MD-2 to MD-4 for Cu, Pb and Fe respectively, all generated using the DINTEST software.

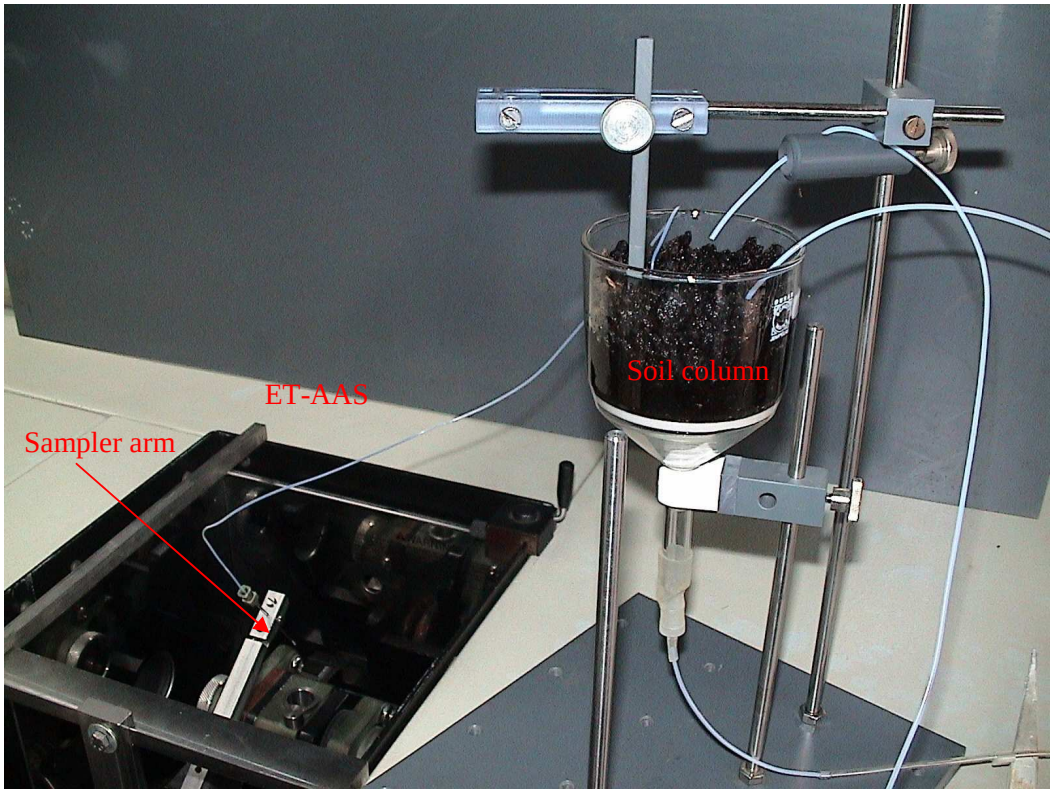


Fig. 7-3: Laboratory implementation of MD-ETAAS for in-situ sampling of metals from soil

At prefixed times the sampler arm was actuated to insert the tip of the perfusate delivery tube into the graphite tube. During this operation (5 s), the microdialysis pump was stopped to assure repeatable injection of microdialysate volumes into the atomizer. Then, the microdialysis pump was actuated to dispense the perfusate solution for 1.0 min, so that a small, well-defined plug of dialysate (10 µl) is introduced into the discontinuously operating detector. Once the sampling step of the ETAAS was concluded, the pump was again stopped for 5 s, and the sampler arm returned to the waste position, whereupon the ETAAS program was started. The total time for a single measurement including sampling and detection was merely 2.5 min, so that a reasonably high temporal resolution (with a delay time of only 6.5 min, i.e. time required to fill injection line) was obtained by the in-line procedure.

7.5 Experimental

The experiments consisted of those carried out to investigate the characteristics of MD in aqueous solution and the in-situ feasibility studies carried out in a model soil column.
First, the effect of flow rate, probe geometry, membrane type on the dialysis efficiency was studied. Thereafter, investigation of the chemical effects like acceptor and donor composition, the presence of other ions and chelating agents, on the performance of the MD probe followed. The applications of the characterised probe to monitoring ion-exchange and metal release processes in laboratory scale (model) soil matrix were investigated. Finally, dynamic stimulus-response in the form of simulated acid spill (leaching circulation) and monitoring of soil metal content on localised input (retro-dialysis) were studied.

7.5.1 Experimental procedures

7.5.1.1 Construction of microdialyser probe

The custom-made capillary microdialyser (shown below in Fig. 7.4) with an effective transfer length of 3 cm was constructed from a single cellulose regenerated hollow fibre (4 cm long, 200 µm i.d., MWCO ≈5 kDa) obtained from a bundle-type probe intended for haemodialysis. Each end was inserted into a short piece of Tygon tubing previously swelled by immersion in toluene for 10 min. After evaporation of the organic solvent, the connection was tight enough to withstand the typical flow rates exploited in microdialysis. To prevent mechanical stress over the membrane surface when inserting into soil matrix, the probe was housed in an extra PVC cylinder. The latter consisted of tubing (4 cm long, 3.0 mm i.d.) into which several holes (400 µm diameter) had been drilled. For easy placement of the hollow fibre, the tubing was cut longitudinally into two parts; a piece of soft wetted polyurethane foam was inserted to fill the gaps between fibre and PVC cover and finally the housing was clamped together with two O-rings. The tubular dialysis membrane was soaked in water prior to implementation into the protective cover.

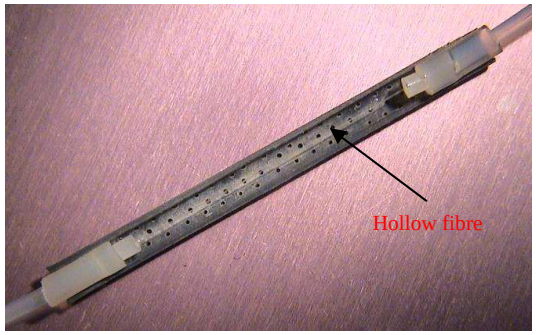


Fig. 7-4: Close up picture of the hollow fibre dialyser

Soil column (open)

This was a standard Buchner funnel (5.0 cm diameter), the outlet of which was connected to the peristaltic pump through Tygon tubing. To avoid loss of solid particles, a cellulose acetate filter was placed at the bottom of the funnel and the weighed soil sample filled to a height of about 3cm below the rim.

Implementation of probe in soil sample matrix

The membrane tubing with the protective cover was fixed on a holder that enabled implantation of the probe at different depths. To avoid metal contamination, all units in contact with the soil were made of plastic (PTFE or PVC) materials.

7.5.1.2 Characterisation of probe

Effect of flow rate, probe geometry on dialysis efficiency

The transfer efficiency of a given metal ion by dialysis depends mainly on the sample pH, ionic strength and the presence of complexing agents.

For the purposes of characterisation of the constructed probe first in solution, 10^{-3}M $CaCl_2$ solution was employed as perfusion liquid, and the probe immersed in 25 µg/L to 5 mg/L mixed standard solutions of Pb, Cu and Fe. Experiments were performed at two different perfusion rates i.e. 2 µl/min and 10 µL/min.

Influence of chemical effects

The effect of complexing agents on the microdialysis of metals in aqueous medium was studied by replacing the sample solution with a solution containing a mixture of metal standards in 1) EDTA and 2) citrate solution. The perfusion liquid remained 10^{-3}M $CaCl_2$ solution

Preparation of quartz sand model sample

Quartz sand used for microdialyser characterization was washed with water and thereafter dilute nitric acid in an ultrasonic bath and the liquid decanted. For spiking, a standard Pb solution was added till the sand was fully submerged. The mixture was left in the ultrasonic bath to “equilibrate”, after which excess solution was decanted.

Characterisation of probe (solid medium)

The dialysis efficiency of metal ions from the spiked quartz sand model sample was investigated under the same conditions as with the aqueous standard above, at a perfusion rate of 10µl/min only.

7.5.1.3 General procedure for metal sampling in solid (interstitial fluid) matrices

For the leaching experiments, 10 g of soil sample were dry packed into the column. The soil material was wetted with the minimum volume of a 10^{-3} M $CaCl_2$ solution, so that the liquid head above the soil column was maintained at a level of about 5 mm. Automated micro-sampling of metals was performed by insertion of the probe at a level of 1.5 cm beneath the soil surface. A multi-channel peristaltic pump furnished with Tygon tubing was used to provide the extracting agents to the soil column and also to maintain a constant flow of fresh solution through the solid material. This was optimised by setting the flow rates at the inlet and effluent streams at 0.5ml/min. To drain the column, the outlet of the funnel had been connected to the peristaltic pump tubing through a Nylon tube adapter.

In these experiments, reagents were provided in sequence ranging from mild 10^{-2} M $CaCl_2$ to acidic solution (10^{-3} M HNO_3). For studying the effect of competing ligands, 10^{-4} and 10^{-2} M EDTA and citrate were used.

Metal leaching studies

For monitoring both the kinetics of metal release in soil samples and the concentration changes at the soil-liquid interface, the analysis was initiated by continuously pumping the perfusion solution (10^{-3} M $CaCl_2$) through the implanted microdialyser at a flow rate of 10 µl/ min, while the diffusate stream was directed to waste, as shown in Fig. 7.7. The peristaltic pump was synchronously turned on and a stream of identical electrolyte concentration as the perfusing liquid delivered a top the implanted probe for a preset time interval. Equilibration times of 30 - 45 min were typically needed to attain a stable baseline level. Thereafter, the diluted electrolyte solution was replaced by an influent leaching reagent stream that was provided to the open soil column at a flow rate of 0.5 ml/ min and allowed to pass through the column at the same rate. At prefixed times, the autosampler arm was actuated to insert the tip of the dialysate delivery tube into the graphite tube and metal content of dialysate measured as described under general analytical procedure. The procedure was repeated till no significant change in absorbance was registered and the next leaching agent was introduced and the cycle of measurements repeated.

As a check on the leaching profiles, in some experiments, for particular releasing agents, the soil column outlet (effluent) fractions were also continuously collected in polyethylene vessels for off-line analysis. In that case, the ashing temperature of the ETAAS program was raised to 850 ºC using the recommended Pd-Mg $(NO_3)_2$ chemical modifier.

Stimulus-response

In the stimulus-response experiments, the dilute electrolytic solution (10^{-3} M $CaCl_2$) employed as a perfusion liquid in this work was replaced by dilute nitric acid solution 10^{-3} M to simulate the effect of

an acid input, such as an accidental spill, onto the soil under quiescent conditions. As such, the ancillary flow of eluent propelled by the peristaltic pump was not used.

Further experiments were conducted aimed at evaluating the capability of stimulus-response schemes for fast in-situ screening. Hence, the concentration of nitric acid in the perfusing stream was increased up to 0.1 M, and the response of the soil sample was continuously followed by analysis of the dialysates at the ETAAS.

In the next chapter, the design and construction of the hollow fibre microdialyser is discussed. The performance of the probe in comparison with commercially available models at detecting small changes in the microenvironment is also reported. Finally the applications of the system for monitoring the leaching of ions from environmental compartments, stimulus-response studies and other environmental studies are described.

7.6 Results and discussion

7.6.1 Microdialysis: more than a continuous sampling method?

Synopsis

This part discusses the design, construction and characterisation of a home made microdialysis probe with a higher surface-to-volume ratio than the commercial probes. The performance characteristics of the dialyser for sampling metal ions were initially evaluated in aqueous matrices. Maximum extraction fractions (EF) expressed as the ratio between the dialysate concentration and the initial metal concentration in the sample were evaluated for several metals.

Further, the development and assessment or suitability of a hyphenated MD-ETAAS method for in-situ (on site) continuous sampling and monitoring of metal ions during leaching processes at real time are also discussed.

The continuous flow system for monitoring of leached trace metals from soils as depicted in *Fig*s. 7.4 and 7.7 comprises a miniaturized flow-through membrane-based separation device hyphenated to ETAAS for sampling purposes and on-line analysis of the microdialysates, and an auxiliary continuous flow system to provide the leaching reagent to the laboratory-made soil column.

7.6.1.1 Probe characterisation (homogenous system)

The dialysis efficiency was found to be much higher for metal ions sampled from standard aqueous solutions. This can be explained by the higher surface area to volume ratio of the constructed probe.

The effect of types of membrane on dialysis efficiency has to be carried out for the type of analyte of interest. Research by various workers [253],[254] show the suitability of regenerated cellulose acetate material (5-30 KDa MWCO) for the dialysis of metal ions.

The dialysis efficiency in a homogeneous system was tested in aqueous and in solid model samples using the general set up illustrated in Fig. 7.5 and 7.7 below. For this, standard solutions of three metals in dilute nitric acid and a spiked quartz sand sample which had been equilibrated in a $CaCl_2$ buffer as interstitial liquid were used as substrates. Perfusion liquid was dilute nitric acid with flow rates of 2µl/min and 10µl/min. In order to be near to real field conditions, dialysis was done under quiescent conditions i.e. no convection contribution to the mass transport. Also no chemical effects were envisaged.

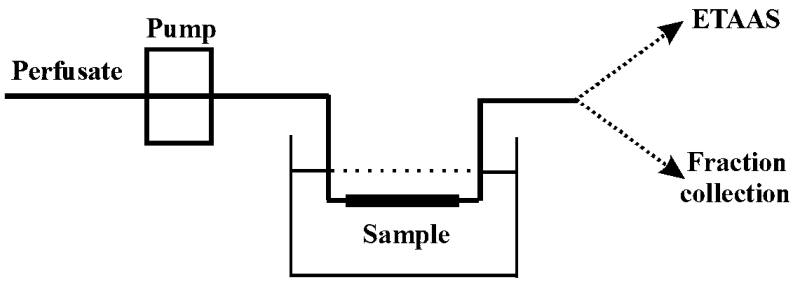


Fig. 7-5: Diagram showing general micro dialysis set up for the sampling of metal ions

As expected, the maximum extraction fraction was smaller with the faster flow rate, [255,256] since there is less time for 'equilibrium'. Relative recovery ranged for the metals studied from 70-98% for the 2 µl/min and 25-50% at 10µl/min. This is indeed an improvement over the commercial probes, [244], probably due to the geometry. As opposed to commercially available configurations, the hollow-fibre linear design allows the use of perfusion flow rates within a wide interval (viz. 1.0-60 µl /min), and thus, near real-time measurements may be accomplished. An exponential relationship between dialysis efficiency and perfusion rate was attained from 1.0 to 10 µl/min (the higher the flow rate the lower the recovery), which is in good agreement with previous observations [256].

7.6.1.2 Probe characterisation in control soil (quartz)

Quartz soil that had been prepared and spiked with metal standards as described in the experimental procedure section above, was used as model for further characterisation of the MD probe.
In the model (quartz) soil containing homogeneous particles of defined morphology i.e. distribution of pore size, the relative recovery was somewhat reduced. This is attributed to turtuosity, since organic matter and other constituents of a 'real soil' were absent. Tortuosity describes the tortuous pathway that a molecule must traverse through the sample (tissue) as a result of which the effective diffusion through the tissue is decreased.

7.6.1.3 Investigation of chemical effects

The positive effect of the presence of other ions (oppositely charged) on the dialysis efficiency of metal analytes can be explained by the 'Donnan' effect i.e. the fast migration of ions of opposite charge causes an accelerated transfer of the analyte ions to preserve electro-neutrality.
For instance, the dialyzable fraction of chloride in soil increased as the pH was lowered using the constructed probe. This was explained by the fact that H^+ ions travel faster across the membrane than the chloride, causing a pull on the chloride ions to preserve neutrality [241].

It is expected that the presence of complexing agent in the sample reduces the free metal ion content thereby decreasing the dialysis efficiency. This is on the assumption that the metal complexes formed are themselves not readily dialyzable. This was tested by introduction of citrate and EDTA into the standard metal solutions used as sample. As expected, the dialysis efficiency was reduced drastically as shown in Fig. 7.6 for an aqueous standard solution.

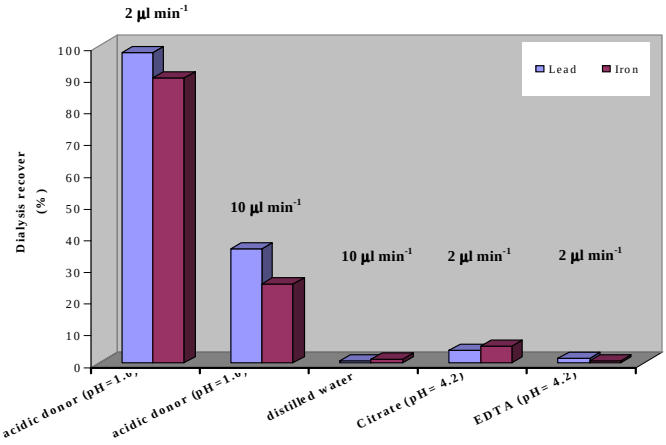


Fig. 7-6: Dialysis of metal ions from aqueous standard solution with and without ligands

7.6.2 Leaching of metals from real soil sample

The application of the microdialyser to in-situ monitoring of heavy metal release and transport was tested on a laboratory scale using a soil sample in a column as model (see Figs. 7.4 and 7.7).

The effects of acid and complexing agents on the recovery of metals from soil were studied using two approaches. In the first approach, acid was pumped through the soil body using an auxiliary line as shown in Fig 7.7 below. For the acid leaching studies, the perfusion liquid was kept as 10^{-3}M $CaCl_2$, while the leaching agents, i.e. neutral electrolyte and dilute nitric acid, were applied successively by continuous pumping through the soil column. Metal content of the dialysate was monitored continuously at the ETAAS

In the stimulus-response (second) approach, the dilute electrolytic solution (10^{-3} M $CaCl_2$) employed as a perfusion liquid in this work was replaced by dilute nitric acid solution 10^{-3} M to simulate the effect of an acid input, such as an accidental spill, onto the soil under quiescent conditions. Further, the concentration of nitric acid in the perfusing stream was increased up to 0.1 M and the response of the soil sample was continuously followed by analysis of the dialysates at the ETAAS

7.6.2.1 Acid leaching

At acidic pH, it is expected that the release of metals is increased, yielding free metal ions for dialysis. However, at the same time, the H^+ ion is faster which causes a net positive charge on the acceptor side of the membrane, repelling the transport of metal ions across the membrane. This hypothesis was tested by introducing nitric acid into the soil column by means of an auxiliary flow (see Fig 7.7 below).

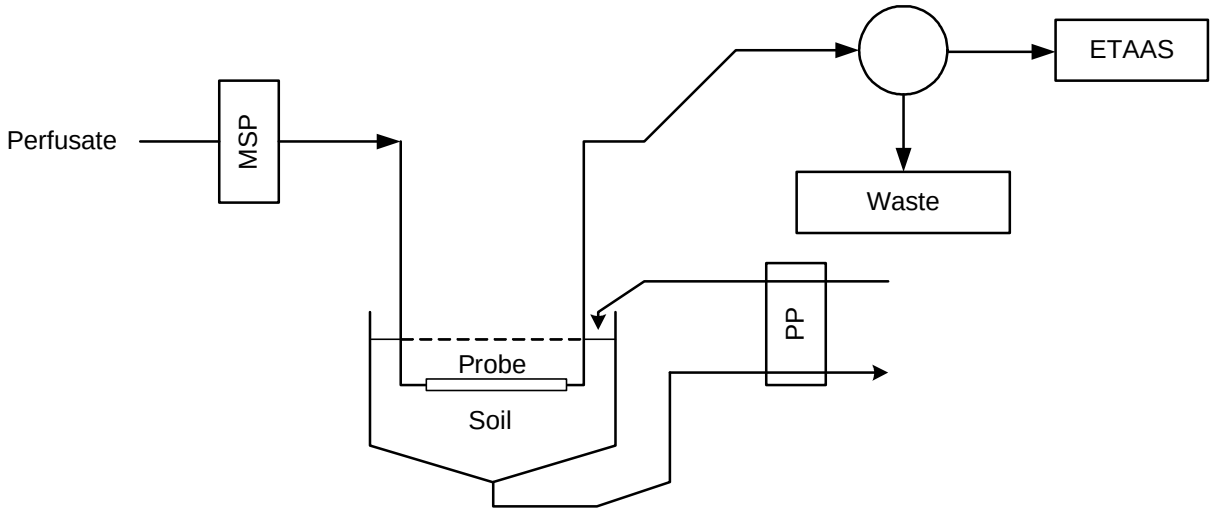


Fig. 7-7: Schematic representation of microdialysis manifold for metal leaching studies

The results obtained here is a net information i.e. integral sum of activities on the soil layers above the probe simulating seepage down the solid column as had been discussed before under Modelistic view (subsection 7.3.2, page 102)

Fig 7.8 below shows the metal release in changing from conditioning buffer to 10^{-2}M strength and then to acid leaching agent. Using $CaCl_2$ solution, a weakly bound, easily accessible fraction was released, reaching a plateau after ca. 60 min.

This indicated that metal was constantly released to compensate for any dilution effects of the flow of extractant. On switching to acid, a peak which could be attributed to a fast dialyzable fraction was observed followed by a slower release of a less accessible (probably bound to humic substances) fraction of higher concentration.

It is of importance to note that the leaching information obtained by analysis of collected effluent fractions from the column as a check on the dialysis experiments gave evidence of only one fraction, probably due to the column geometry and longer sampling interval. This suggests that the probe is well suited to detect changes in metal concentration at the microenvironment, where leaching took place.

It is plausible that the observed increase in dialysed Pb could be a result of a fresh release of soil-bound Pb or only a manifestation of the now favourable (ionic composition) conditions of dissolved Pb. This was tested by acidifying a filtered dialysate (using $CaCl_2$ as perfusion and leaching agent) to pH 4 and then pH 3. No significant change was observed, indicating that the increase of dialysed Pb was indeed from fresh release of soil-bound species.

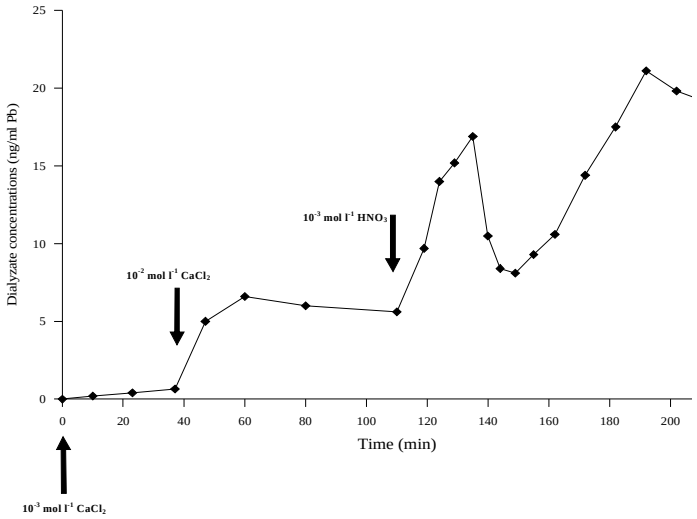


Fig. 7-8: Monitoring of leaching profiles of lead from a sewage sludge-amended soil by sequential application of different extracting reagents.

The results of this experiment could be compared with those of stimulus-response described below. The information obtained in the latter is as a result of local introduction of acid (as perfusion liquid) through the membrane.

7.6.2.2 Influence of ligand

Fig 7.9 below shows the change in concentration of Pb in the perfusate in the presence of EDTA as auxiliary flow. Contrary to expectation, a significant amount of Pb was dialyzable. Also, a higher recovery of Pb was seen on increasing the concentration of EDTA.

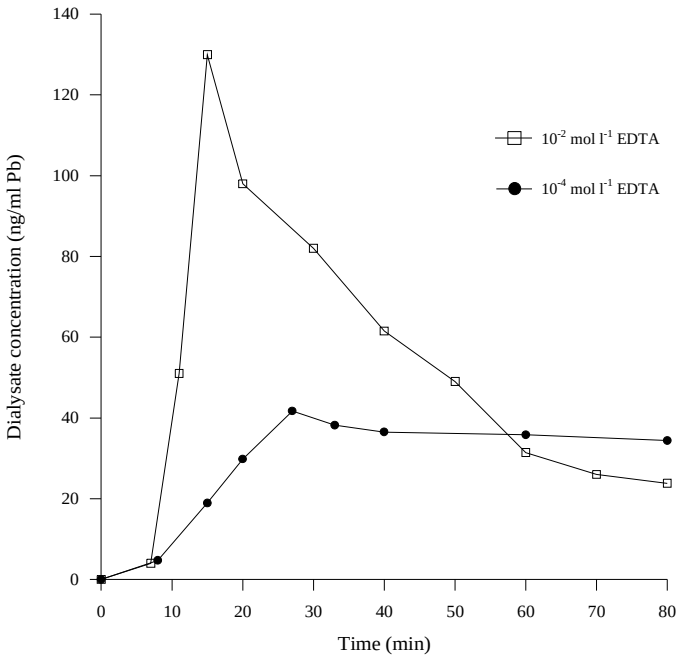


Fig. 7-9: Lead mobility in a high-organic-content soil sample in the presence of different concentrations of EDTA.

It is well known that the soil components include metal binding ligands which may affect the dialysis of metals. Two counter processes are to be considered in the leaching of metals in the presence of strong complexing agent like EDTA. Firstly, the competition between applied ligands and the chelating sites of the soil phase for the target metal ion, and secondly dissolution of amorphous iron oxo-hydroxides by complexation of Fe with EDTA, with subsequent mobilization/release of occluded trace metals [257,258]. The presence of dialyzable Pb can be explained by the formation of Fe-EDTA complex in preference to Pb-EDTA (log K Fe-EDTA=25.1 versus log K Pb-EDTA= 18.3). Only when the available Fe is exhausted is the dialyzable fraction of Pb reduced. Furthermore, the amount of Pb leached using EDTA (10^{-4}M) was much higher (ca. 80% more) than that obtained using dilute nitric acid (10^{-2}M), complementing results by former workers [259,260].

It is to be noted that the amount of free metal ions sensed at the microdialyser probe in the presence of EDTA is only a fraction of the actual amount present. Dialysis yield in the presence of EDTA was found to be about 9% in aqueous medium, and physical effects of the soil matrix i.e. tortuosity led to a basic decrease (to 30%) in dialysis efficiency. This decrease is reflected in the results shown in Fig.7.6.

In comparison with the acid extractant, a 1.7-fold higher metal release is attained with EDTA, even at the lowest concentration assayed. It can be concluded that fixed (non-labile) elements, such as lead, are more easily remobilized by complexation rather than by acidification processes. These results are

in good agreement with recent observations made by Sahuquillo et al. [259] and Song and Greenway [260] exploiting leaching tests in batch procedures.

7.6.2.3 Stimulus-response

Here, the dilute electrolytic solution (10^{-3} M $CaCl_2$) employed as a perfusion liquid in this work was replaced by nitric acid solutions of different concentration (10^{-3} and 10^{-1} M HNO_3) to simulate the effect of an acid input, for example an accidental spill, on the soil. Since dialysis is bi-directional, it is expected that the H^+ ions are transferred from the perfusing liquid to the soil microenvironment around the probe and that the effect of this transfer be measured in the dialysate by ETAAS. Results (see Table 7.1) show a decrease in leaching efficiency compared to the other direct pumping of acid eluent through the soil column. However, increasing the acid concentration of the perfusing liquid was reflected immediately by an increase in the dialysed metal showing the sensitivity of the probe to changing environmental conditions.

Table 7-1: Comparison of the influence of various leaching agents on the dialysis efficiency of Pb

Leaching agent	**Maximum microdialysate concentration (μg l^{-1} Pb)**	**Accumulated release (ng Pb)**	**Dialyzable fraction (%) ****
10^{-2} M $CaCl_2$	6.6	3.8	0.011
10^{-3} M HNO_3	21.1	12.1	0.035
10^{-4} M EDTA	41.7	20.8	0.18
10^{-2} M EDTA	130	40.0	0.35

Given values are normalized to a 80 min-monitoring interval

** It is calculated with respect to the total lead content of the soil layer a top the inserted probe

When the perfusion liquid itself contains a complexing agent, it is expected that higher efficiency results due to the high affinity for the substances of interest. Mogopodi and Torto [261] very recently reported significantly higher RR of metals when several complexing agents were added to the perfusion liquid.

Analogy to and differences with the microcolumn experiments

Due to the geometry of the soil column, it takes about 30 min to completely change the soil environment (interstitial liquid), thus monitoring analytes at the column effluent does not provide much fractionation information. A better "sensor" is the implanted microdialyser probe which senses changes at its immediate vicinity and thus gives more time resolved information of the leaching process.

The open soil column is on a 1000 times larger scale than the microcolumn. The leaching profile obtained at the microdialyser probe differs from that at the microcolumn because the former senses the minute changes at the micro environment (pore water) while the latter measures changes at the effluent stream.

Further applications

Although the execution of such projects was not in the scope of the present work, future possible applications of the developed microdialyser probe are presented below.

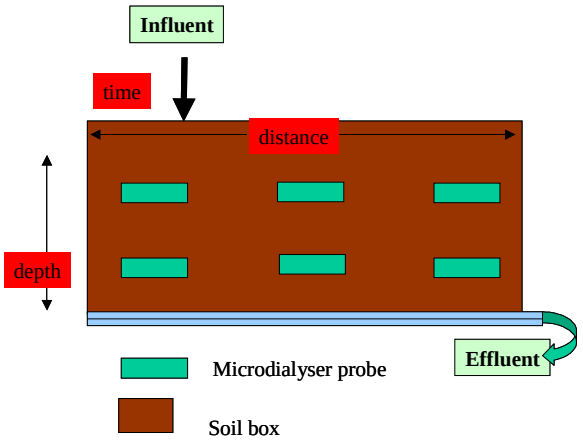
a) Applicability in fate and transport studies

The fate and transport of pollutant (or nutrients) introduced on a site can be monitored by an arrangement of probes at different depths and across distances (see Fig 7.10a below). This would supply highly time resolved information as to the mobility, transport and fate of such pollutants in the area under investigation. Since the microdialysis fibres used in this work are stable over months, long term in-situ monitoring could be carried out for risk assessment purposes.

b) In-situ stimulus-response schemes

An alternative method of assessing remediation of contaminated sites is offered by an arrangement of microdialyser probes in different locations on the affected site. In this case, a stimulus in form of the active agent (e.g. releasing, sorptive agent) is introduced via the first probe into the microenvironment. The response in the form of increase or decrease of pollutant concentration can be monitored via a secondary microdialyser placed at a distance from the first probe. This kind of arrangement is illustrated in the Fig 7.10b.

a)



b)

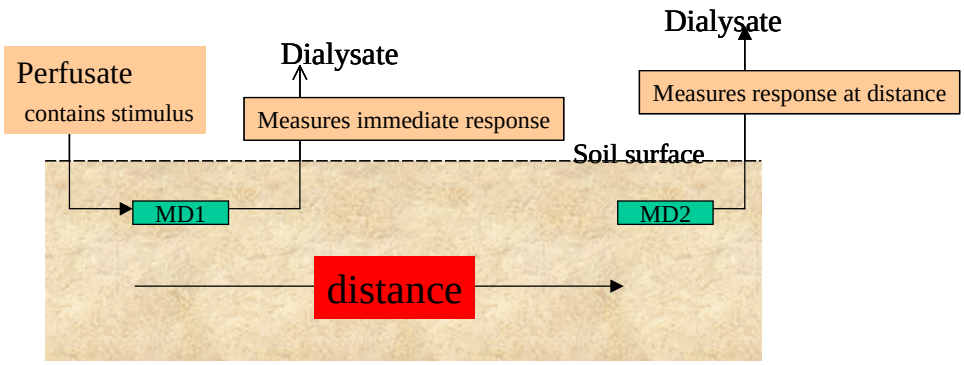


Fig. 7-10: Possible arrangement of probes in soil a) array for 3D metal transport b) array for in-situ stimulus-response

7.7 Concluding remarks

Feasibility of the in-situ application of microdialyser probe for the continuous sampling of metal ions

The in-vivo application of MD has been well documented. A novel application of MD for environmental monitoring of heavy metal release was presented. The advantages of on site analysis are manifold, ranging from the absence of laboratory transportation, sample contamination, storage and handling problems to simplicity and ease of information gathering. Sampling itself is a disturbance to natural environment, causing changes in equilibria. The microdialysis probe provides a solution since it is almost non-invasive by nature. The implanted probe is capable of detecting changes in the micro environment, but quantification of metal content is relative and not absolute, since the total weight of the soil being analysed is unknown. In other words, the probe is sensitive to events occurring at the surface up to depths immediately above and spaces surrounding it. However, the sample must be sufficiently wet to allow diffusion of analytes from the interstitial fluid (pore water).

The applicability of microdialyser probe to the study of fate and transport of pollutants (or nutrients) on a long term basis has also been discussed.

Features:

- Non-invasive -no disturbance of equilibria
- Semi-permeable barrier
- Chemical composition of sample is not usually modified, when this is needed, the principle of retro-dialysis is involved.
- Complementary information to common leaching studies

Conclusion

It is a proven fact that the mobility, bioavailability, toxicity or essentiality and fate of an element depends on the form in which it occurs, rather than its total concentration. Exposure to some forms of an element may be harmless, while other species of the same element may be toxic, carcinogenic or mutagenic.

The direct speciation in solids is limited to substances which have been identified, stable enough to be isolated in the original form and for which sensitive methods are available. Fractionation methods are the preferred alternative and usually involve some form of chemical extraction scheme. Furthermore, fractionation allows assessment of mobility and (bio) availability with respect to a particular matrix and experimental conditions can be selected to mimic those of the natural environment.

Classical sequential extraction methods are based on the principle of selective dissolution of predefined phases and analysing the released metals with an element specific detector. However these schemes which are equilibrium based (meaning that the reagent has to be in contact with the solid for prolonged times, normally 8-16 hrs) suffer generally the problems of non-selectivity of reagents used, re-adsorption and re-distribution. Kinetic methods on the other hand give enhanced information about the behaviour of metal forms during leaching and other processes. Continuous extraction methods are more suitable for risk assessment studies since the environmental processes they simulate are also dynamic in nature.

The thesis concerned the development of methods suitable for obtaining enhanced information about the forms of metal binding in solid environmental matrices basically following two approaches: microcolumn leaching and in-situ microdialysis. The design of a microanalytical system allowing the near real time monitoring of metal release from the various compartments of solid samples was an improvement over and extension of existing fractionation (sequential extraction) methods. Thus the handicaps of the traditional batch schemes like lack of kinetic information, readsorption and redistribution problems but also the high time and reagent demands were effectively overcome using the developed on-line microanalytical system. Further advantages of on-line coupling of sample extraction and analysis techniques in a closed system include minimal sample consumption, time saving factor, avoidance of contamination especially through laboratory atmosphere and other human errors. The system is easy to calibrate and can be automated. Flexibility in choice of detection is also in-built. The analytical results obtained in the leaching studies clearly demonstrate the feasibility of the approach with respect to practical aspects and the suitability for simultaneous determination of several elements thus receiving leaching fingerprints. The investigation of the real samples evidences a different leaching behaviour for different elements and also variability in the leaching characteristics of a given element for samples of structural difference (i.e. soil and plant materials). However, it became also apparent that interpretation of results in terms of identification of fractions with particular

phases remains difficult to obtain. Still, rapid screening information as to immediate and potential risk could be conveniently obtained for a wide variety of solid environmental samples.

Although the system was used for leaching studies, the concept could be expanded to metal sorption and indeed the simultaneous analysis of anions/ binding partners.

Sample introduction via injection valve and pump stop-and-go cycles was done manually. Probably all the manipulations could eventually be fully automated such that the system could become an attractive standard method. Commercial application of such system could be on-site collection of fresh vegetable samples, filling into the micro-cartridges and extraction in mobile laboratory vehicle for an immediate assessment of market or garden produce. The same holds for soil, dust or other solid samples of environmental interest.

Industrial applications of the microanalytical flow through system could include investigation of corrosion and wearing properties of materials (building, construction) under various pH conditions.

The second approach concerned the use of membrane based techniques in the development of microanalytical methods for studying metal binding forms in environmental samples. The suitability of a microdialysis probe (constructed for the continuous sampling of metal ions in the interstitial fluid of solid samples), for ‘sensing’ changing environmental conditions was demonstrated. Onsite sampling and analysis provides an easy way to avoid the problems encountered due to traditional sampling, sample transport to laboratory, subsequent treatment, storage and analysis. Such problems include the effect of temperature changes, alterations in speciation results due to the use of different sample pre-treatment etc.

Implantation of a linear-type microdialyser into a real soil sample containing high levels of organic matter allowed the study of the dynamics of metal desorption from the soil body under simulated environmental scenarios, such as rainfall and discrete anthropogenic actions.

Analytical characterization of the probe showed that operation under near steady-state regime was feasible with almost no perturbation of the microenvironment. As such the microdialyser serves as an analytical tool for automated sampling and sample clean-up of trace metals from complex aqueous matrices (e.g. wastewater and effluent streams) without the inherent recovery limitations of available commercial types. The extremely low perfusing rates handled assure compatibility between available dialysate volumes and detector requirements.

The on-line hyphenation of the miniaturized device with ETAAS allowed the analysis of dialysates without collection of fraction or addition of chemical modifier (the inherent molecular size-discrimination feature of microdialysis prevented spectral interferences caused by organic matter).

In order to correctly estimate the metal (bio) availability to biota, investigation of the resistance to mass transfer through semi-permeable membranes under various experimental conditions was carried out.

Finally, the application of an acidic stimulus through the implanted microdialysis probe offered a simple and convenient means of continuous assessment of the efficiency of metal remediation processes in contaminated solid materials, such as compost, industrial solid residues and sludges containing high concentration of organics.

Future research work should focus on the implementation of the probe into real sites for in-situ assessment of metal pollution and real-time monitoring of the kinetics of metal release at the time scale of environmental events, without disturbance of the natural equilibria. Stimulus-response (retrodialysis) schemes presented in the work could be applied to the on-line evaluation of metal adsorption profiles of various types of soils, thus offering a straightforward means to predict their potential capabilities as remediation materials for metal contaminated waste.

Further environmental application of the microdialysis probe would be the spatial, 3D sensing of changes in the microenvironment around implanted probes according to distance, depth and time, the simultaneous assay of other ions apart from metals and multi element detection possibilities.

Outlook

Although the system was used for leaching studies, the concept could be expanded to metal sorption and indeed the simultaneous analysis of anions/ binding partners.

Sample introduction via injection valve and pump stop and go cycles was done manually. Probably all the manipulations could eventually be fully automated such that the system could become an attractive standard method. Commercial application of such system could be on-site collection of fresh vegetable samples, filling into the micro-cartridges and extraction in mobile laboratory vehicle for an immediate assessment of market or garden produce. The same holds for soil, dust or other solid samples of environmental interest.

Industrial applications of the microanalytical flow through system include investigation of corrosion and wearing properties of materials (building, construction) under various pH conditions.

8 Materials and Experimental Procedures

8.1 Materials

8.1.1 Water, Reagents and Chemicals

Water was in-house treated by reverse osmosis followed by demineralisation in a Seradest S 600 system for the AAS studies while Millipore grade water Milli-Q purification system (>18 MOhm cm^{-1}) was necessary for experiments involving ICP-MS and ICP-AES measurements.

8.1.1.1 Reagents and Chemicals

Hydrogen peroxide, nitric, hydrochloric and acetic acids were of suprapur grade (Merck).

EDTA disodium salt, sodium citrate, sodium chloride, sodium thiocyanate, sodium bicarbonate, sodium sulphate, potassium chloride, potassium hydrogen phosphate, calcium chloride, magnesium chloride, ammonium acetate, urea, uric acid and hydroxylamine hydrochloride were p.a. grade purchased from Merck. The enzymes mucin and pepsin (from porcine stomach mucosa), pancreatin (from porcine pancreas) and trypsin (108 367 200 fip/u) were also purchased from Merck. Bile extract (EC no 232-369.0) and α-Amylase VI-B were purchased from Sigma.

Stock reagents:

A stock solution of 10^{-1} M $CaCl_2$ was prepared by dissolving 1.470 g of calcium chloride dihydrate in 100 ml of deionised water.

Working solutions:

0.11M acetic acid solution,

0.5 M hydroxylamine hydrochloride solution (prepared fresh daily)

2 M HNO_3,

1 M ammonium acetate solution

Perfusion liquid- 10^{-3} M $CaCl_2$

Leaching agents-10^{-2} M $CaCl_2$, 10^{-1} and 10^{-3} M HNO_3

Ligands- 10^{-4} and 10^{-2} M EDTA, and 10^{-4} M citrate were prepared by dissolving an appropriate amount of the corresponding sodium salt in water, and adjusting the pH to 4.2.

Working standard solutions of lead and other metals for ETAAS calibration were obtained by serial dilution of the corresponding 1000 ± 2 mg l^{-1} stock solution (Merck) in a 1.0% (v/v) aqueous nitric acid solution.

8.1.1.2 Reference materials

BCR 701, lake sediment reference sample was obtained from Standards Methods and Testing programme formally BCR, Brussels. SRM 1648, urban particulate matter and SRM 1570a, spinach leaves were obtained from NIST Gaithersburg, USA. CRM 3616, pyrrhotine ore was obtained from the Institute of Ore Deposits, Petrography, Mineralogy and Geochemistry (IGEM) of the RF Academy of Sciences, Moscow, Russia.

8.1.1.3 Plant samples

Parsley, dandelion, onion, bean, cucumber, lettuce, potato, asparagus, zucchini and carrot purchased from the open market.

8.1.1.4 Soil samples

BAM 2b contaminated soil for inter-laboratory assessment was kept as obtained from Bundesanstalt für Materialforschung und –prüfung (BAM). Private garden soil (A-horizon) was collected near a highway in Lübbenau, near Berlin. For microdialysis experiments, a sludge amended forest soil was collected on the outskirts of Berlin. All soil samples were kept in polyethylene bags and stored at room temperature in the dark and prepared before use as described below (sample preparation).

8.1.2 Instrumentation / Apparatus

Soil and plant extracts and digests were analysed using either an inductively coupled plasma mass spectrometer (Perkin Elmer, Elan 6000, Norwalk, CO, USA) stationed at ISAS, Berlin or an inductively coupled plasma atomic emission spectrometer (Model CCD Ciros, Spectro Analytical, Kleve, Germany) stationed at AZBA GmbH, Berlin.

Flame and electrothermal atomic absorption spectrometry was performed using a Varian Spectraa 400 dual instrument equipped with an air-acetylene flame and graphite furnace, respectively. The AAS conditions were optimised according to manufacturer’s recommendation for maximum sensitivity of detection of the various elements and given in Appendix Table MD-1.

8.2 Experimental procedures

8.2.1 Sample preparation (Washing, drying and grinding)

Plant material

Various plant materials from different sources were randomly collected. Generally the samples were washed with distilled water, chopped and oven-dried at 60°C for about 2-3 days. Few samples were freeze dried. The dried samples were finally pulverised in an agate ball mill (particle size approx. 50 µm) and stored in polyethylene bottles until use. For reasons of comparison, some vegetables and spices have also been used without drying and pulverisation. In this case the materials had only been cut up with scissors and the coarse material filled into the extraction cartridge by careful stuffing.

Soil samples

Soil samples collected from the field in Lübbenau and in the forest zone on the outskirts of Berlin were stored at room temperature, in the original sample bags in the dark until required. The soil was manually broken up and spread into a thin layer on brown paper sheeting. With occasional turning over, the soil was allowed to air-dry over several days. Pre-sieving through a 2 mm stainless steel mesh was done to remove rock fragments, plant litter, and other non-soil debris. A finer 63 µm mesh was then used to produce the final soil samples for analysis.

8.2.1.1 Effect of sample preparation on the extraction of heavy metals in biomatrices

a) Effect of cutting on extractability of metals

Vegetable samples were washed with distilled deionised water, drained of wash water, and further prepared by the following methods:

1. air drying, knifed
2. fresh, scissored
3. blended (home homogeniser), oven dried 60°C, powdered in an agate ball mill.

Aliquots of the thus prepared samples were subjected to on-line real time monitoring of the metal leaching. Further aliquots were also partially digested with nitric acid in a microwave (details in Table 8.1) for the determination of total metal content using ICP-MS.

b) Effect of cooking on heavy metal content of potatoes

A potato sample was divided in two portions. One portion was oven dried (60°C), ground and kept for total content determination by aqua regia extraction described below (section 8.2.2). The other portion

was prepared for cooking by peeling before boiling in distilled water. The peel was dried, ground and kept for analysis. The cooked part was also homogenised, dried in the oven at 60°C and ground.

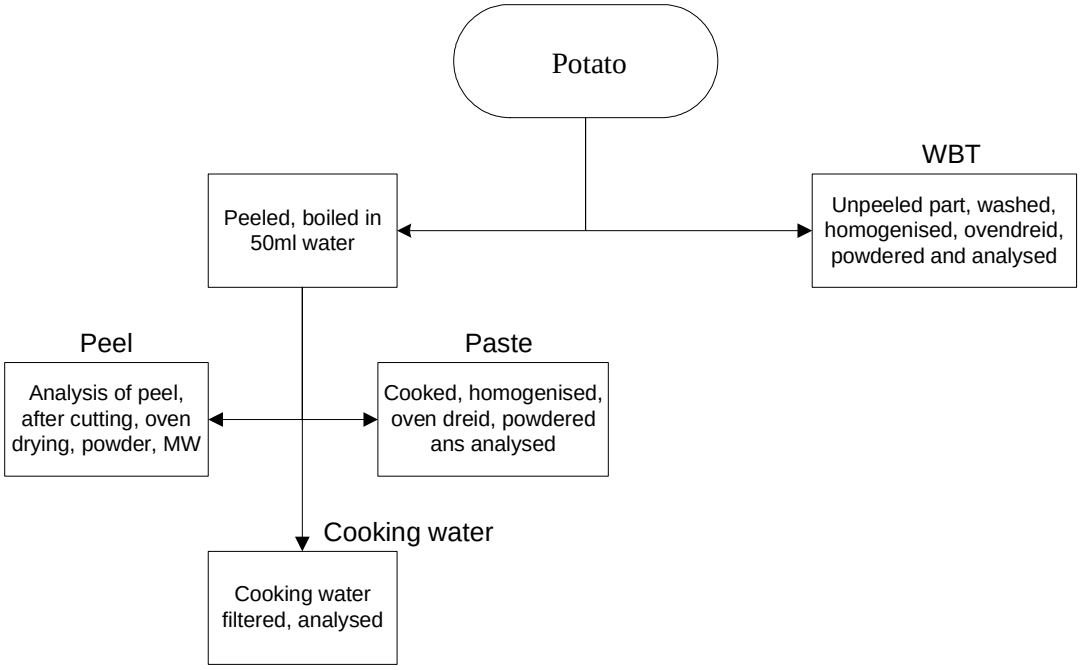


Fig. 8-1: Scheme for the study of effect of sample preparation on metal content in potato

8.2.1.2 Moisture content

A separate portion of the sample material was analysed for moisture content by heating 3g sample for 4 hours at 105°C in an oven. Moisture content was then calculated as % weight loss.

8.2.2 Sample digestion

Various methods were compared for the pseudo total digestion of samples. These included the use of mineral acid (classical acid digestion), hot water extraction and enzymatic hydrolysis. Methods for the microwave digestion (with different programs) besides the classical aqua regia digestion and variants using different mixtures of mineral acids are described below and summarised in Tables 8.1 and 8.2.

Table 8-1: Acid combinations and digestion protocols

Method	Conditions	
KW1	aqua regia ($HCl+HNO_3$ /3+1) 2hr digestion under reflux at 120°C	0.5g sample + 7ml acid mixture
KW2	Nitric acid 2hr digestion under reflux at 120°C	0.5g sample + 7ml acid
KW3	2hr acid digestion under reflux at 120°C	0.5g sample + 10ml HNO_3 + 5ml H_2O_2
MW	microwave digestion program modified from Application Note AG-2, rev. date 11-91 Alfafa leaves using CEM MDS 2000	0.5g sample + 7ml HNO_3 +3ml H_2O_2

MW digestion

The digestion program used in the sample digestions (Microwave CEM) is given in Table 8.2 below.

Table 8-2 : MW digestion program

Stage	1	2	3
%Power *	100	100	100
PSI	40	40	75
Time (min)	6	6	10
Tap	3	3	5
Fan speed	100	100	100

8.2.2.1 Aqua regia extraction

To 0.5g sample in the digestion vessel, 7ml of aqua regia (7ml HNO_3 +21ml HCl) were added and the mixture left either an hour or overnight to pre-react. The vessel was then placed on the heating block, fitted with a cold finger and heated at 120°C for 2 hours under reflux.

8.2.2.2 Dilute nitric acid extraction

2.5g of sample was extracted with 100ml of 1% nitric acid for 30 minutes in an ultrasonic bath. The extract was filtered and kept in polyethylene bottles for analysis.

8.2.2.3 Hot water extraction

100ml of water at 60°C was added to 2.5g sample and the mixture kept in ultrasonic bath for 30minutes. The extract was centrifuged at about 4000g, after which the supernatant was removed for metal analysis. To differentiate dissolved from fine particulate originating analytes, some extract was filtered through 0.45µm membrane filter (Sartorius).

8.2.2.4 Enzymatic digestion

Enzymatic hydrolysis of plant material was attempted using trypsin. Different sample to enzyme ratios (2:1, 5:1, 10:1 and 20:1) were studied at pH 7, 8-9 and 10-12 and monitored by measuring the the yield of Cd and Pb. For this, appropriate amount of trypsin was added to 2g sample in a plastic bottle. 50 mL water was added and the mixture shaken and adjusted to required pH with few drops of NaOH solution. The mixture was stirred for 10min with magnetic stirrer after which the bottle was placed in a water bath at 37°C for 2 hours. The effect of ultrasonic energy instead of agitation on the digestion yield was also studied.

The optimum conditions were found to be 20:1 at pH 8-9. Five min of ultrasonic bath enhanced the extraction yield. The enzymatic digestion of dandelion samples gave higher yield of Pb than the dilute nitric acid extraction. Cadmium yield by enzymatic digestion was about 100% and about 60% by acid extraction.

8.2.3 Sequential (Batch) extraction procedures

8.2.3.1 Laboratory internal reference material (first results)

In the preliminary test, a spiking suspension was made by dispersing PbO_2 solid in 50% alcohol to give a concentration of 200 ppm Pb. This stock was diluted 50 µl to 5ml (i.e. 2 ppm suspension) in 50% alcohol. About 70 mg of cellulose was spiked with 10 µl of the 2ppm suspension i.e. 20 ng Pb absolute. The prepared cellulose material was then subjected to on-line nitric acid sequential extraction using both GFAAS and ICP-MS detection. Good recovery of Pb was obtained in both cases as shown in Table 8.3 below.

Table 8-3: Recovery of PbO_2 using GFAAS and ICP-MS

	GFAAS	ICP-MS
amount added (ng)	20	20
amount found (ng)	20,1	14,4
% yield	100,3	72

8.2.3.2 BCR Batch Sequential Extraction scheme

The revised BCR sequential extraction [137] was carried out as described below:

Step 1 (adsorbed, exchangeable, carbonate fraction):

40 ml of a 0.11M acetic acid solution was added to 1 g sample. The mixture was shaken for 16 h (overnight) at room temperature in an end-over-end shaker and then centrifuged 20 min at 3000 g, supernatant was removed for analysis (extract A), while the solid residue was shaken for 15 min with 20 ml water, centrifuged and this washing discarded. The residue was kept for further extraction as below

Step 2 (reducible fraction, Fe- and Mn-oxyhydroxides):

40 ml of 0.5 M hydroxylamine hydrochloride solution as extractant, previously adjusted to pH 2 by incorporation of 2.5 ml 2 M HNO_3 in 100 ml reagent solution, was added to the washed residue from step 1 and shaken as in step 1 for 16 h. After centrifuging, supernatant was removed for analysis, as extract B. The solid residue was washed as described above and kept for step 3

Step 3 (oxidisable fraction):

10 ml of hydrogen peroxide was added to the residue and digested at room temperature for 1 h, with occasional manual shaking. Further digestion was done in a water bath at 85°C for 1 h, shaking occasionally in the first half hour. The digest was reduced to about 2 ml, and then 10 ml of fresh hydrogen peroxide was added and digested finally for 1 h at 85°C. The digest was then concentrated to 1 ml and allowed to cool. 50 ml of 1 M ammonium acetate solution was then added and the mixture shaken as before for 16 h, centrifuged, and supernatant (extract D) removed for analysis.

Residual

The residue from the above extractions was washed as before and digested with 7 ml aqua regia. This digest was also analysed.

8.2.3.3 E DIN 19738: 2000-05 Method [230]

Synthetic digestive juices were prepared by dissolving first the inorganic components in water and thereafter, (just before use), the enzymes, with water (in the proportions indicated in Appendix Table D-1).

2 g of sample was first extracted with the 30ml synthetic saliva under constant stirring in a water bath at 37°C for 30 min. Then 70 ml of the stomach fluid was added and the extraction carried out for 2 h under the same conditions as before except that pH of 2 was maintained using 10% HCl. Finally, 100ml of the intestinal fluid was added to the sample and extracted at pH 7.5 (adjusted where necessary with saturated $NaHCO_3$ solution) for 6 h.

The mixture was centrifuged for 10 min at 7000 g and the supernatant collected for analysis. Residue was washed with 30 ml water and centrifuged. The washing solution was combined with the supernatant for analysis. The residue was also analysed for metals as a quality control measure. In addition to the described procedure, an aliquot (1 ml) was taken from each extraction stage for separate analysis, aimed at studying the fate of metals along the simulated digestive tract.

8.3 Comments

The determination of total content of metals in the various plant samples served two purposes. On the one hand, the suitability of the mild extraction methods for the quick assessment of heavy metal load in plants was tested against the aqua regia (ISO method) method. Most elements especially the major constituents like Na, K and Ca could be easily determined using any of the mild extractions. Fe, Al and Pb however in some cases required more stringent conditions. On the other hand, the selection of suitable plant types for the speciation studies was facilitated using the total metal content. The criteria set included metal content above LOQ, ease of sample handling and more importantly, the possibility of fractionation of metals. For instance if the amount of a metal extracted was different using water, dilute acid and other method, the suitability of the sample for later fractionation schemes was given.

Results of the study of batch and on-line leaching behaviour of metals in plant material using carrot as an example are presented below.

Batch extraction- Study of the leaching behaviour of different metals in plant material (carrot as example matrix)

Mean total content of elements in carrot (Table 8.4) was determined using aqua regia digestion and compared to a 1% nitric acid ultrasonic assisted extraction both carried out as described under methods and materials.

The concentrations (mg/L) measured at different extraction times for 13 elements using ICP-AES (GFAAS for Pb and Cd with values in µg/L) are given in Table 8.5 below.

Table 8-4: Aqua regia and 1%HNO3 extraction of metals from carrot

Metal (mg/kg)	1% nitric acid	total digest
Al	9	11
Ca	2989	3769
Cr	0,2	1
Cu	4	6
Fe	22	46
K	30842	30393
Mg	844	1083
Mn	5	5
Mo	<LOD	<LOD
Na	1350	2171
Ni	<LOD	<LOD
Zn	18	18
Cd	0,2	0,22
Pb	0,1	0,5

Table 8-5: Time series extraction of metals from carrot sample

Sample	Time (min)	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	Al	Cd	Pb
T2	2	147	0.005	0.14	0.58	782	41	0.25	24	0.014	0.8	0.2	0.04	0.15
T5	5	150	0.006	0.14	0.68	801	45	0.25	25	0.015	0.,84	0.3	0.05	0.19
T 10	10	159	0.005	0.15	0.9	795	43	0.25	24	0.02	0.86	0.3	0,05	0.30
T30	30	159	0.008	0.18	0.99	793	43	0.26	25	0.016	0.89	0.3	0.05	0.37
T 60	60	152	0.01	0.2	1	808	44	0.3	23	0.02	1	0.3	0.1	0.35
T120	120	153	0.02	0.2	1	811	44	0.2	23	0.02	1	0.3	0.1	0.35
T inf	2880	151	0.02	0.2	1	746	37	0.3	60	0.02	1	0.5	0.1	0.32

Element concentrations in mg/L and for Cd, Pb in µg/L, 5g sample extracted in 100ml of 1% nitric acid, 5ml aliquots of extract were taken at time intervals, filtered and analysed by ICP-AES and for Cd, Pb by GF-AAS.

Elements like Ca, K, Mg, Na were mainly extracted within the first 5 min while Al, Pb and Fe required over 30min for maximum extraction to be attained. Still, the amount extracted in all cases was less than the total content. In order to further characterise the elements, pre-defined physico-chemical properties like fraction accessible to water and dilute acid were studied. This was done by first evaluating the amount extractable by hot water and 1% acid in comparison to the total aqua regia extractable content. However, the quality of results obtained was poor due to high variability of real samples used (parallel extractions were carried out) and the high detection limits of the ICP-AES method used. Values for the water extractable content for the heavy metals studied were either below LOD or so close to incorporate errors. Thus mass balance was not upheld for the fractions studied.

Batch vs. in-line method

Using ICP-MS as detector yields better sensitivities for the elements under study. The in-line sequential extraction of elements from plant samples using water, 1%, 10 and 30% nitric acid again showed better sensitivity with this instrument. Generally, similar extraction patterns were observed for the metals as in the batch time series discussed above. The extraction profiles obtained show two distinct groups of metals – a water accessible group of usually essential elements like K, Na, Mg, but also Cd, Cu depending on matrix and another group of less accessible metals like Pb, Fe and Al.
In addition however, more detailed information as to the kinetics of the leaching processes was available. Also since the method offered less matrix interference problems and much lower LOD using ICP-MS, the quality of results was highly enhanced and mass balance could be established i.e. sum of metal fractions extracted agreed well with the total extractable content.

9 Appendix

Symbols, Abbreviations, Acronyms

AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectrometry
ASE	accelerated solvent extraction
BCR	Bureau Community of Reference now Standards Measurements and Testing programme (SM&T)
CCD	charge coupled detector
CEC	cation exchange capacity
CUES	continuous ultrasound-assisted extraction system
DOM	dissolved organic matter
DPTA	dipentene triacetic acid
EDTA	ethylene diamine tetraacetic acid
ETV	electro-thermal vaporisation
ESI-MS	electrospray ionisation mass spectrometry
FI-FAAS	flow injection flame atomic absorption spectrometry
GFAAS	graphite furnace atomic absorption spectrometry
GSC	Geological Survey of Canada
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ISAB	ionic strength adjustment buffer
IUPAC	International Union of Pure and Applied Chemistry
LOQ	Limit of determination or quantification
MAE	microwave assisted extraction
MD	microdialysis
MFS	microcolumn field sampling
MWCO	molecular weight cut-off
MSF	multi component spectral fitting
PACE	pressure assisted chelating extraction
PAH	polycyclic aromatic hydrocarbon
PBET	physiologically based extraction technique
PFA	perfluoroalkoxy
PLE	pressurised liquid extraction
POP	persistent organic pollutant

PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
SEM-EDX	scanning electron microscopy combined with X-ray emission
SES	sequential extraction scheme
SFE	supercritical fluid extraction
SRM	standard reference materials
USE	ultrasonic assisted extraction
XAS	x-ray absorption spectroscopy
XAFS	x-ray absorption fine structure
XANES	x-ray absorption near edge structure
δ	thickness of the liquid film surrounding the particle

List of Tables

List of figures

9.1 Additional Tables

Appendix Table 1:(Table L-1): Permissible limits for metals in drinking water, hazardous waste and sewage sludge for land application (USEPA)

Metal	Primary drinking water standard (MCL) mg/L	Secondary drinking water Standard mg/L	Hazardous waste screening criteria (TCLP) mg/L	TCLP Hazardous waste limit mg/L	Common range in soils[b] mg/kg	Land application of sewage sludge ppm
Al		0.05-0.2			10,000-300,000	
As	0.05		100	5.0	1-50	75
Sb	0.006					
Ba	2.0		2000	100	100-3000[c]	
Be	0.004					
Cd	0.005		20	1	0.01-0.7	85
Cr	0.1		100	5	1-1,000	3,000
Cu	1.3	1.0			2-100	4,300
Fe		0.3			7,000-550,000	
Pb	0.015		100	5	2-200	840
Mn		0.05			20-3,000	
Hg	0.002		4	0.2	0.01-0.3	57
Ni					5-500	420
Se	0.05		20	1	0.1-2.0	100
Ag		0.1	100	5	0.01-5	
Tl	0.002					
Zn		5.0			10-300	

*MCL-Maximum Contaminant Level for drinking water from a public water supply system From “Current Drinking Water Standards”, E.P.A.Office of Water

**Hazardous Waste Screening Criteria (TCLP) refers to limits that are allowed in solid waste or soil for disposal in a landfill. Limits developed by ODEQ under authority given by “Test Methods for Evaluating Solid Waste, Physical/Chemical Methods”, SW-846 Manual, Section 8.4, E.P.A. Office of Solid Waste.

TCLP - Toxicity Characteristic Leaching Procedure is a test to determine the mobility of contaminants in solid wastes or soils. These are the limits allowed to leach out of soil or solid waste in a landfill. From 40 CFR 261.24.

a Naturally occurring in the soil. Limits taken from Lindsay 1979

b Metal concentration limits allowed in the use or disposal of sewage sludge. From 40 CFR 503.13

c Naturally occurring in the soil. From Deuel and Holliday, 1994, “Soil Remediation for the Petroleum Extraction Industry”.

Appendix Table 2 (Table –M-1): ICP-MS operating conditions

Instrument	Elan 6000 (Perkin–Elmer)
Rf power	1000 W
Nebuliser	Standard concentric nebuliser
Cones	Ni
Data acquisition	Peak hopping
Number of sweeps per reading	1
Dwell time	50 ms
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	1 L min^{-1}
Nebuliser gas flow rate	0.88 L min^{-1}
Elements /Isotopes	Ni (60, 61), Cu (63, 65), Zn (66, 68), Cd (111, 114), Pb (206, 208), Al (27)

9.1.1 Analytical calibration data for MD-ETAAS system

Appendix Table 3 (Table MD-1): Temperature programs for Pb and Fe

a) ETAAS temperature program for Pb (prog51)

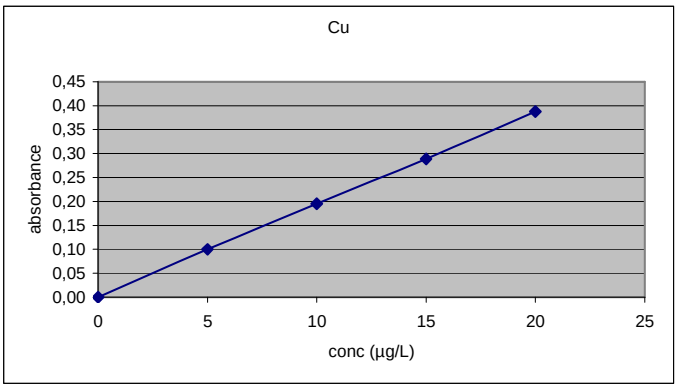
Stage	Temperature (°C)	Time (sec)	Read	Flow (3L/min)
1	90	5	-	yes
2	120	10	-	yes
3	400	10	-	yes
4	600	10	-	-
5	700	5	-	-
6	2100	0.8	yes	-
7	2100	2.0	yes	-
8	2400	2.0	-	yes

b) ETAAS temperature program for Fe (prog46)

Stage	Temperature	Time (sec)	Read	Flow (3L/min)
1	85	5	-	yes
2	95	20	-	yes
3	120	10	-	yes
4	700	5	-	yes
5	800	3	-	yes
6	800	2	-	-
7	2300	0.8	yes	-
8	2300	2.0	yes	-
9	2400	2.0	-	yes

Appendix Table 4 (Table MD-2): Calibration data for Cu (MD-ETAAS system)

Analyte	Cu calibration curve
Matrix	standard solution
Analytical device	GFAAS
Remarks	



Calibration points

No.	Calibrator	Response
1	0,00	0,00
2	5,00	0,10
3	10,00	0,195
4	15,00	0,289
5	20,00	0,388

Characteristics

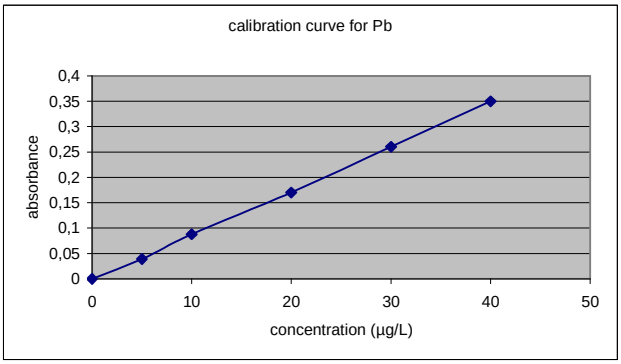
Slope a	0,019		Number of measurements n	1
Intercept b	0,001		Standard error of estimate Sy	0,002
Correlation coefficient r	0,9999		Standard error of procedure Sx	0,098
Result uncertainty	33,33	%	Sum of squared deviations	250,000
Probability of error (alpha)	5,00	%	Quantile (one-sided)	2,353
			Quantile (two-sided)	3,182

Analytical limits according to DIN 32645

Limit of detection	0,291	
Limit of quantitation	1,157	(approximation)
	1,149	(exact)

Appendix Table 5 (Table MD-3): Calibration data for Pb (MD-ETAAS system)

Analyte	Pb
Matrix	standard solutions
Analytical device	GFAAS
Remarks	



Calibration points

	Calibrator	Response
No.	µg/L	abs
1	0,00	0,00
2	5,00	0,039
3	10,00	0,088
4	20,00	0,17
5	40,00	0,35

Characteristics

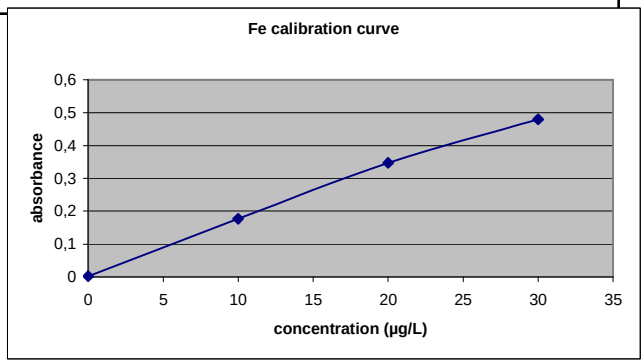
Slope a	0,009		Number of measurements n	1
Intercept b	-0,002		Standard error of estimate Sy	0,003
Correlation coefficient r	0,9998		Standard error of procedure Sx	0,362
Result uncertainty	33,33	%	Sum of squared deviations	1000,000
Probability of error (alpha)	5,00	%	Quantile (one-sided)	2,353
			Quantile (two-sided)	3,182

Analytical limits according to DIN 32645

Limit of detection	1,018	µg/L	
Limit of quantitation	4,009	µg/L	(approximation)
	3,978	µg/L	(exact)

Appendix Table 6 (Table MD-4): Calibration data for Fe (MD-ETAAS system)

Analyte	Fe
Matrix	standard solution
Analytical device	GFAAS
Remarks	



Calibration points

	Calibrator	Response
No.		
1	0,00	0,002
2	10,00	0,177
3	20,00	0,347
4	30,00	0,48

Characteristics

Slope a	0,016		Number of measurements n	1
Intercept b	0,011		Standard error of estimate Sy	0,016
Correlation coefficient r	0,9981		Standard error of procedure Sx	0,978
Result uncertainty	33,33	%	Sum of squared deviations	500,000
Probability of error (alpha)	5,00	%	Quantile (one-sided)	2,920
			Quantile (two-sided)	4,303

Analytical limits according to DIN 32645

Limit of detection	3,724	
Limit of quantitation	14,280	(approximation)
	14,125	(exact)

9.1.2 Tables on analytical characteristics of the microcolumn extraction system

Appendix Table 7 (Table A-1): Composition of stdmix 1-4 used for calibration at the ICP-MS

	Solution 1	Solution II	Solution III	Solution IV
Al	0.1	0.2	10	100
Cd	0.03	0.1	0.3	3
Cu	0.1	0.5	10	100
Ni	0.01	0.2	1	10
Pb	0.01	0.3	1	10
Zn	0.1	5	10	100

Appendix Table 8 (TableA-2): Comparison of peak height with peak area (integral) for Pb (1ppm multi iv standard) using two different sample loop sizes

	Integral Pb208	Integral Pb208	PeakheightPb208	PeakheightPb208
Peak number	Small size loop	Large size loop	Small size loop	Large size loop
1	2418289	5625089	14764056	21143397
2	2424394	5583970	12687108	21420527
3	2421522	5636820	12566373	21893279
4	2420666	5600906	13432150	21925882
5	2460453	5624225	13759968	22871385
6	-	5612051	-	22480142
7	-	5567740	-	21632450
Average	2429064	5607257	13441931	21909580
Std deviation.	17682	24654	892415	599187
rel. std deviation	0,007	0,004	0,066	0,027

Appendix Table 9 (Table A-3) :Typical calibration data at ICP-MS

std conc (ng)	2.1	21	210	2100	
	10ppb	100ppb	1ppm	10ppm	R^2
Al27	1.92E+05	1.11E+06	7.18E+06	7.08E+07	0.999985
Cr52	1.65E+05	1.37E+06	1.19E+07	1.18E+08	0.999999
Cr53	2.55E+04	1.37E+05	1.48E+06	1.41E+07	0.999976
Ni60	4.69E+04	3.16E+05	3.13E+06	2.95E+07	0.999972
Ni61	2.28E+03	1.41E+04	1.45E+05	1.39E+06	0.999988
Cu63	1.69E+05	8.29E+05	7.47E+06	7.51E+07	0.999997
Cu65	8.38E+04	4.04E+05	3.63E+06	3.59E+07	1.000000
Zn66	2.19E+05	5.82E+05	2.37E+06	2.15E+07	0.999950
Zn68	1.64E+05	2.43E+05	1.75E+06	1.58E+07	0.999974
Cd111	5.42E+04	4.86E+05	3.54E+06	3.71E+07	0.999955
Cd114	1.32E+05	1.14E+06	8.04E+06	8.33E+07	0.999964
Pb206	2.05E+05	2.23E+06	1.56E+07	1.73E+08	0.999864
Pb208	4.94E+05	4.66E+06	3.64E+07	3.83E+08	0.999959

Appendix Table 10 (Table A-4): Calibration data for Ni, Zn, Cd, Pb and Al

conc mg/L	**Ni60**	**Ni61**
0,01	5,10E+04	3,51E+03
0,2	6,60E+05	3,03E+04
1	3,99E+06	1,84E+05
10	4,00E+07	1,92E+06
slope	8784621	192880
intercept	-1246476	-5217
3sd	7630	932
yb	65399	1643
yDL	73028	2576
xDL (mg/L)	0,150	0,040

conc mg/L	**Zn66**	**Zn68**
0,1	2,33E+05	1,71E+05
5	1,07E+07	7,56E+06
10	2,55E+07	1,82E+07
100	2,56E+08	1,78E+08
slope	2572799	1785946
intercept	-791272	-364521
3sd	20179	14939
yb	56005	38828
yDL	76183	53767
xDL (mg/L)	0,337	0,234

conc mg/L	Cd111	Cd114
0,003	8,57E+03	2,01E+04
0,1	3,80E+05	8,85E+05
0,3	9,83E+05	2,27E+06
3	1,10E+07	2,47E+07
slope	3668509	8242486
intercept	-33201	-45089
3sd	4680	9323
yb	4114	9514
yDL	8793	18837
xDL (mg/L)	0,011	0,008

conc mg/L	Pb206	Pb208
0,01	2,39E+05	4,81E+05
0,3	5,87E+06	1,16E+07
1	2,44E+07	4,69E+07
10	2,47E+08	4,91E+08
slope	24787445	49289753
intercept	-641738	-1853241
3sd	19653	32784
yb	20262	48759
yDL	39915	81543
xDL (mg/L)	0,027	0,039

conc mg/L	Al27
0,1	1,13E+06
0,2	1,98E+06
10	9,21E+07
100	9,61E+08
slope	9614574
intercept	-1169728
3sd	137544
yb	377549
yDL	515093
xDL (mg/L)	0,175

Appendix Table 11 (Table A-5): Replicate analysis (n=19) of 1ppm multi standard solution in 1%HNO_3

intensities	mean	sd	% rsd
Al27	7299597	141902	2
Cr52	12033158	208168	2
Cr53	1499238	31568	2
Ni60	3167351	64513	2
Ni61	146993	2801	2
Cu63	7577374	119626	2
Cu65	3685818	80613	2
Zn66	2398461	95238	4
Zn68	1770889	44621	3
Cd111	3581998	94985	3
Cd114	8187873	180364	2
Pb206	15829258	358561	2
Pb208	36905263	807355	2

Appendix Table 12 (Table A-6): Development of measuring strategy at the Spectro CCD ICP-AES

Method_Name	Strategie	Gesamtzeit
Flowinjection1 (FI1)	schnell	3.0sec
Flowinjection2 (FI2)	manuell	1.3 sec
Flowinjection3 (FI3)	manuell	0.3 sec
Flowinjection4 (FI4)	manuell	24 sec

Summary	FI1	FI2	FI3	FI4
Meßzeit	3	0.3	1.3	24
Number of points per peak	4	7	6	4
Mean peak height	493219	218586	186348	162023
rsd %	32	7	3	7
Continuous flow*	433165	413358		
Reading interval(sec)	6.94	3.47	4.63	8.10

* intensity reading on continuous aspiration of a standard solution

9.1.3 Analytical data on soil, plant and reference materials

Appendix Table 13 (Table B-1) -Mass content of elements in pyrrhotine ore (CRM 3616-87) from the Institute of Ore Deposits, Petrography, Mineralogy and Geochemistry (IGEM) of the RF Academy of Sciences, Moscow, Russia.

Element	Content % (w/w)	Element	Content % (w/w)
Al	0.16	Ni	4.74
Cu	5.56	Pb	0.014
Fe	49.68	Zn	0.063
Mn	0.03		

Appendix Table 14 (Table B-1 cont'd): Certified metal content in SRM 1648 urban particulate matter

Element	Content %	Element	Content(mg/kg)
Al	3.42(0.11)	Cd	75 (7)
Fe	3.91 (0.1)	Cu	609 (27)
Pb	0.655 (0.008)	Mn	786 (17)
Zn	0.476 (0.014)	Ni	82 (3)

(±uncertainty)

Appendix Table 15 (Table B-1 cont'd) : Certified metal content in SRM 1570a spinach leaves

Element	Content mg/kg	Element	Content mg/kg
Al	319 (11)	Mn	75.9 (1.9)
Cd	2.89 (0.07)	Ni	2.14 (0.1)
Cu	12.2 (0.6)	Pb	0.2 not certified
Fe	n. a	Zn	82 (3)

n.a. not available, Date of revised certificate 31.08. 2001

Appendix Table 16 (Table B-2): Soil characteristics

Soil property	BAM2b	Lübbenau soil
Moisture content	-	8.8%
Al (% wt)	0.6	0.57
Ca(%wt)	2	2.1
Fe (% wt)	7	1.7
Cd (mg/kg)	11	0.3
Cu (mg/kg)	276	23
Cr (mg/kg)	107	13
Mn (mg/kg)	621	595
Pb (mg/kg)	1740	21
Zn (mg/kg)	4509	49

Appendix Table 17 (Table B-3): Mean total concentration of elements in various plants (mg/kg)

	Parsley	Lettuce	Carrot	Dandelion
Al	89	4	11	179
Ca	17318	3250	3769	7202
Cr	1	3	1	1
Cu	11	9	6	14
Fe	127	76	46	315
K	42987	38504	30393	21364
Mg	2501	2141	1083	1527
Mn	104	19	5	23
Mo	<LOD	<LOD	<LOD	<LOD
Na	6495	4300	2171	2776
Ni	0.05	0.1	<LOD	0.4
Zn	17	35	18	58
Cd	0.13	0.22	0.22	0.14
Pb	0.7	0.5	0.5	3.4

	Asparagus	Zucchini	Onion	Potato	Bean
Al	23	7	2	18.8	30
Ca	2078	5339	2816	481	7054
Cr	<LOD	1	3	<LOD	<LOD
Cu	17	27	5	5.8	11
Fe	53	108	22	24	75
K	30206	90649	16174	21837	35641
Mg	1423	5201	1187	1534	2919
Mn	14	25	9	7	24
Mo	<LOD	1	<LOD	<LOD	6
Na	132	251	344	265	135
Ni	8.5	1	2	<LOD	<LOD
Zn	55	88	13	17	25
Cd	0.01	0.04	0.07	0.04	0.01
Pb	0.7	0.1	0.6	0.4	0.3

Appendix Table 18 (Table B-4): Cd and Pb content in Dandelion samples using various extraction methods

Element	Concentration (mg/kg)			
	Hot water extract	1% nitric acid	enzymatic	Aqua regia digest
Cd	0.02	0.082	0.09	0.1
Pb	0.17	0.664	1.08	3.4

Appendix Table 19 (Table B-5): Effect of sample preparation on the metal content in parsley (aqua regia extraction)

Element mg/kg	PWBT	PDK	PWT	Pfresh	Pfresh1	Pstem
Al	60	147	147	21	22	28
Ca	13585	37959	12073	12446	18666	8465
Cu	7	14	6	nd	nd	3
Cd	nd	nd	nd	nd	nd	nd

Cr	nd	nd	nd	nd	nd	nd
Fe	111	345	166	40	46	42
K	8581	44469	35507	20981	25630	36139
Mg	1210	1304	1263	1055	1088	866
Ni	nd	nd	nd	nd	nd	nd
Mn	45	83	40	27	32	18
Na	2874	3359	2516	3191	3517	3007
Zn	27	31	22	nd	nd	11
Si	102	296	202	37	74	58

PDK parsley, dirty knived

PWBT parsley, washed,blended, oven-dried, powdered

PWT parsley, washed,whole,ovendried, powdered

Pfresh parsley, washed whole, fresh

Pstem parsley stems,washed,blended, oven-dried powdered

Appendix Table 20 (Table B-6): Effect of cooking on the distribution of metals in potato

See sample preparation scheme (Fig.8.1) for details

Element / mg/kg	Cooked Paste (mean)	Cookwater	Potato powder (WBT)	Peel (by difference)
Al	7	0.04	20	12
Ca	125	20	203	58
Cu	7	0,1	8	1
Fe	21	0.3	36	14
K	26886	1484	36556	8186
Mg	1126	32	1346	188
Mn	4	0.09	6	2
Na	107	34	159	19
Zn	9	0.4	15	5

9.1.4 Additional BCR Results

Appendix Table 21 (Table C-1): Recovery study using a mixed standard solution BCR stdmix with ICP-AES and ICP-MS detection

Metal	Composition, mg/L	% Recovery ICP-AES	% Recovery ICP-MS
Ni	5	105	105
Al	900	114	105
Cu	10	108	115
Zn	15	101	120
Cd	1	110	146
Pb	5	105	100
Cr	10	105	107

Appendix Table 22 (Table C-2): Limits of quantification for simBCR procedure (ICP-MS)

LOQ (ng)	step 1	step 2
Al	1.1	1.9
Cr	0.5	0.5
Ni	0.5	0.5
Cu	0.5	0.7
Zn	0.6	0.6
Cd	0.05	0.05
Pb	0.2	0.2

solnA	ng LOQ
Al	1.01
Cr 52	1.87
Ni 60	0.19
Cu 63	0.13
Cu 65	0.25
Zn 68	1.16
Cd 114	0.03
Pb 206	0.03
Pb 208	0.02

Appendix Table 23 (Table C-3): Kinetic time series of BCR extraction step 2 of BCR 701 with solution B

				batch extractions	
mg/kg metal	10 min	60 min	960 min	experimental	certified
Cd	1	2	3	5	3.77
Cr	14	26	44	47	45.7
Cu	48	74	100	125	124
Ni	8	12	24	28	26.6
Pb	90	94	115	130	126
Zn	28	38	78	122	114
Al	1621	770	1529	1187	
Ca	41	629	747	1370	
Fe	2649	2433	5431	5158	
K	110	88	232	253	
Mg	138	147	627	690	
Mn	58	63	109	136	
Na	27	21	56	42	

Appendix Table 24 (Table C-4): Kinetic time series of BCR extraction step 3 of BCR 701 with solution D

				batch extractions	
mg/kg metal	10 min	60 min	960 min	experimental	certified
Cd	0.4	0.2	0.3	0.4	0.27
Cr	117	117	112	132	143
Cu	29	24	24	49	55.2
Ni	11	13	13	15	15.3
Pb	9	5	3	6	9.3
Zn	34	35	38	42	45.7

Appendix Table 25 (Table C-5): Comparative study using different extraction modes on analysis of BCR701

(Element concentrations are given in mg/kg)

Element /given total content (mg/kg)		Batch/ ref.	Batch/ exptal	online-MS	online-AES	recycle	contflow	offlineA
Ni 103	step 1	15	14	10	15	119	43	129
	step 2	27	28	15	26	n.a	70	
	sum	42	42	25	41	n.a	113	
	ratio step1/step 2	0.6	0.5	0.7	0.6		0.6	
Zn 454	step 1	205	194	149	247	194	360	417
	step 2	114	122	70	66	31	41	
	sum	319	316	219	313	225	401	
	ratio step1/step 2	1.8	1.6	2.1	3.7	6.3	8.8	
Cr 272	step 1	2	2	4	30	7	55	11
	step 2	46	47	28	17	16	118	
	sum	48	49	32	47	23	173	
	ratio step1/step 2	0.04	0.04	0.14	1.76	0.44	0.47	
Pb 143	step 1	3	2	4	42	28	n.a	5
	step 2	126	130	138	83	15	n.a	
	sum	129	132	142	125	43	-	
	ratio step1/step 2	0.02	0.02	0.03	0.51	1.87		
Cd 12	step 1	7	7	6	5	16	n.a	10
	step 2	4	5	3	7	10	n.a	
	sum	11	12	9	12	26	-	
	ratio step1/step 2	1.8	1.4	2	0.7	1.6	-	
Cu 275	step 1	49	48	34	28	93	174	103
	step 2	124	125	111	94	42	45	
	sum	173	173	145	122	135	219	
	ratio step1/step 2	0.4	0.4	0.3	0.3	2.2	3.9	

n.a. not available; recycle, recirculating manifold; contflow, continuous flow extraction; offlineA, offline detection involving 10ml of solutionA only; (extraction modes are explained in detail in Chapter four)

9.1.5 Data on DIN 19738 method for the assessment of bioaccessibility

Appendix Table 26 (Table D-1): Composition of synthetic digestive juices used in the DIN procedure [138]

Components (weight in mg) dissolved in 30 ml, 70 ml and 100 ml water for saliva, gastric and intestinal juices respectively.

Synthetic saliva (mg)		synthetic gastric juice (mg)		synthetic intestinal juice (mg)	
NaCl	50	NaCl	290	KCl	30
NaSCN	15	KCl	70	CaCl2	50
Na_2SO_4	55	KH_2PO_4	27	MgCl2	20
$NaHCO_3$	15	Pepsin	100	$NaHCO_3$	100
KCl	45	Mucin	300	Trypsin	30
KH_2PO_4	60			Pancreatin	900
$CaCl_2$	15			Bile, lyophilised	900
Mucin	75			Urea	30
a-Amylase	25				
Urea	10				
Uric acid	1				

Appendix Table 27 (Table D-2): Extraction of metals from BCR 701 with synthetic digestive fluids using DIN 19738 batch method

(Metal concentrations (mg/kg) in simulated digestive tract after ingestion of BCR 701

BCR 701	saliva	stomach	intestine	% available*
Ni	4	16	10	10
Cu	18	81	63	23
Zn	23	87	31	7
Pb	1	34	8	6
Al	641	1560	248	1

* % available is calculated as % of total metal content of solid sample material

Appendix Table 28 (Table D-3): Extraction of metals from parsley with synthetic digestive fluids using DIN 19738 batch method

mg/kg	saliva	gastric juice	intestinal fluid	residue	% availability
Al	4	7	6	25	4
Cu	2	2	5	3	91
Fe	16	27	49	47	30
Mn	11	12	20	10	50

Appendix Table 29 (Table D-4): Extraction of metals from Lübbenau garden soil with synthetic digestive fluids using DIN 19738 batch method

mg/kg	saliva	gastric juice	intestinal fluid	Residue	% available
Al	151	393	69	4661	1
Cu	1.2	nd	3	15	12
Fe	470	502	125	#	1
Mn	22	262	29	445	5

n d not detected

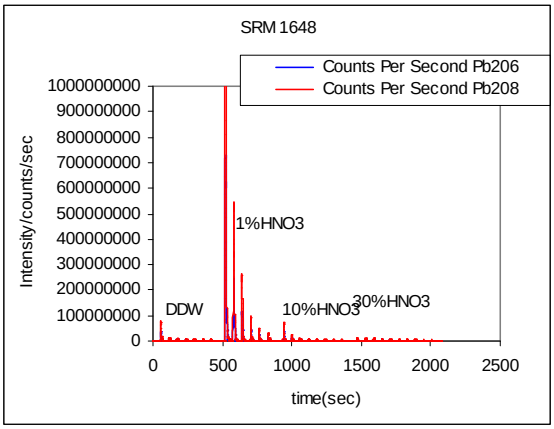
not determined

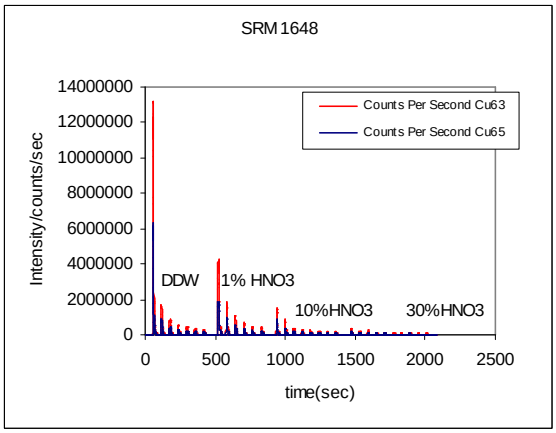
9.2 Figures

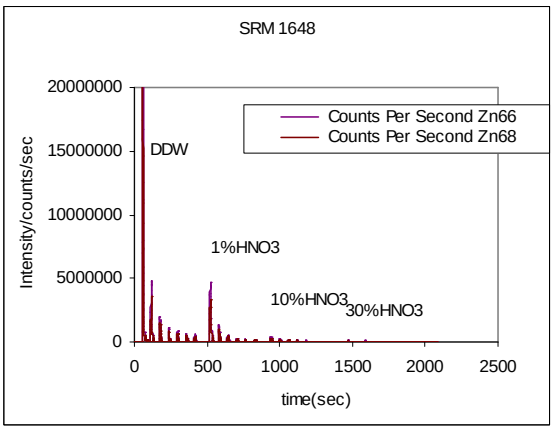
9.2.1 Leaching profiles of metals extracted from various environmental samples

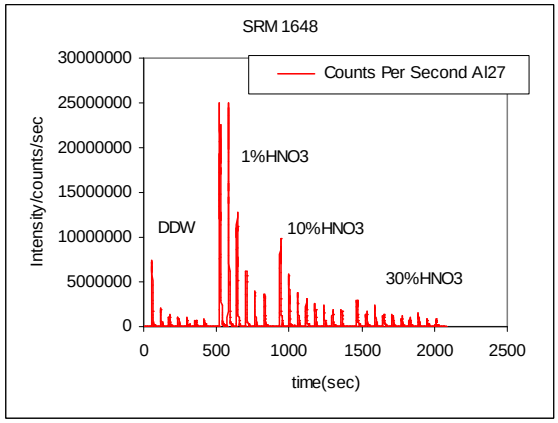
9.2.1.1 Extraction of metals from SRM 1648, urban dust reference material

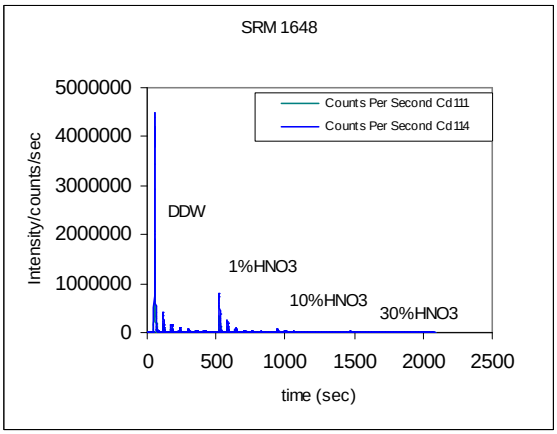
a) SRM 1648 acid scheme

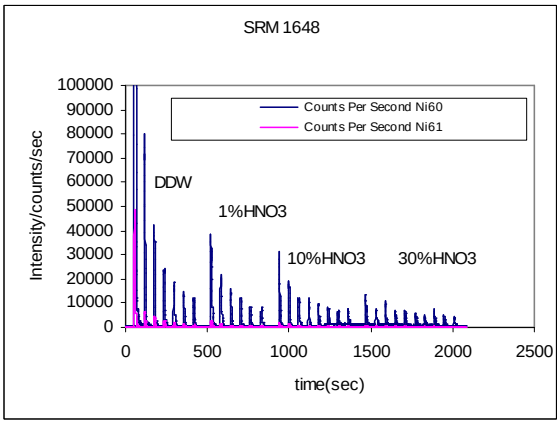




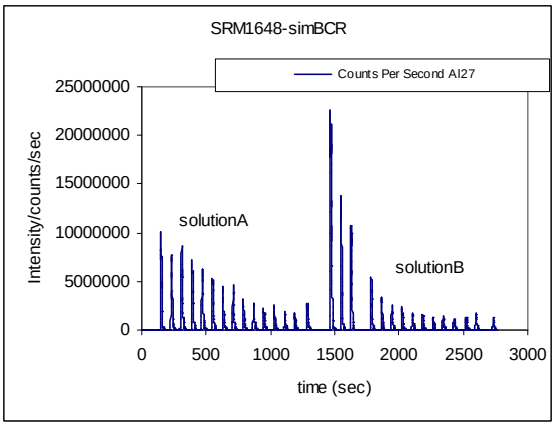


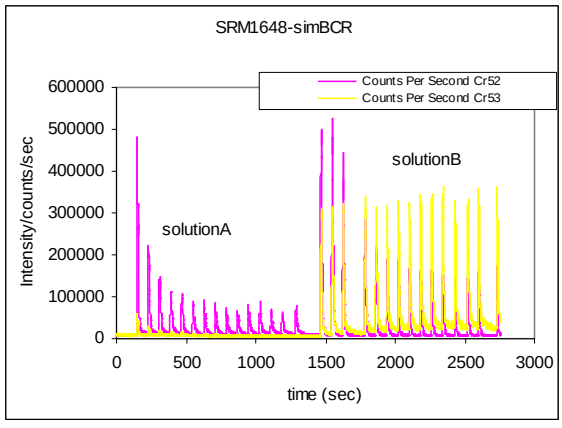


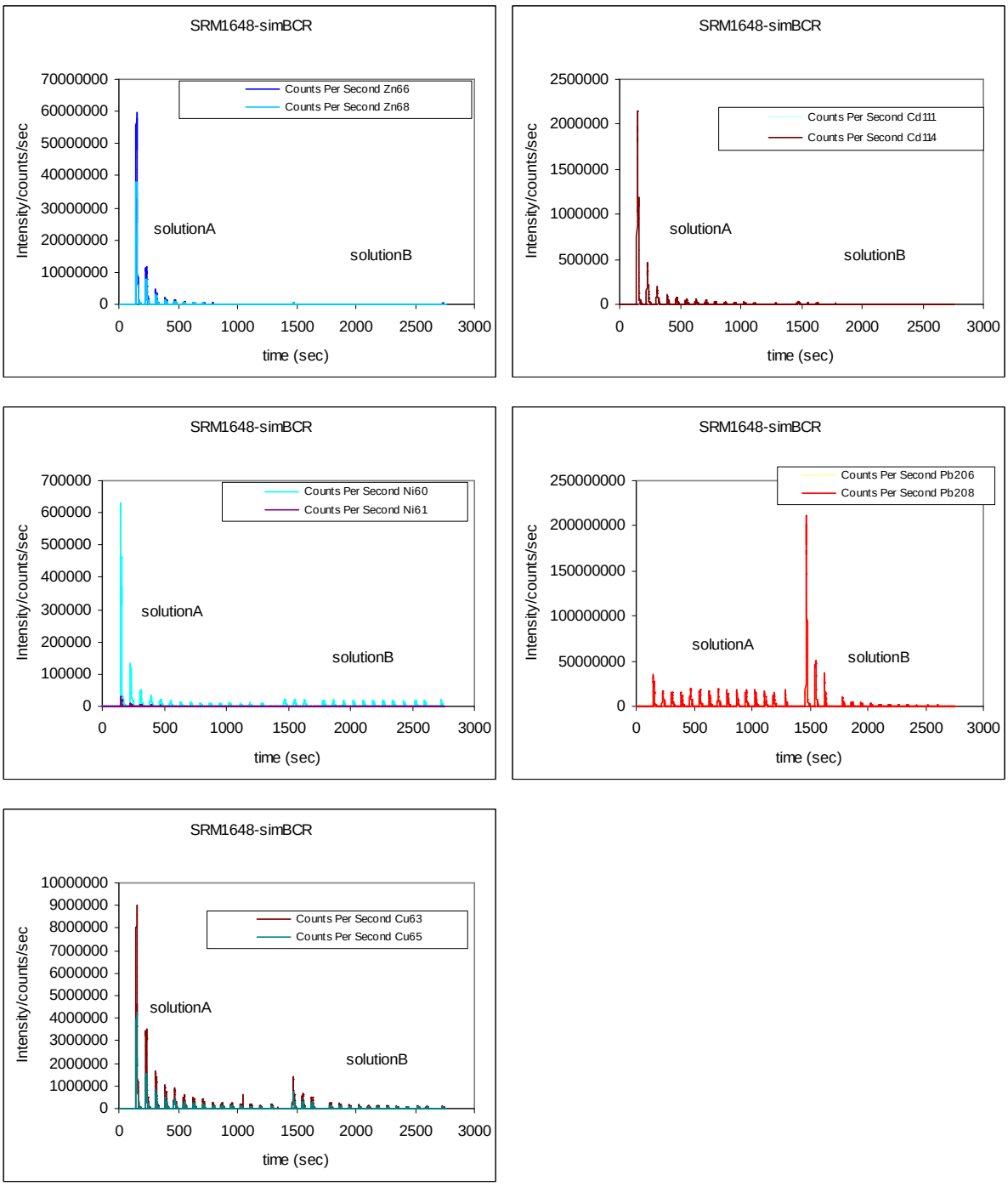




b) simBCR scheme



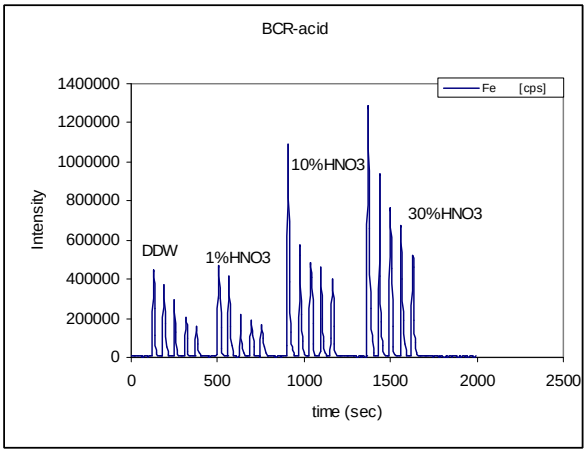


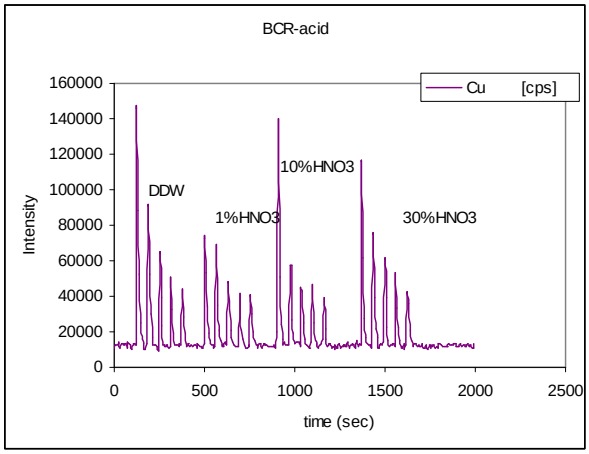


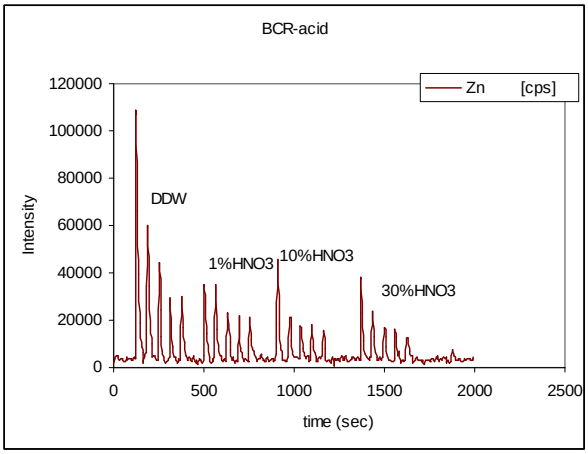
Appendix Fig. F- 1: Extraction behaviour of metals in SRM 1648, urban particulate matter, using a) nitric acid scheme and b) simBCR scheme

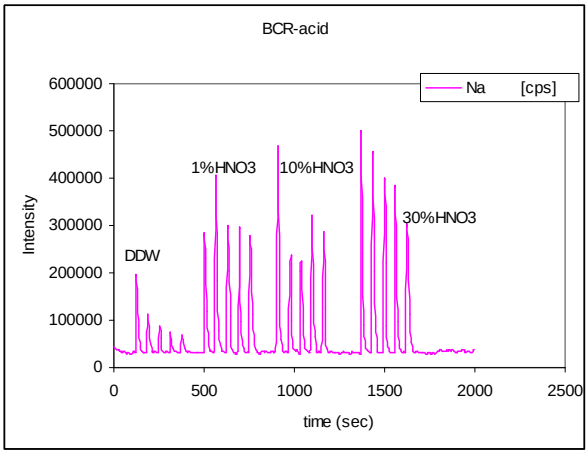
9.2.1.2 Extraction of metals from BCR 701, reference material

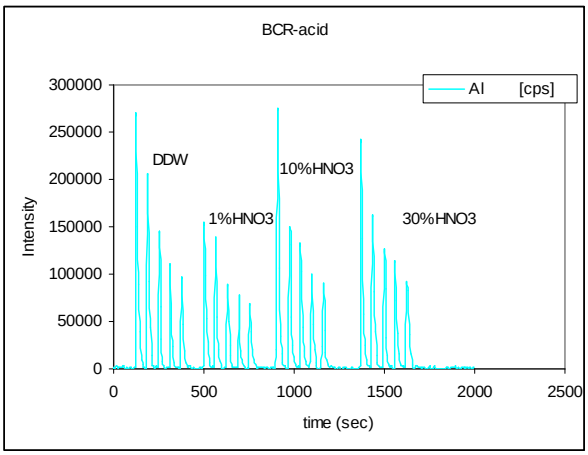
a) Nitric acid scheme with ICP-AES detection



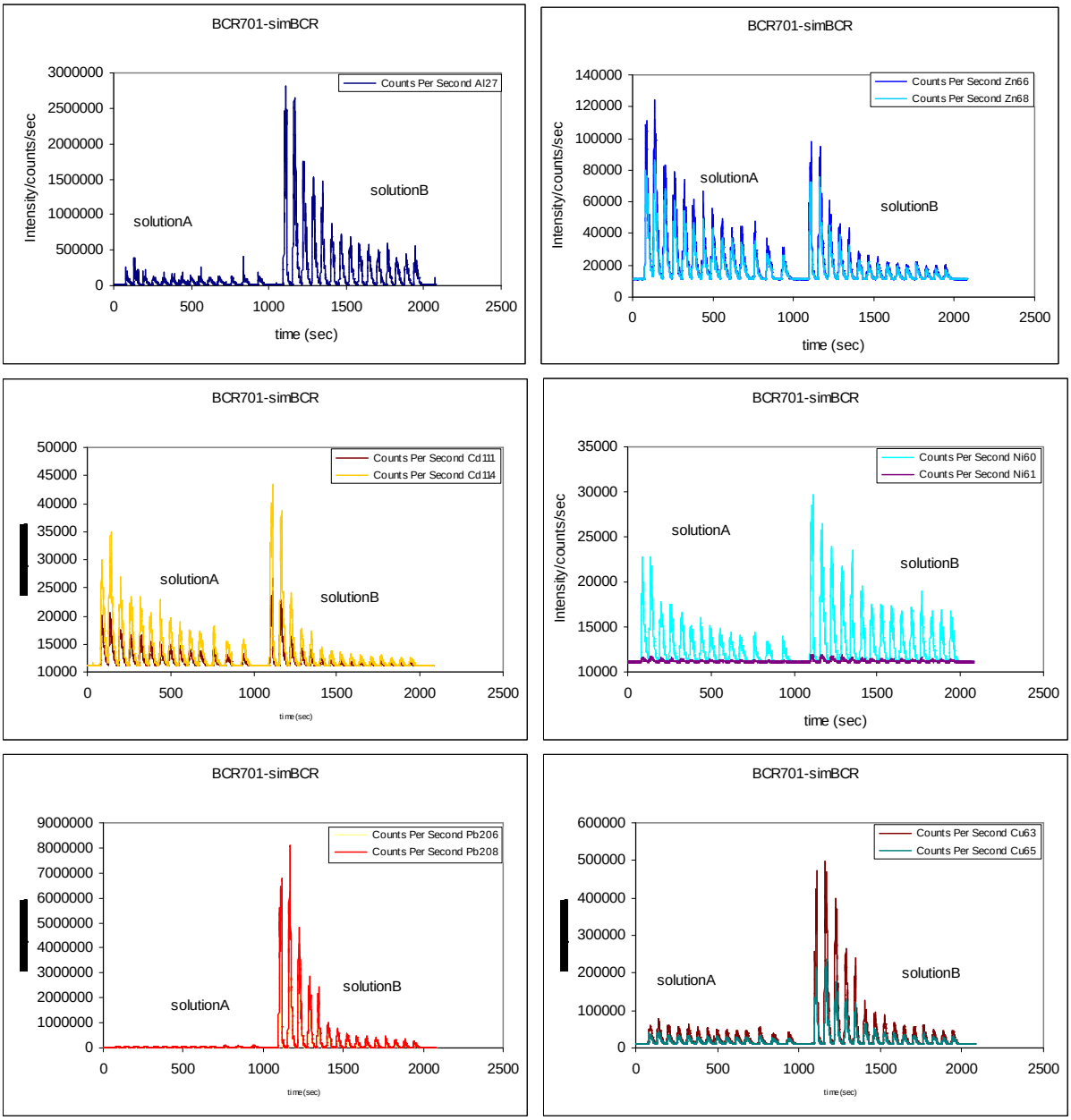








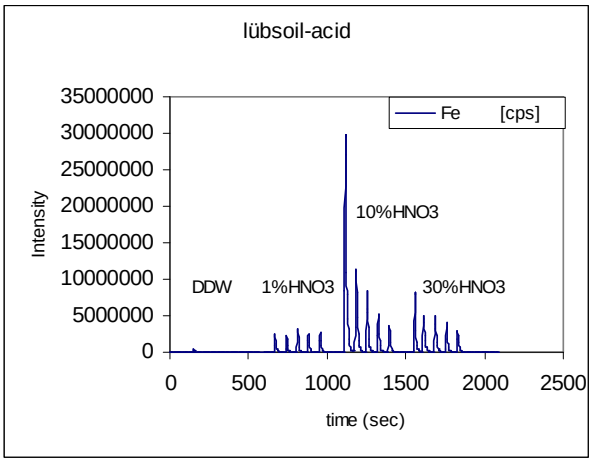
b) simBCR scheme

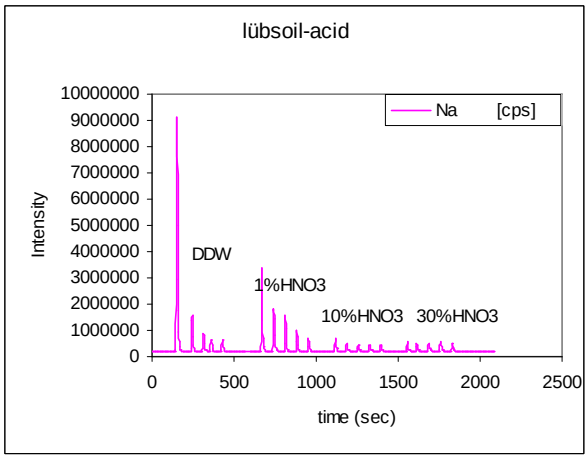


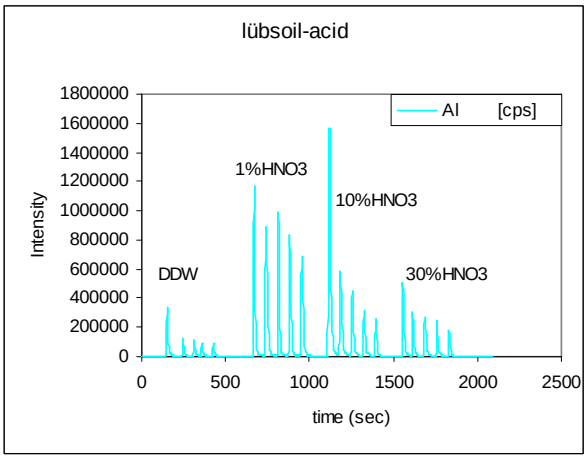
Appendix Fig. F- 2: Leaching behaviour of metals in BCR 701 lake sediment, under a) nitric acid and b) simBCR extraction conditions

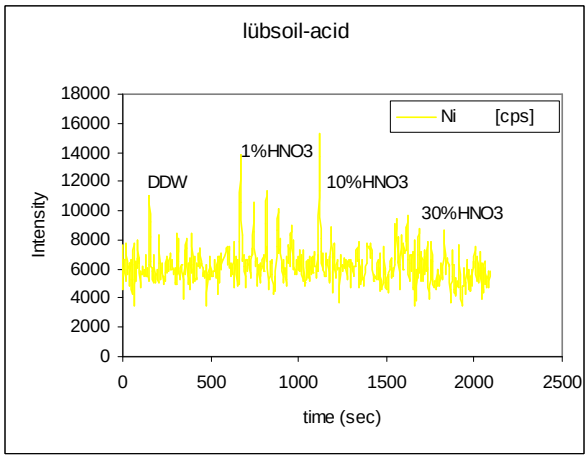
9.2.1.3 Extraction of metals from garden soil (50-100m from Autobahn in Brandenburg) using various schemes

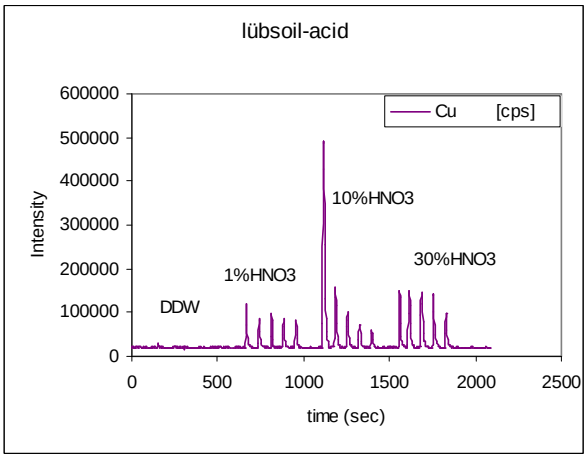
a) Nitric acid scheme

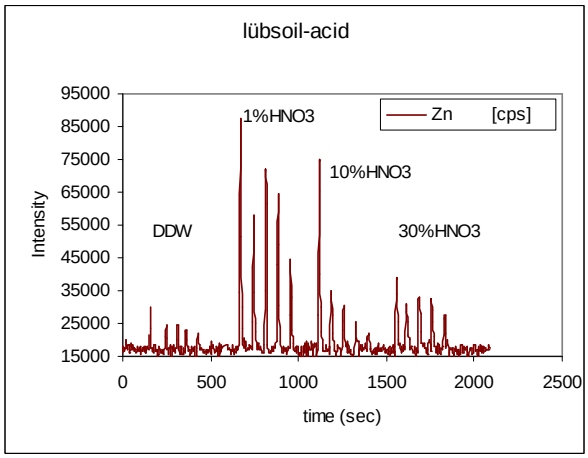


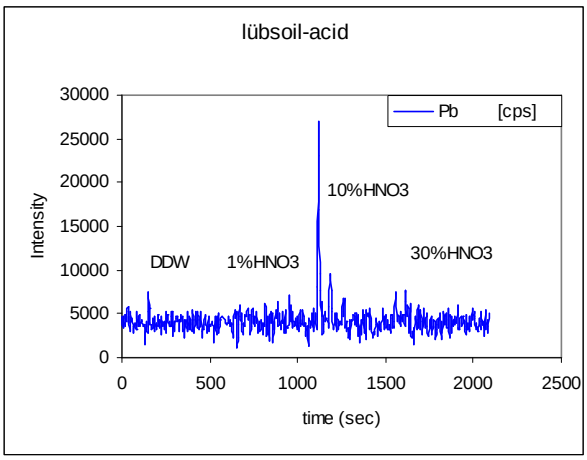


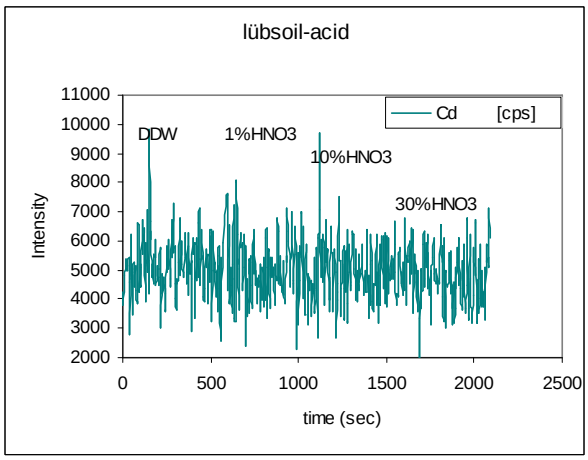




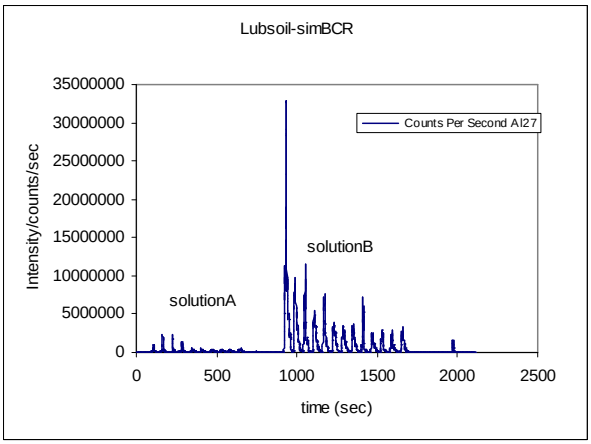


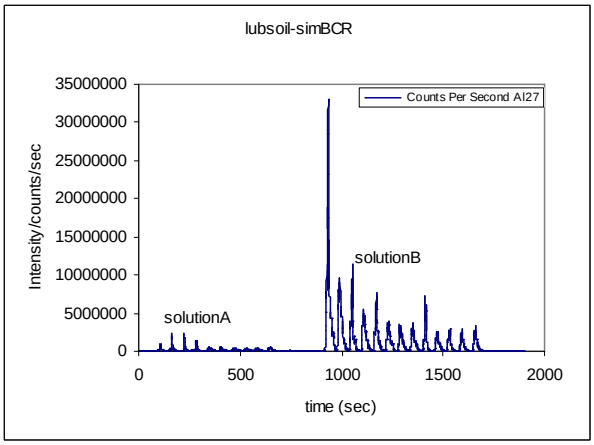


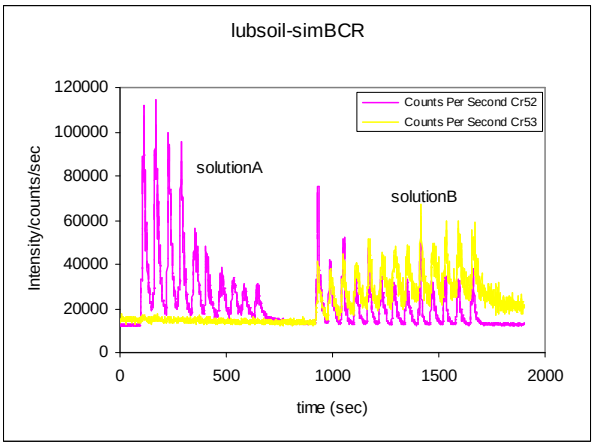


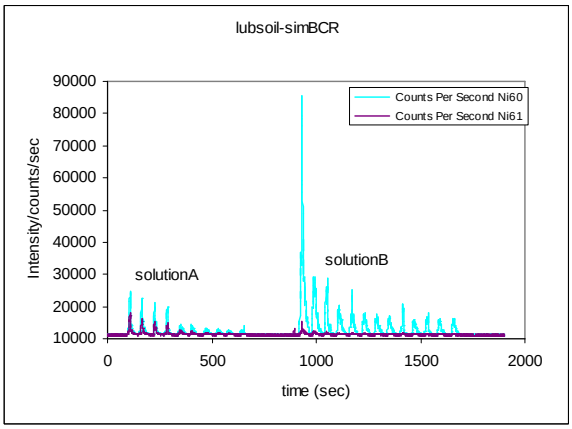


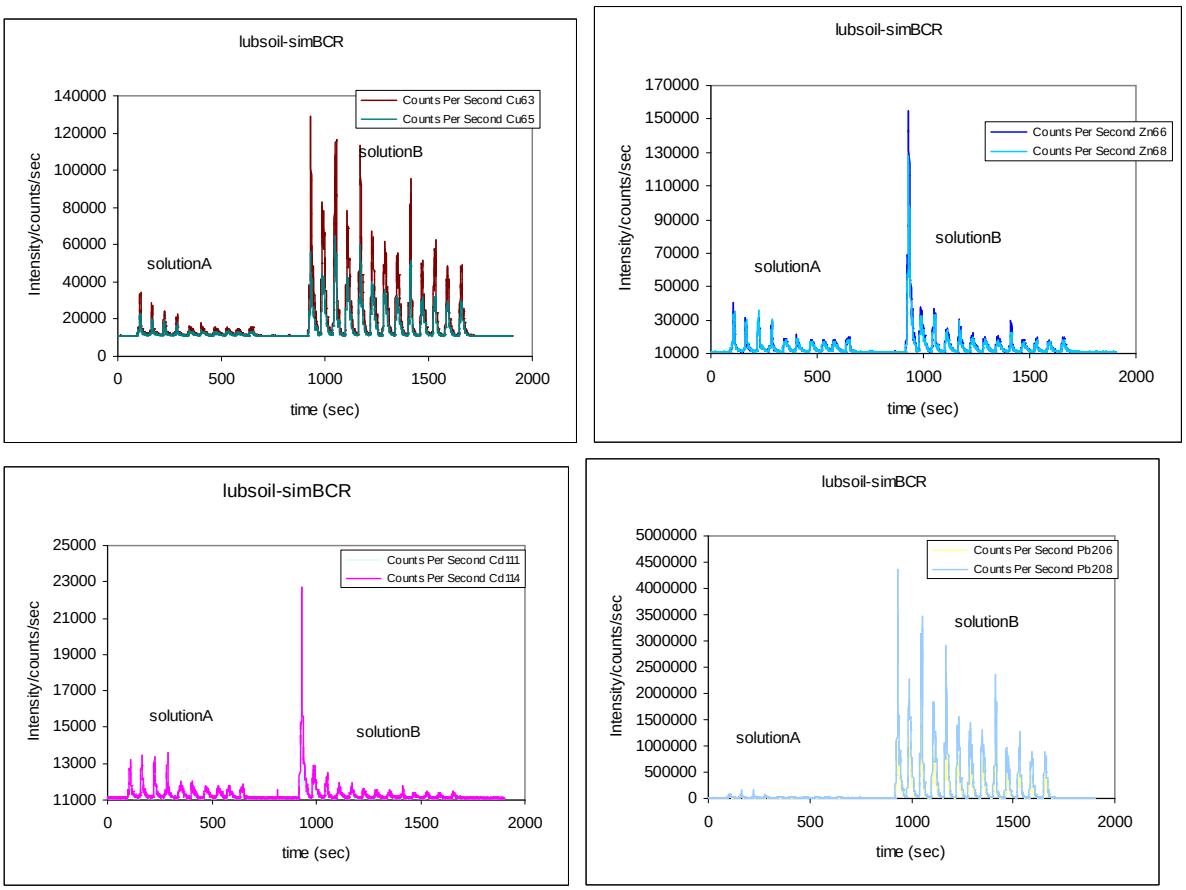
b) simBCR scheme









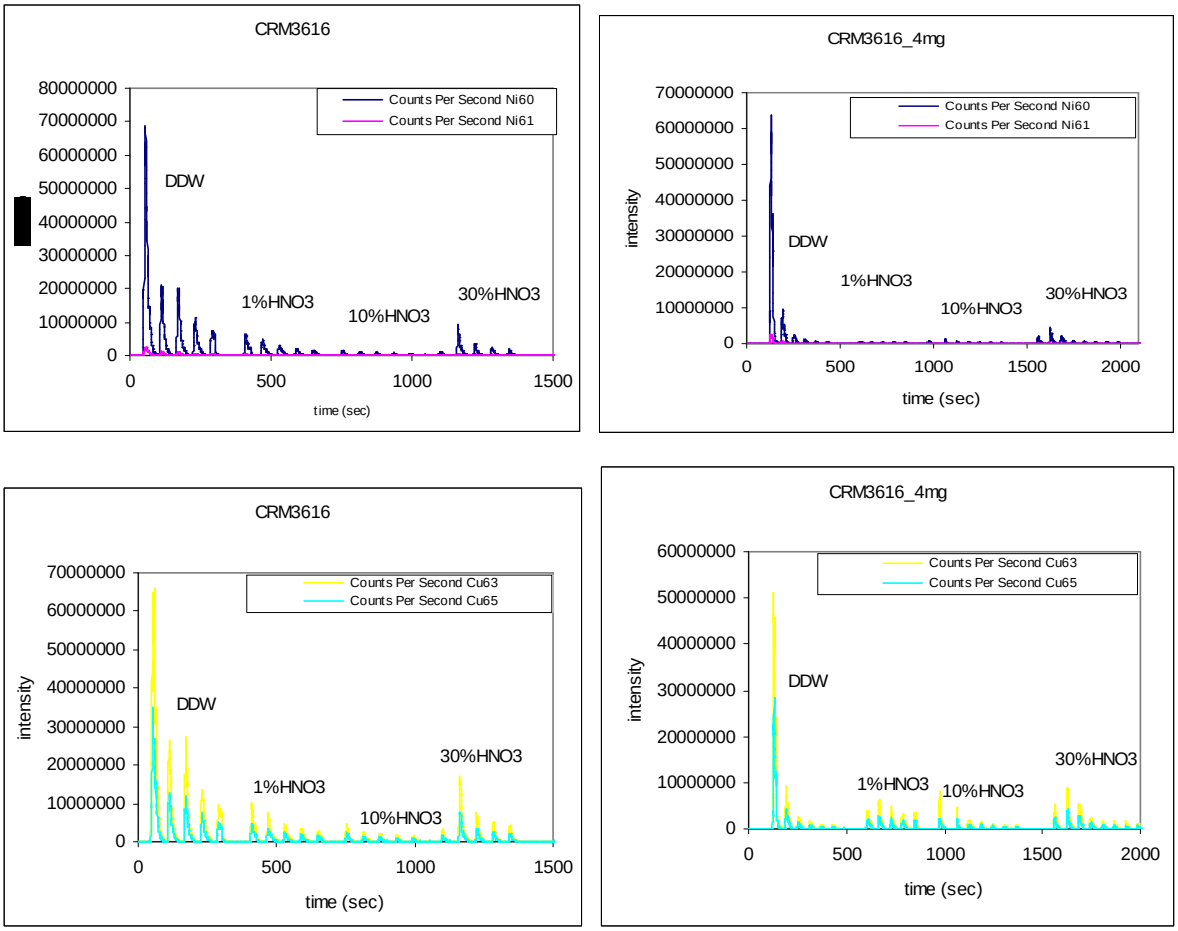


Appendix Fig. F- 3: Extraction profiles for metals in garden soil from Lübbenau a) acid scheme b) simBCR scheme

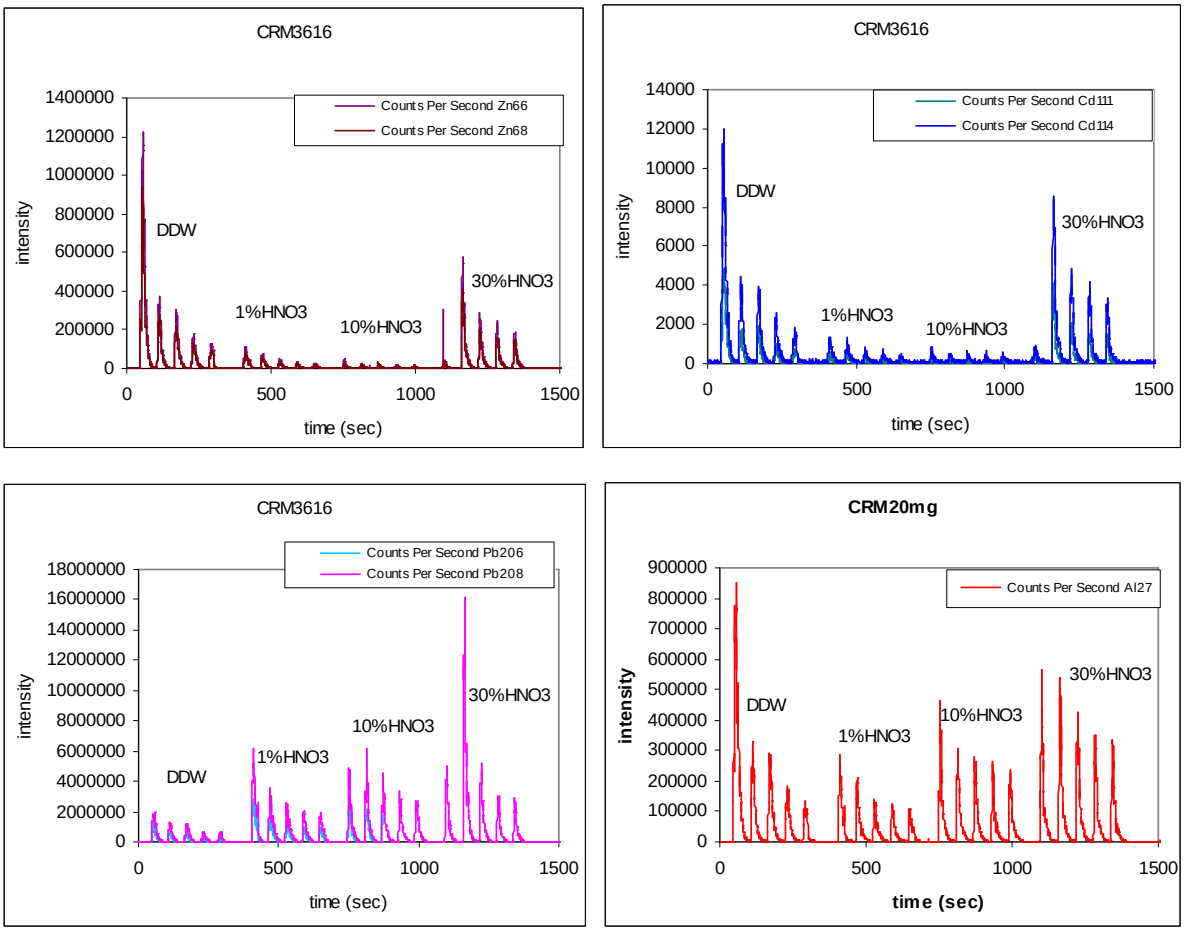
9.2.1.4 Extraction profiles of metals from CRM 3616 pyrrhotine ore using the nitric acid scheme

a) 20 mg sample

b) 4 mg sample

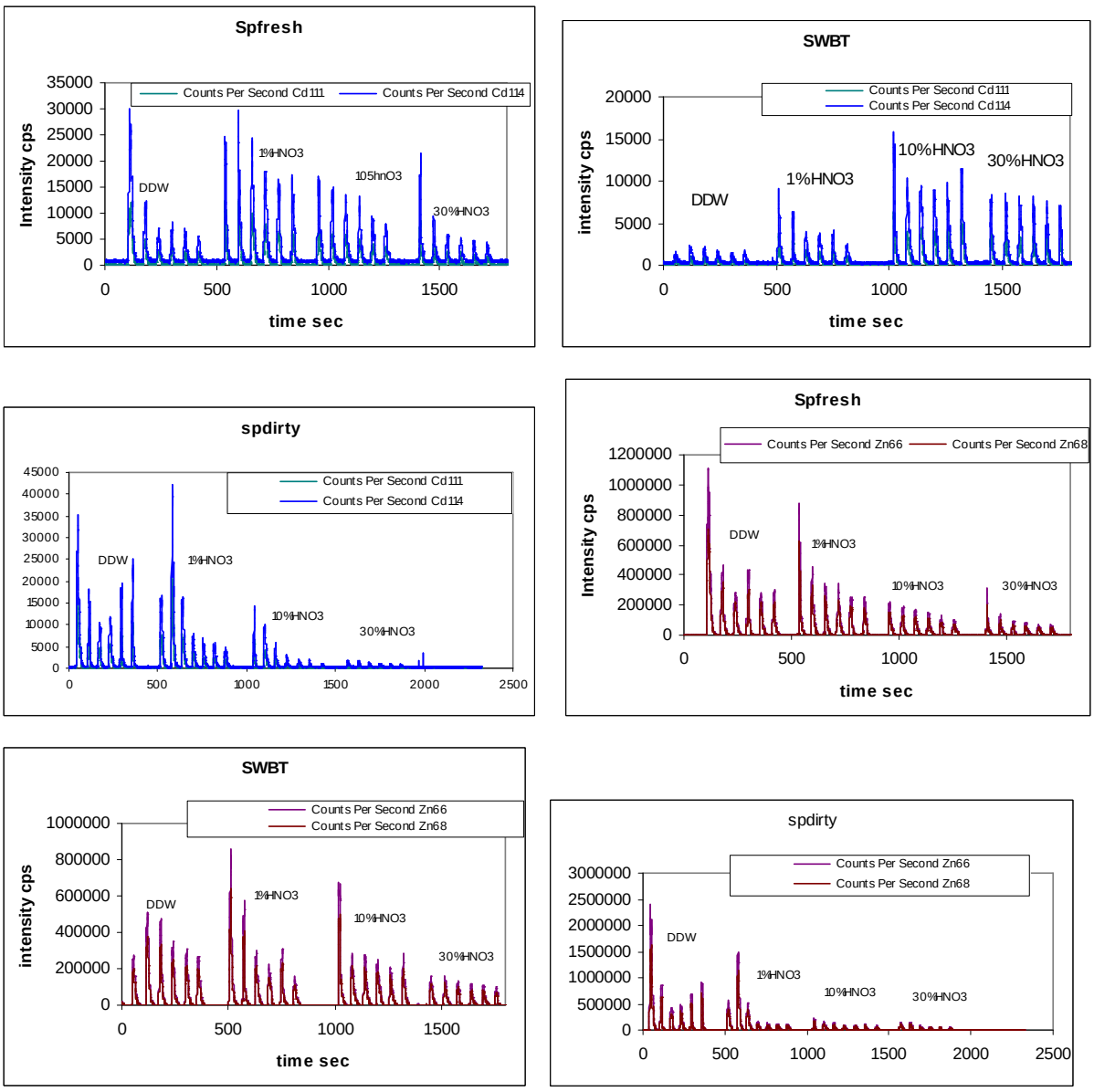


Appendix Fig. F- 4: Effect of sample weight on extraction profiles of Ni and Cu from CRM 3616



Appendix Fig. F- 5: Leaching profiles of metals from CRM 3616 pyrrhotine ore using the nitric acid scheme

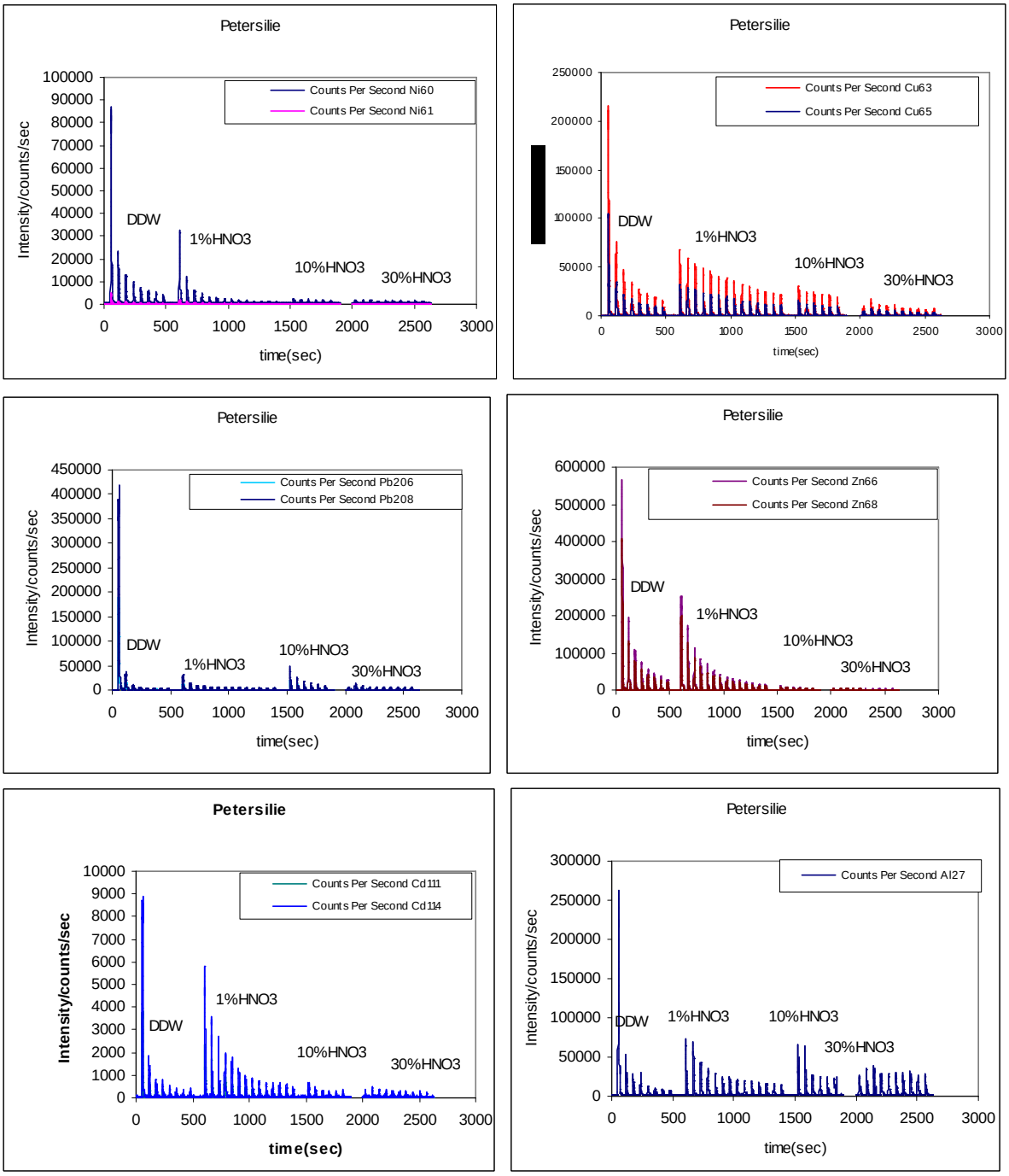
9.2.1.5 Effect of sample weight and preparation method on the leaching behaviour of metals from plant material



Appendix Fig. F- 6: Extractability of metals in spinach samples that have been prepared in different ways

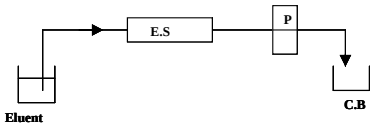
SWBT spinach, washed blended and dried; spdirty spinach unwashed, blended and dried; spfresh spinach washed, fresh sample

9.2.1.6 Leaching profiles of metals from parsley samples

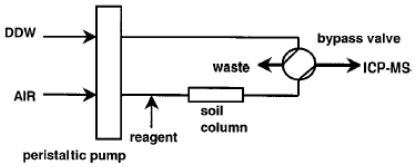


Appendix Fig. F- 7: Extraction behaviour of metals in parsley samples (nitric acid scheme)

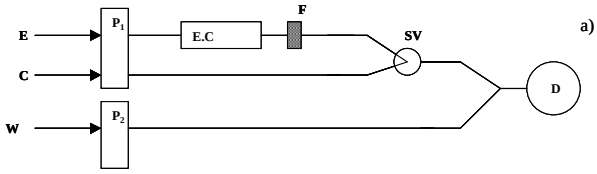
9.2.2 Continuous extraction configurations found in the literature



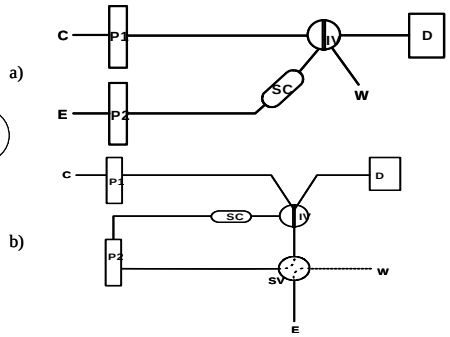
E.S extraction system consisting of extraction chamber, magnetic stirrer and glass microfibre filter; C.B collection bottle for sample extract; P peristaltic pump.
adapted from: W. Tiyapongpattana, P. Pongsakul J. Shiowatana, D. Nacapricha . Sequential extraction of phosphorus in soil and sediment using a continuous-flow system Talanta 62 (2004) 765–771



From Diane Beauchemin, Kurt Kyser, and Don Chipley
Inductively Coupled Plasma Mass Spectrometry with On-Line Leaching: A Method To Assess the Mobility and Fractionation of Elements Anal. Chem.2002, 74,3924-3928



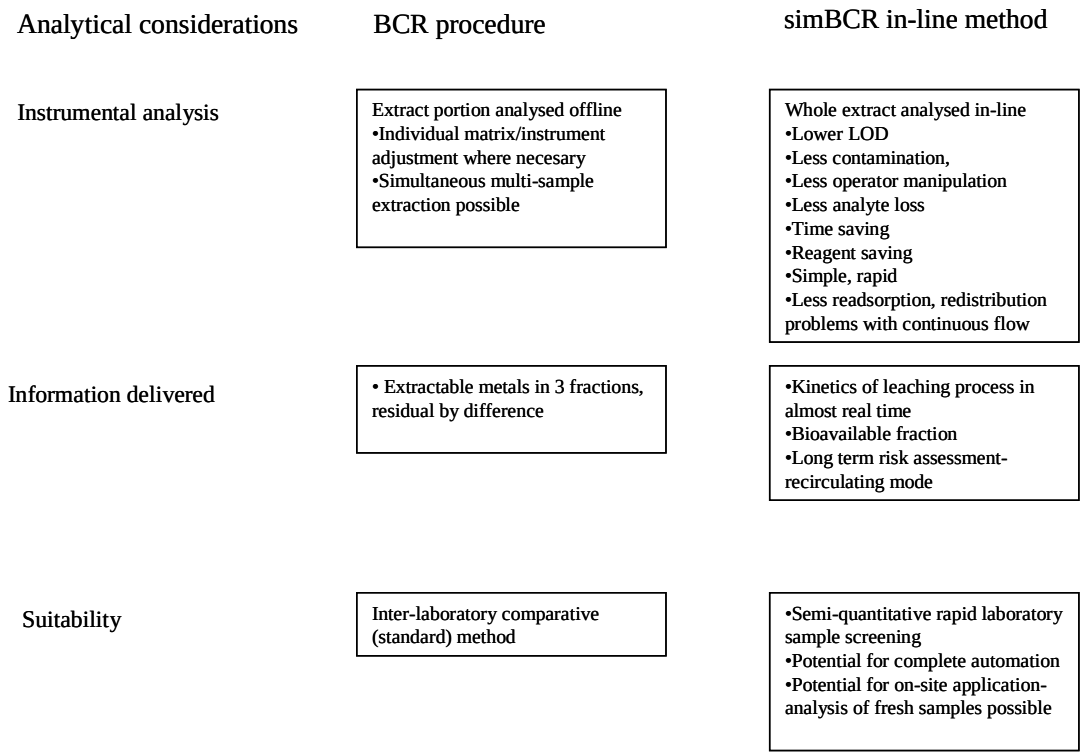
P1 peristaltic pump; P2 ICP pump; E.C extraction column; E extraction solution; C calibration solution; W water; SV 2way valve; F filter; D ICP-AES detector.
Adapted from PO Scokart, K Meeus-Verdinne and R. De Borger Speciation of heavy metals in polluted soils by sequential extraction and ICP Spectrometry Int. J. Environ. Anal. Chem 1987, 29, 305-315



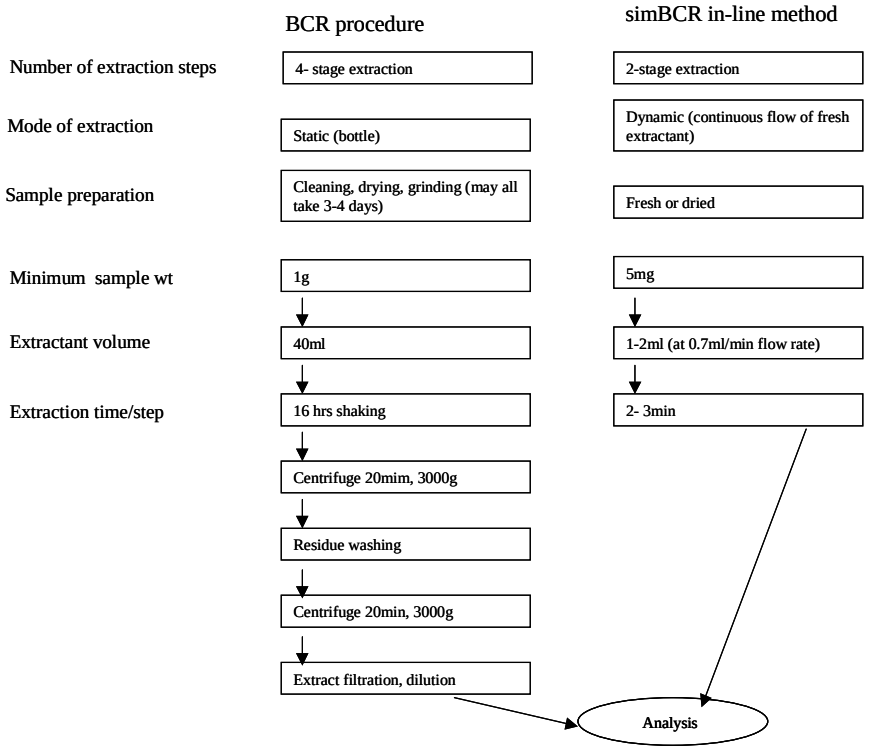
Flow manifolds for studying a) fast and b) slow leaching kinetics
P1 und P2 = Pumps, SV = Switching valve, IV = Injection valve, SC = Sample column, C = Carrier, E = Eluent, W = Waste, AS = Atomic spectroscopic detector
From this work

Appendix Fig. F- 8: Configurations found in literature for continuous extraction (off- and in-line detection)

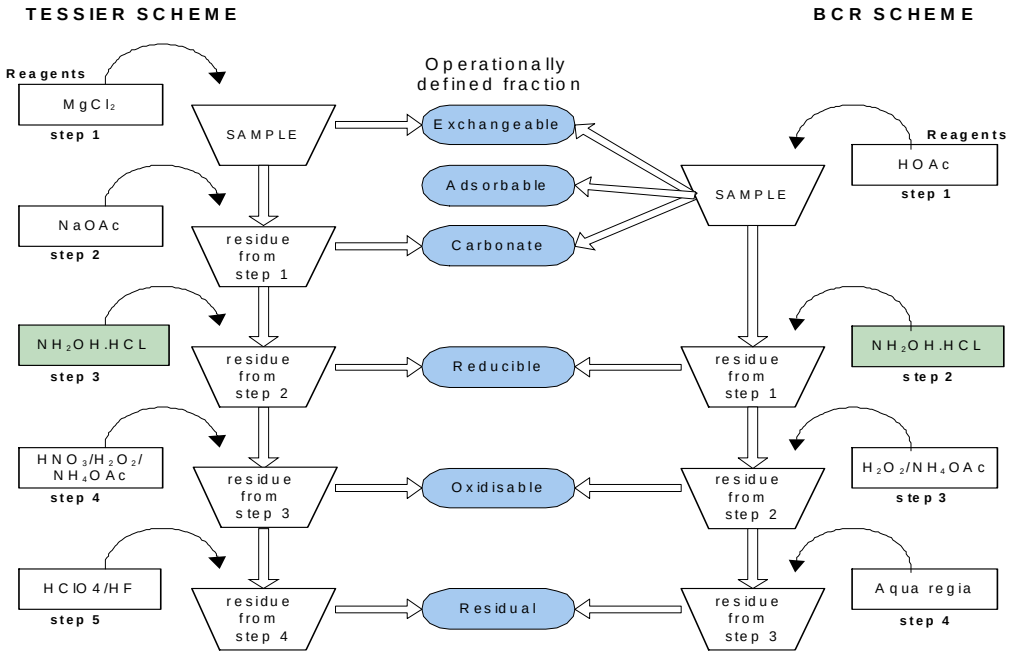
9.2.3 Schematic comparison of some sequential extraction schemes



Appendix Fig. F- 9: Schematic comparison of batch BCR and on-line simBCR



Appendix Fig. F- 10: Scheme of extraction steps used in BCR batch and simBCR on-line methods



Appendix Fig. F- 11: The BCR and Tessier schemes in comparison.

10 References

[1] E. Merian, M. Anke (ed.)
Elements and their compounds in the environment : occurrence, analysis and biological relevance,
Weinheim Wiley-VCH ISBN: 3-527-30459-2

[2] K C. Berger
Micronutrient Deficiencies in the United States
J. Agric. Food Chem 10 (3), 178 (1962)

[3] A. Mehlich
Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant
Commun. Soil Sci. Plant Anal. 15, 1409-1416 (1984)

[4] W. Smith, A. Smith (ed.)
Minamata
Rinehart and Winston, New York, 1975

[5] P. Laszlo
Tools, instruments and concepts: the influence of the second chemical revolution. In P.Morris (ed),
From classical to modern chemistry- the instrumental revolution
London, RSC Publishers 2002

[6] R A. Kelly , J C. Andrews , J G. DeWitt
An X-ray absorption spectroscopic investigation of the nature of the zinc complex accumulated in
Datura innoxia plant tissue culture
Microchem. J 71, 231–245 (2002)

[7] D E. Salt , R C. Prince, I J. Pickering
Chemical speciation of accumulated metals in plants: evidence from X-ray absorption spectroscopy
Microchem J 71, 255–259 (2002)

[8] G. Köllensperger, G. Friedbacher, R. Kotzick, R. Niessner, M. Grasserbauer
In-situ AFM investigation of aerosols exposed to different humidities
Fresenius J. Anal Chem 364, 296- 304 (1999)

[9] J. D. Winefordner, I. B. Gornushkin, D. Pappas, O. I. Matveev, B. W. Smith
Novel uses of lasers in atomic spectroscopy-Plenary Lecture
J. Anal. At. Spectrom. 15, 1161-1189 (2000)

[10] K J. Lamble, S.J. Hill, 1998
Microwave digestion procedures for environmental matrices
Analyst Vol. 123 (103R–133R)

[11] K Ashley, R N. Andrews, L Cavazos, M Demange
Ultrasonic extraction as a sample preparation technique for elemental analysis by atomic spectrometry
J. Anal. At. Spectrom. 16, 1147–1153 (2001)

[12] D M Templeton
Trace element speciation in toxicology and clinical sciences
Analusis 26(6), M68-71 (1998)

[13] DM Templeton
Biomedical aspects of trace element speciation
Fresenius J. Anal Chem. 363, 505-511 (1999)

14 S.J. Fairweather-Tait
The importance of trace element speciation in nutritional sciences
ibid :536-540 (1999)

15 A Paschke
Consideration of the physicochemical properties of sample matrices – an important step in sampling and sample preparation
Trends Anal. Chem. 22 (2), 78-89 (2003)

16 V. H. Kennedy, A. L. Sanchez, D. H. Oughton, A. P. Rowland
Use of Single and Sequential Chemical Extractants to Assess Radionuclide and Heavy Metal Availability From Soils for Root Uptake
Analyst 122, R–100R (1997)

17 IUPAC Nomenclature -Guidelines for terms related to chemical speciation and fractionation of elements.
Pure Appl Chem 72, 1453–70 (2000)

18 Rachel L. Carson
Silent Spring
Houghton Mifflin Books Boston First published 1962

19 A.A. Meharg, D. Osborne, D.J. Pain, A. Sanchez
The Donana ecological disaster: contamination of a world heritage estuarine marsh ecosystem with acidified pyrite mine waste.
Sci Total Environ. 222(1-2), 45-54 (1998)

20 http://duna.org/cyanidespill/3rd-spill-news.shtml accessed on 21. April 2005

21 http://www.iso.org/iso/en/CatalogueListPage.CatalogueList?scopelist=

22 http://www.bam.de/service/referenzverfahren/inhalt_verfahren_i.htm

23 http://www.normung.din.de

24 ISO 18412:2005: Water quality - Determination of chromium(VI) - Photometric method for weakly contaminated water

WHO Guidelines for drinking-water quality, third edition Volume 1 – Recommendations ©World Health Organization 2004 ISBN 92 4 154638 7

26 R. Cornelis, C. Camara, L. Ebdon, L. Pitts, M. Sperling, R. Morabito, O.F.X. Donard, H. Crews, E.H. Larsen, B. Neidhart, F. Ariese, E. Rosenberg, O. Berrouiguet, G.M. Morrison, G. Cordier, F. Adams, B. Dero, J. Marshall, B. Stojanik, A. Ekvall, P. Quevauviller
The EU network on trace element speciation in full swing
Trends Anal. Chem. 19, 210-215 (2000)

27 D. Peakall, J Burger
Methodologies for assessing exposure to metals: speciation, bioavailability of metals, and ecological host factors
Ecotoxicology and Environmental Safety 56, 110–121 (2003)

MV. Ruby, R. Schoof , A Davis, S Eberle, C Sellstone
Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test
Environ. Sci. Technol. 30,422-430 (1996)

29 B. K. Kramer, P. B. Ryan
Soxhlet and microwave extraction in determining the bioaccessibility of pesticides from soil and model solids
Proceedings of the 2000 Conference on Hazardous Waste Research, pp 196–210

30 M. Chu, D. Beauchemin
Simple method to assess the maximum bio-accessibility of elements from food using flow injection and inductively coupled plasma mass spectrometry
J. Anal. At. Spectrom. 19, 1213-1216 (2004)

31 K. M. Ellickson, R.J. ,Meeker, M.A. Gallo, B.T. Buckley, P.J. Lioy
Oral bioavailability of lead and arsenic from a NIST standard reference soil material
Arch. Environ. Contam toxicol 40(1), 128-135 (2001)

32 A.G. Oomen, J. Tolls, A.J.A.M. Sips, J.P. Groten
In vitro intestinal lead uptake and transport in relation to speciation
Arch. Environ Contam Toxicol, 44(1) 116-124 (2003)

33 K. T. Semple, K. J. Diock, K.C. Jones, P. Burauel, A. Craven, H. Harms
Defining Bioavailability and Bioaccessibility of Contaminated Soil and Sediment is Complicated
Environ. Sci. Technol. 38, 229A-231A (2004)

34 W.J.G.M. Peijnenburg, T. Jager
Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues
Ecotoxicology and Environmental Safety 56, 63–77 (2003)

J.S. Rieuwerts, I. Thornton, M.E. Farago, M.R. Ashmore
Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals
Chem. Spec. and Bioavail. 10(2), 61-75 (1998)

36 D.C. Adriano
Trace elements in terrestrial environments Biogeochemistry, bioavailability and risks of metals
2nd Edition Springer-Verlag 2001 pp 224 ISBN: 0-387-98678-2

37 M. Chen, L. Q. Ma, W. G. Harris
Arsenic Concentrations in Florida Surface Soils: Influence of Soil Type and Properties
Soil Sci. Soc. Am. J. 66, 632–640 (2002)

38 M.C. Yebra, N. Carro, M. F. Enríquez, A. Moreno-Cid, A. García
Field sample preconcentration of copper in sea water using chelating minicolumns subsequently incorporated on a flow-injection–flame atomic absorption spectrometry system
Analyst 126 (6), 933-937 (2001)

39 Xiu-Ping Yan, Yan Li, Yan Jiang
A flow injection on-line displacement/sorption preconcentration and separation technique coupled with flame atomic absorption spectrometry for the determination of trace copper in complicated matrices
J. Anal. At. Spectrom. 17 (6), 610 – 615 (2002)

40 J. L. Burguera, M. Burguera
Flow Injection for Automation in Atomic Spectrometry
J. Anal. At. Spectrom. 12 (6), 643 – 651 (1997)

41 Handbook of elemental speciation-techniques and methodology
Cornelis, Crews, Caruso and Heumann (ed.)
John Wiley & Sons Ltd 2003

J. D. Winefordner, I. B. Gornushkin, D. Pappas, O. I. Matveev, B. W Smith
Novel uses of lasers in atomic spectroscopy, Plenary Lecture
J. Anal. At. Spectrom 15, 1161-1189 (2000)

43 R. E. Sturgeon
Future of atomic spectrometry for environmental analysis-Invited Lecture
J Anal At Spectrom 13, 351–361 (1998)

44 J. D. Winefordner, I. B. Gornushkin, T. Correll, E. Gibb, B. W. Smith, N. Omenetto
Comparing several atomic spectrometric methods to the super stars: special emphasis on laser induced breakdown spectrometry, LIBS, a future super star
J Anal At Spectrom 19, 1061-1083 (2004)

45 M. Stoeppler
Probennahme und Aufschluss. Basis der Spurenanalytik
Springer Verlag 1994

46 S. B. Niazi, D Littlejohn, D J. Halls
Rapid partial digestion of biological tissues with nitric acid for the determination of trace elements by atomic spectrometry
Analyst 118 (7), 821-825 (1993)

47 A K. Wanekaya, S Myung, O A. Sadik
Pressure assisted chelating extraction: a novel technique for digesting metals in solid matrices
Analyst 127, 1272–1276 (2002)

48 J. Kösters, R. A. Diaz-Bonea, B. Planer-Friedrich, B. Rothweiler, A. V. Hirner
Identification of organic arsenic, tin, antimony and tellurium compounds in environmental samples by GC-MS
Journal of Molecular Structure 661-662, 347-356 (2003)

49 K. Bunzl
Probability for Detecting Hot Particles in Environmental Samples by Sample Splitting
Analyst 122, 653–656 (1997)

Deutsches Institut fuer Normung. *Sludge and sediments (Group S) digestion using aqua regia for subsequent determination of the acid soluble portion of metals DIN 38414 1984b; S7*
Beuth Verlag GmbH Berlin (1984)

51 T.T. Gorsuch
Dissolution of organic materials. In Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis-
Vol 1 Proceedings of the 7th Materials Research Symposium held at National Bureau of Standards Oct. 7-11 1974 issued Aug. 1976

52 A. Abu-Samra, S.J. Morris, S.R. Koirtyohann
Wet ashing of some biological samples in a microwave oven
Anal. Chem. 47: (8), 1475– 1477 (1975)

53 A.V Hirner
Elutions- und Extraktionsverfahren zur Bestimmung mobiler anorganischer und organischer Kontaminanten in Feststoffen
Analytiker Taschenbuch, Band 21 (2000) Springer-Verlag Berlin

54 M. C. Yebra, A. Moreno-Cid
Continuous ultrasound-assisted extraction of iron from mussel samples coupled to a flow injection-atomic spectrometric system
J. Anal. At. Spectrom., 2002, 17, 1425–1428

55 G. Wibetoe, D.T. Takuwa, W. Lund, G. Sawula
Coulter particle analysis used for studying the effect of sample treatment in slurry sampling electrothermal atomic absorption spectrometry,
Fresenius J. Anal. Chem. 363, 46-54 (1999)

J-M. Lévêque, J-L. Luche, C. Pétrier, R. Roux, W. Bonrath
An improved preparation of ionic liquids by ultrasound
Green Chemistry 4(4), 357 – 360 (2002)

57 C. Santos de Sousa, M. Korn
Effects of ultrasonic irradiation on the spectrophotometric determination of nickel with dimethylglyoxime
Anal. Chim. Acta 444, 309-315 (2001)

58 K. Wolnik, FL Fricke, CM Gaston
Quality assurance in the elemental analysis of foods by inductively coupled plasma spectroscopy
Spectrochimica Acta 39B(5), 649-655 (1984)

59 H. El Azouzi, M.L. Cervera, M. de la Guardia
Multi-elemental analysis of mussel samples by atomic absorption spectrometry after room temperature sonication
J. Anal. At. Spectrom. 13 533- 538 (1998)

60 F. Lazaro, M. D. Luque de Castro, M. Valcarcel
Direct introduction of solid samples into continuous-flow systems by use of ultrasonic irradiation
Anal. Chim.Acta 242, 283-289 (1991)

61 K Ashley, R N. Andrews, L Cavazos, M Demange
Ultrasonic extraction as a sample preparation technique for elemental analysis by atomic spectrometry
J. Anal. At. Spectrom. 16, 1147–1153 (2001)

62 C. Gerbersmann, M. Heisterkamp, F. C. Adams, J.A.C. Broekaert
Two methods for the speciation analysis of mercury in fish involving microwave-assisted digestion and gas chromatography-atomic emission spectrometry
Anal Chim Acta 350 (3), 273-285 (1997)

63 V. Nischwitz, B. Michalke, A. Kettrup
Investigations on extraction procedures for Pt species from spiked road dust samples using HPLC–ICP–MS detection
Anal Chim Acta 521 (1), 87-98 (2004)

64 S. Morales-Muñoz, J. L. Luque-García, M. D. Luque de Castro
A continuous approach for the determination of Cr(VI) in sediment and soil based on the coupling of microwave-assisted water extraction, preconcentration, derivatization and photometric detection
Anal Chim Acta 515 (2), 343-348 (2004)

65 B. Pérez-Cid, I. Lavilla, C. Bendicho
Aplication of microwave extraction for partitioning of heavy metals in sewage sludge
Anal Chim Acta 378 (1-3), 201-210 (1999)

66 B. Zunk
Mikrowellenaufschluss zur Bestimmung von Spurenelementen in Pflanzenmaterial
Anal Chim Acta 236, 337-343 (1990)

67 M. Bettinelli, U. Baroni, N. Pastorelli
Microwave oven sample dissolution for the analysis of environmental and biological materials
Anal Chim Acta 225, 159-174 (1989)

68 C. S. Eskilsson, E. Björklund
Analytical-scale microwave-assisted extraction
J Chromatog A 902 (1), 227-250 (2000)

69 F. E. Smith, E. A. Arsenault
Microwave-assisted sample preparation in analytical chemistry
Talanta 43 (8), 1207-1268 (1996)

70 M. Al-Harahsheh, S. W. Kingman
Microwave-assisted leaching—a review
Hydrometallurgy 73(3-4), 189-203 (2004)

71 S. Morales-Muñoz, J.L. Luque-Garcia, M.D. Luque de Castro
A continuous approach for the determination of Cr(VI) in sediment and soil based on the coupling of microwave-assisted water extraction, preconcentration, derivatization and photometric detection
Anal Chim Acta 515, 343–348 (2004)

72 G. Heltai, B. Fehér, K. Percsich, B. Barabás, I. Fekete
Application of sequential extraction with supercritical CO_2, subcritical H_2O, and an H_2O/CO_2 mixture for estimation of environmentally mobile heavy metal fractions in sediments
Anal Bioanal Chem 373, 863–866 (2002)

73 P Bermejo-Barrera , A Moreda-Pineiro, A Bermejo-Barrera
Sample pre-treatment methods for the trace elements determination in seafood products by atomic absorption spectrometry
Talanta 57, 969–984 (2001)

74 S. Sasi, Kannamkumarath, K Wrobel, K Wrobel , A Vonderheide, J. A. Caruso
HPLC–ICP–MS determination of selenium distribution and speciation in different types of nut
Anal Bioanal Chem 373, 454–460 (2002)

75 K.-D. Wenzel, B. Vrana, A. Hubert, G. Schuurmann
Dialysis of persistent organic pollutants and polycyclic aromatic hydrocarbons from semipermeable membrane A procedure using an accelerated solvent extraction device
Anal Chem 76, 5503-5509 (2004)

76 A. K. Das, M. de la Guardia, M. L. Cervera
Literature survey of on-line elemental speciation in aqueous solutions –a review
Talanta 55, 1–28 (2001)

77 J. Pawliszyn
Sampling and sample preparation in field and laboratory; Fundamentals and New Directions in Sample Preparation
Comprehensive Analytical Chemistry Series Vol 37 Elsevier 2003

78 J. Szpunar, R. Lobinski
Sample preparation techniques for elemental speciation studies; In Elemental Speciation, new approaches for trace element analysis Caruso, J.A.; Sutton, K.L.; Ackley, K.L (eds)
Comprehensive Analytical Chemistry Series Vol 33 Elsevier 2000

79 J. Szpunar, R. Lobinski
Hyphenated techniques in speciation analysis
RSC Chromatography monographs RSC 2004 ISBN 0854045457

S. Slaets, F. Adams
Determination of organomercury compounds with a miniaturised automated speciation analyser
Anal. Chim. Acta 414, 141-149 2000

81 T.M. Florence
Electrochemical approaches to trace element speciation in waters a review.
Analyst 111, 489-505 (1986)

82 T.M. Florence, G.E. Batley
Determination of the chemical forms of trace metals in natural waters, with special reference to copper, lead, cadmium and zinc.
Talanta 24, 151-158 (1977)

83 M T. Lam, J. Murimboh, N M. Hassan, C. L. Chakrabarti
Competitive ligand exchange/adsorptive cathodic stripping voltammetry (CLE/AdCSV) for kinetic studies of nickel speciation in aqueous environmental samples containing heterogeneous, macromolecular, organic complexants
Anal Chim Acta 402(1-2), 195-209 (1999)

84 G. Branica, M. Lovric
Pseudopolarography of totally irreversible redox reactions
Electrochim Acta 42(8), 1247-1251 (1997)

85 J. Buffle, M.-L. Tercier-Waeber
Voltammetric environmental trace-metal analysis and speciation: from laboratory to in situ measurements
Trend Anal Chem 24(3), 172-191 (2005)

86 B. Kolb, G. Kemmer, F.H. Schelser, E. Wideking
Elementspezifische Anzeige gaschromatographisch getrennter Metallverbindungen mittels Atom-Absorptions-Spektroskopie (AAS)
Fresenius Z. Anal. Chem. 221, 166-175 (1966)

87 K. Gunther , H. Waldner
Speziesanalytik von Zink und Cadmium in handelsüblichen pflanzlichen Lebensmitteln
Anal. Chim. Acta 259, 165-173 (1992)

88 A Sanz-Medel
Trace element analytical speciation in biological systems: importance, challenges and trends
Spectrochimica Acta Part B 53, 197-211 (1998)

89 Joanna Szpunar
Bio-inorganic speciation analysis by hyphenated techniques
Analyst 125, 963–988 (2000)

90 J.L. Gómez-Ariza, T. Garcıa-Barrera, F. Lorenzo, V. Bernal, M.J. Villegas, V. Oliveira
Use of mass spectrometry techniques for the characterization of metal bound to proteins (metallomics) in biological systems –Review
Anal Chim Acta 524, 15–22 (2004)

91 F. Adams
Elemental speciation: where do we come from? Where do we go?
J. Anal At Spectrom 19, 1090-1097 (2004)

92 J. Ruzicka, E.H. Hansen
Flow injection analyses: Part I. A new concept of fast continuous flow analysis
Anal Chim Acta 78 (1), 145-157 (1975)

93 Z.L. Fang
Flow Injection Atomic Absorption Spectrometry
Wiley, New York, 1995

J. Ruzicka, E.H. Hansen
Flow Injection Analysis
2nd Edition, Wiley, New York, 1988 ISBN (0-471-81355-9)

95 M. Valcarcel, M.D. Luque de Castro
FIA principles and applications
1987 Ellis Horwood ltd Chichester

96 W. Frenzel
Methodische Möglichkeiten der Fließinjektionsanalyse
In H. Günzler (Hrsg.) Analytiker Taschenbuch Band 20, Springerverlag, Heidelberg 1999

97 M Miro, W Frenzel
What flow injection has to offer in the environmental analytical field
Microchim. Acta. 148, 1-20 (2004)

J. L. Burguera, M. Burguera
Flow Injection for Automation in Atomic Spectrometry
J Anal At Spectrom 12, 643–651 (1997)

99 E. V. Alonso, A. G de Torres, J.M. C Pavon
Flow injection on-line electrothermal atomic absorption spectrometry
Talanta 55, 219–232 (2001)

100 E. H. Hansen, J. Wang
Implementation of suitable flow injection/sequential injection-sample separation/preconcentration schemes for determination of trace metal concentrations using detection by electrothermal atomic absorption spectrometry and inductively coupled plasma mass spectrometry
Anal Chim Acta 467, 3–12 (2002)

101 D. S. de Jesus, R. J. Cassella, S. L. Costa Ferreira, A. C. S. Costa, M. S. de Carvalho, R. El Santelli
Polyurethane foam as a sorbent for continuous flow analysis: Preconcentration and spectrophotometric determination of zinc in biological materials
Anal Chim Acta 366, 263-269 (1998)

102 H. Karami, M. F. Mousavi, Y. Yamini , M. Shamsipur
On-line preconcentration and simultaneous determination of heavy metal ions by inductively coupled plasma-atomic emission spectrometry
Anal Chim Acta 509, 89–94 (2004)

S. Vicente, N. Maniasso, Z. F. Queiroz, E. A.G. Zagatto
Spectrophotometric flow-injection determination of nickel in biological materials
Talanta 57, 475–480 (2002)

104 M.C. Yebra, N. Carro, M. F. Enríquez, A. Moreno-Cid, A. García
Field sample preconcentration of copper in sea water using chelating minicolumns subsequently incorporated on a flow-injection–flame atomic absorption spectrometry system
Analyst 126, 933–937 (2001)

J E. T. Andersen
A novel method for the filterless preconcentration of iron
Analyst 130, 385–390 (2005)

106 M. Miro, W. Frenzel
A novel flow-through microdialysis separation unit with integrated differential potentiometric detection for the determination of chloride in soil samples
Analyst 128, 1291-1297 (2003)

107 W. Frenzel
Gas diffusion separation and flow injection potentiometry -a fruitful alliance of analytical methods
Fresenius J. Anal. Chem 336, 21-28 (1990)

108 J.L. Gómez-Ariza, T. Garc´ıa-Barrera, F. Lorenzo, V. Bernal, M.J. Villegas, V. Oliveira
Use of mass spectrometry techniques for the characterization of metal bound to proteins (metallomics) in biological systems
Anal Chim Acta 524, 15–22 (2004)

109 M. Waisberg, W.D. Black, C.M. Waisberg, B. Hale
The effect of pH, time and dietary source of cadmium on the bioaccessibility and adsorption of

cadmium to/from lettuce(Lactuca sativa L. cv. Ostinata)
Food Chem Toxicol 42, 835–842 (2004)

110 B. Markert
Sample preparation (cleaning, drying, homogenisation) for trace element analysis in plant matrices
Sci Total Environ 176, 45-61 (1995)

111 F. Bordas, A. M. Bourg
A Critical Evaluation of Sample Pretreatment for Storage of Contaminated Sediments to be Investigated for the Potential Mobility of their Heavy Metal Load
Water Air Soil Pollut 103, 137–149 (1998)

112 Shuzhen Zhang, Songxue Wang, Xiao-quan Shan
Effect of sample pre-treatment upon the metal speciation in sediments by a sequential extraction procedure
Chemical Speciation and Bioavailability 13(3), 69-74 (2001)

113 F M.G. Tack, M G. Verloo
Leaching behaviour and solid phase fractionation of selected metals as affected by thermal treatment of a polluted sediment
Intern J Environ Anal Chem 51, 167-175 (1993)

114 C M. Davidson, L E. Wilson, A M. Ure
Effect of sample preparation on the operational speciation of cadmium and lead in a freshwater sediment
Fresenius J Anal Chem 363, 134–136 (1999)

115 T. Hjorth
Effects of freeze-drying on partitioning patterns of major elements and trace metals in lake sediments
Anal Chim Acta 526, 95-102 (2004)

116 J. Wragg, M. R. Cave, B. Palumbo, B.A. Klinck
A Study of the Geochemical Controls of Arsenic, Cadmium, Lead and Zinc Bioaccessibility from Mine Waste using a Respiratory Uptake Model
Nottingham, British Geological Survey 2002.

117 J. Pawliszyn
Sample Preparation: Quo Vadis?
Anal. Chem 75, 2543-2558 (2003)

118 F.G. Viets
Chemistry and Availability of Micronutrients in Soils
J. Agric Food Chem 10, 174-178 (1962)

119 A. Tessier, P. G. C. Campbell, M. Bisson
Sequential extraction procedure for the speciation of particulate trace metals
Anal Chem 51, 844-851 (1979)

120 A. Ure, Ph. Quevauviller, H.Muntau, B.Griepink
Speciation of heavy metals in soils and sediments, an account of the improvement and harmonisation of extraction techniques undertaken under the auspices of the BCR of the commission of the European communities
Int. J. Environ. Anal. Chem. 51, 135–151 (1993)

121 M. Pueyo, J.F. Lopez-Sanchez, G. Rauret
Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils
Anal. Chim. Acta 504, 217-226 (2004)

122 Handbuch der Bodenuntersuchung, Band 4,
Beuth Verlag GmbH Berlin, (2000) ISBN: 3-410-14590-7

123 Handbuch der Bodenuntersuchung, Band 5
Ibid. 2000

124 J. Hlavay, T. Prohaska, M Weisz, W. W. Wenzel, G.J. Stingeder
Determination of trace elements bound to soils and sediment fractions (IUPAC Technical Report)
Pure Appl. Chem 76 (2), 415–442 (2004)

125 V.H. Kennedy, A.L. Sanchez, D.H Oughton, A.P. Rowland
Use of single and sequential chemical extractants to assess radionuclide and heavy metal availability from soils for root uptake
Analyst 122, 89R–100R (1997)

126 I. Novozamsky, T.M. Lexmond, V.J.G. Houba
A single extraction procedure for evaluation of uptake of some heavy metals by plants
Int. J. Environ. Anal.Chem. 51, 47-58 (1993)

V.J.G. Houba, T.M. Lexmond, JJ. Novozamsky, J. J. van der Lee
State of the art and future developments in soil analysis for bioavailability assessment
Sci Total Environ 178, 21-28 (1996)

128 A. Sahuquillo, A. Rigol, G. Rauret
Overview of the use of leaching/ extraction tests for risk assessment of trace metals in contaminated soils and sediments
Trends Anal Chem 22 (3), 2003

129 M. Ure
Single extraction schemes for soil analysis and related applications
Sci Total Environ 178, 3-10 (1996)

130 S.K. Gupta, C. Aten
Comparison and evaluation of extraction media and their suitability in a simple model to predict the biological relevance of heavy metal concentrations in contaminated soils
Int. J. Anal. Chem 51, 25-46 (1993)

131 Ph. Quevauviller, G Rauret, B Griepink.
Single and sequential extraction in sediments and soil
Conclusions of the workshop on sequential extraction in sediments and soils Sitges, Spain , 29th March-1st April 1992
Int. J. Environ. Anal. Chem.51, 231-235 (1993)

Deutsches Institut für Normung. Schlamm und Sedimente (Gruppe S) Bestimmung der Eluierbarkeit mit Wasser (S4) DIN 38414 1984a; Teil 4: 1-10.
Beuth Verlag GmbH Berlin (1984)

133 S. Van Herreweghe, R. Swennen, V. Cappuyns, C. Vandecasteele
Chemical associations of heavy metals and metalloids in contaminated soils near former ore treatment plants: a differentiated approach with emphasis on pH stat-leaching
J. Geochem. Exploration 76(2), 113-138 (2002)

134 R. Chester, M. J. Hughes
A chemical technique for the separation of ferro-manganese minerals, carbonate minerals and adsorbed trace elements from pelagic sediments
Chem. Geol. **2**, 249-262 (1975)

135 G.E.M. Hall, G. Gauthier, J.C. Pelchat, P. Pelchat, J.E. Vaive
Application of a sequential extraction scheme to ten geological certified reference materials for the

determination of 20 elements.
J. Anal. Atom. Spectrom. 11, 787–796 (1996)

136 M. Kersten, U. Förstner
Chemical fractionation of heavy metals in anoxic estuarine and coastal sediments
Wat. Sci. Tech 18: 121-130 (1986)

C. M Davidson, R. P Thomas, S. E Mc Vey, R Perala, D Littejohn, A. M Ure
Evaluation of a sequential extraction procedure for the speciation of heavy metals in sediments
Anal.Chim. Acta 291(3), 277-286 (1994)

138 M. Pueyo, G. Rauret, D. Lück, M. Yli-Halla, H. Muntau, Ph. Quevauviller, J. F. Lo´pez-Sa´nchez
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, 243–250 (2001)

139 F M.G. Tack, M G. Verloo
Single extractions versus sequential extraction for the estimation of heavy metal fractions in reduced and oxidised dredged sediments
Chem. Spec and Bioavail. 11(2), 43-50 (1999)

140 A. V. Filgueiras, I. Lavilla, C. Bendicho
Comparison of the standard SM&T sequential extraction method with small-scale ultrasound-assisted single extractions for metal partitioning in sediments
Anal Bioanal Chem 374, 103–108 (2002)

141 RG McLaren, DV Crawford
Studies on soil copper. 1. Fractionation of copper in soils.
J. Soil Sci., 24, 171-181 (1973)

142 G.S.R. Krishnamurti, P.M. Huang, K.C.J. Van Rees, L.M. Kozak, L.M. Rostad
Speciation of particulate-bound Cadmium of soils and its bioavailability
Analyst 120, 659-6 (1995)

L.M. Shuman
Separating soil iron- and manganese oxide fractions for microelement analysis
Soil Sci. Soc. Am. J. 46, 1099 (1982)

F.M.G. Tack, M.G. Verloo
Chemical speciation and fractionation in soil and sediment heavy metal analysis: a review.
Int. J. Environ. Anal. Chem. 59, 225-238 (1995)

145 T.T. Chao
Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride
Soil Sci. Soc. Am. Proc. 36, 764-768 (1972)

L.M. Shuman,
Fractionation method for soil microelements
Soil Sci. 140, 11-22 (1985)

W.R. Berti, S.D. Cunningham
In-Place Inactivation of Pb in Pb-Contaminated Soils
Environ. Sci. Technol. 31, 1359-1364 (1997)

148 C. Kheboian, F. Bauer
Accuracy of selective extraction procedures for metal speciation in model aquatic sediments
Anal. Chem. 59, 1417-1423 (1987)

149 M.J. La Force, S. Fendorf
Solid-Phase Iron Characterization During Common Selective Sequential Extractions
Soil Sci. Soc. Am. J. 64, 1608-16 (2000)

150 C. Gleyzes, S. Tellier, M. Astruc
Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures.
Trend Anal Chem 21 (6+7), 451-467 (2002)

J. Thöming, W. Calmano
Applicability of Single and Sequential Extractions for Assessing the Potential Mobility of Heavy Metals in Contaminated Soils
Acta Hydrochim. Hydrobiol. 26, 338-34 (1998)

152 G.G.S. Holmgren
A rapid citrate-dithionite extractable iron procedure
Soil Sci. Soc. Am. Proc. 31, 210–211 (1967)

153 M. R. Cave, K. Harmon
Determination of Trace Metal Distributions in the Iron Oxide Phases of Red Bed Sandstones by Chemometric Analysis of Whole Rock and Selective Leachate Data
Analyst 122, 501–512 (1997)

154 S. Gao, R. Fujii, A. T. Chalmers, K. K. Tanji
Evaluation of Adsorbed Arsenic and Potential Contribution to Shallow Groundwater in Tulare Lake Bed Area, Tulare Basin, California
Soil Sci. Soc. Am. J. 68, 89–95 (2004)

155 C. S. E. Papp, L. H. Filipek, K. S. Smith
Selectivity and effectiveness of extractants used to release metals associated with organic matter
Appl. Geochem. 6, 349-353 (1991)

156 L Campanella, D. D'Orazio, BM. Petronio, E. Pietratonio
Proposal for a metal speciation study in sediments
Anal chim. Acta 309, 387-393 (1995)

157 Ph. Quevauviller, G. Rauret, H. Muntau, A.M. Ure, R. Rubio, J-F Lo´pez-Sanchez, H.D. Fiedler, B. Griepink
Evaluation of a sequential extraction procedure for the determination of extractable trace metal contents in sediments
Fresenius J. Anal. Chem. 349, 808–814 (1994)

158 J.-F. Lo´pez-Sa´nchez, A. Sahuquillo, R. Rubio, G. Rauret, H. Fiedler, H. Muntau, Ph. Quevauviller
CRM 601, A stable material for its extractable content of heavy metals
Analyst 123, 1675-77 (1998)

159 A. Sahuquillo, J.-F. Lopez-Sanchez, R. Rubio, G. Rauret, R. P. Thomas, C. M. Davidson, A. Ure
Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure
Anal. Chim. Acta 382, 317-327 (1999)

160 G. Rauret, J. F. Lo´pez-Sa´nchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure b, Ph. Quevauviller
Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials
J. Environ. Monit. 1, 57–61 (1999)

161 Ph. Quevauviller
Operationally-defined extraction procedures for soil and sediment analysis. Part 3: New CRMs for

trace-element extractable contents
TrAc 21(11), 774-785 (2002)

162 Christine M. Davidson, Andrew S. Hursthouse, Donna M. Tognarelli,Allan M. Ure, Graham J. Urquhart
Should acid ammonium oxalate replace hydroxylammonium chloride in step 2 of the revised BCR sequential extraction protocol for soil and sediment?
Anal Chim Acta 508 (2004) 193–199

163 M D. Ho, G J. Evans
Operational Speciation of Cadmium, Copper, Lead and Zinc in the NIST Standard Reference Materials 2710 and 2711 (Montana Soil) by the BCR Sequential Extraction Procedure and Flame Atomic Absorption Spectrometry
Analytical Comm. 34, 363–364 (1997)

164 C M. Davidson, G Delevoye
Effect of ultrasonic agitation on the release of copper, iron, manganese and zinc from soil and sediment using the BCR three-stage sequential extraction
J. Environ. Monit. 3, 398–403 (2001)

165 J. Dodd, D.J. Large, N.J. Fortey, A.E. Milodowski, S. Kemp
A Petrographic Investigation of two Sequential Extraction Techniques Applied to Anaerobic Canal Bed Mud
Environmental Geochemistry and Health 22 (4), 281-296 (2000)

166 W.W. Wenzel, N Kirchbaumer, T Prohaska , G Stingeder , E Lombi, D C. Adriano
Arsenic fractionation in soils using an improved sequential extraction procedure
Anal Chim Acta 436, 309–323 (2001)

167 M B. Alvarez, M E. Malla, D A. Batistoni
Comparative assessment of two sequential chemical extraction schemes for the fractionation of cadmium, chromium, lead and zinc in surface coastal sediments
Fresenius J Anal Chem 369, 81–90 (2001)

168 Ianni C, N Ruggieri, P Rivaro, R Frache
Evaluation and comparison of two selective extraction procedures for heavy metal speciation in sediments
Anal Sci 17, 1273-8 (2001)

169 K. Wallmann, M. Kersten, J. Gruber, U. Forstner
Artifacts in the determination of trace metal binding forms in anoxic sediments by sequential extraction
Int. J. Environ. Anal. Chem. 51(1-4), 187-200 (1993)

170 H. Farrah, W.F. Pickering
Factors influencing the potential mobility and bioavailability of metals in dried lake sediments
Chem Speciat Bioavail 5(3), 81-96 (1993)

171 A.F. Albores, B.P. Cid, E.F. Gomez, E.F. Lopez
Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples
Analyst 125, 1353-1357 (2000)

K.F. Mossop, CM. Davidson
Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments
Anal Chim Acta 478(1), 111 –118 (2003)

173 M. Pueyo, J. Sastre, E. Herna´ndez, M. Vidal, J. F. Lopez-Sanchez, G. Rauret
Prediction of Trace Element Mobility in Contaminated Soils by Sequential Extraction
J. Environ. Qual. 32, 2054–2066 (2003)

174 R. A. Sutherland, F. M.G. Tack
Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimised BCR sequential extraction procedure.
Anal. Chim. Acta 454, 249–257 (2002)

175 R. A. Sutherland, F. M.G. Tack
Fractionation of Cu, Pb and Zn in certified reference soils SRM 2710 and SRM 2711 using the optimized BCR sequential extraction procedure
Adv Environ Res 8, 37–50 (2003)

176 S. Tokalioglu, S. Kartal, L. Elci
Determination of heavy metals and their speciation in lake sediments by flame atomic absorption spectrometry after a four-stage sequential extraction procedure
Anal Chim Acta 413 33-40 (2000)

177 A. Guevara-Riba, A. Sahuquillo, R. Rubio, G. Rauret
Assessment of metal mobility in dredged harbour sediments from Barcelona, Spain
Sci Total Environ 321, 241–255 (2004)

178 J.F. Lopez-Sanchez, R. Rubio, G. Rauret
Comparison of two sequential extraction procedures for trace metal partitioning in sediments
Int. J. Environ. Anal. Chem. 51, 113–121 (1993)

179 J. Hlavay, K. Polyak
Chemical speciation of elements in sediment samples collected at Lake Balaton
Microchem. J. 58, 281–290 (1998)

B. A. Perez Cid, Fernandez Albores, E. Fernandez Gomez, Falque Lopez
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 (2001)

181 E Dabek-Zlotorzynska, M Kelly, H Chen, C L. Chakrabarti
Evaluation of capillary electrophoresis combined with a BCR sequential extraction for determining distribution of Fe, Zn, Cu, Mn, and Cd in airborne particulate matter.
Anal Chim Acta 498, 175–187 (2003)

182 J. Hlavay, K. Polyak, M. Weisz
Monitoring of the natural environment by chemical speciation of elements in aerosol and sediment samples
J. Environ. Monit 3, 74-80 (2001)

183 M. Villar, F Alava, I Lavilla, C Bendicho
Operational speciation of thallium in environmental solid samples by electro- thermal atomic absorption spectrometry according to the BCR sequential extraction scheme
J Anal At Spectrom 16, 424–1428 (2001)

184 A. Bermond
Limits of sequential extraction procedures re-examined with emphasis on the role of H+ ion reactivity
Anal Chim Acta 445, 79–88 (2001)

185 G.E.M. Hall, P Pelchat
Comparability of results obtained by the use of different selective extraction schemes for the determination of element forms in soils
Water Air Soil Pollut. 112, 41–53 1999

186 J. L. Gómez Ariza, , I. Giráldez, D. Sánchez-Rodas, E. Morales
Comparison of the feasibility of three extraction procedures for trace metal partitioning in sediments

from south-west Spain
Sci Total Environ 246, (2-3) 271-283 (2000)

187 E D. van Hullebusch, S Utomo, M H. Zandvoort, P N. L. Lens
Comparison of three sequential extraction procedures to describe metal fractionation in anaerobic granular sludges
Talanta 65 (2), 549-558 (2005)

188 A. Raksasataya, A. G. Langdon, N. D. Kim
Assessment of the extent of lead redistribution during sequential extraction by two different methods
Anal. Chim. Acta 332, 1–14 (1996)

189 I. Maiz, M.V. Esnaola, E. Millan
Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure
Sci Total Environ. 206 (2-3): 107-115 (1997)

190 X. Q. Shan, B. Chen
Evaluation of Sequential Extraction for Speciation of Trace Metals in Model Soil Containing Natural Minerals and Humic Acid
Anal. Chem. 65, 802-807 (1993)

191 B. Pérez Cid, M. de Jesús González, E. Fernández Gómez
Comparison of single extraction procedures, using either conventional shaking or microwave heating, and the Tessier sequential extraction method for the fractionation of heavy metals from environmental samples
Analyst 127 (5), 681-688 (2002)

192 P. S. Rendell, G. E. Batley, A.J. Cameron
Adsorption as a Control of Metal Concentrations in Sediment Extracts
Environ Sci Technol 14(3), 314-318 (1980)

193 Tu Qiang, Shan Xiao-Quan, Qian Jin, and Ni Zhe-Ming
Trace Metal Redistribution during Extraction of Model Soils by Acetic Acid/Sodium Acetate
Anal. Chem 66, 3562 – 3568 (1994)

194 A. Tessier, P.G.C. Campbell
Comment on "Pitfalls of sequential extractions"
Wat. Res. 25 (1), 115-117 (1991)

195 A.P. Bermond
Thermodynamics Applied to the Study of the Limits of Sequential Extraction Procedures Used for the Speciation of Trace Elements in Sediments and Soils
Environ. Technol. 13, 1175-1179 (1992)

196 A.B. Ribeiro, J.T. Mexia
A dynamic model for the electrokinetic removal of copper from a polluted soil
J. Hazard Mater. 56, 257-271 (1997)

197 R. G. McLaren, L. M. Clucas
Fractionation of Copper, Nickel, and Zinc in Metal-Spiked Sewage Sludge
J. Environ. Qual. 30,1968–1975 (2001)

198 J. L. Gómez-Ariza, I. Giráldez, D. Sánchez-Rodas, E. Morales
Metal readsorption and redistribution during the analytical fractionation of trace elements in oxic estuarine sediments
Anal Chim Acta 399(3), 295-307 (1999)

199 A. Bermond, I. Yousfi, J.P. Ghestem
Kinetic approach to the chemical speciation of trace metals in soils
Analyst 123, 785–789 (1998)

200 M. J. Gismera, J. Lacal, P. da Silva, R. Garcı´, M. T. Se villa, J. R. Procopio
Study of metal fractionation in river sediments A comparison between kinetic and sequential extraction procedures
Environ Pollut 127, 175–182 (2004)

201 O. Sukreeyapongse, P. E. Holm, B. W. Strobel, S. Panichsakpatana, J. Magid, HC. B. Hansen
pH-Dependent Release of Cadmium, Copper, and Lead from Natural and Sludge-Amended Soils
J. Environ. Qual. 31, 1901–1909 (2002)

202 R. Meissner, H. Rupp, M. Schubert
Novel lysimeter techniques- a basis for the improved investigation of water, gas and solute transport in soils
J. Plant Nutr. Soil Sci. 163, 603-608 (2000)

203 J. Ruiz-Jiménez, J.L. Luque-Garc´ıa, M.D. Luque de Castro
Dynamic ultrasound-assisted extraction of cadmium and lead from plants prior to electrothermal atomic absorption spectrometry
Anal Chim Acta 480 (2003) 231–237

204 J.L. Luque-Garcia, M.D. Luque de Castro
Continuous ultrasound-assisted extraction of hexavalent chromium from soil with or without on-line preconcentration prior to photometric monitoring
Analyst 127(8), 1115-1120 (2002)

205 F. Priego-Capote, MD. Luque de Castro
Dynamic ultrasound-assisted leaching of essential macro and micronutrient metal elements from animal feeds prior to flame atomic absorption spectrometry
Anal Bioanal Chem 378(5),1376-81 (2004)

206 J. R. Dean
Extraction methods for environmental analysis
Chichester Wiley, 1998, ISBN 0-471-98287-3

207 D Fangueiro, A Bermond, E Santos, H Carapuça and A Duarte
Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extraction: search for optimal experimental conditions
Anal. Chim. Acta 459, 245–256 (2002)

208 J. Shiowatana, RG. McLaren, N. Chanmekha, A. Samphao
Fractionation of arsenic in soil by a continuous flow sequential extraction method
J Environ Qual 30, 1940-1949 (2001)

209 J. Shiowatana N. Tantidanai, S. Nookabkaew, D. Nacapricha
A Novel Continuous-Flow Sequential Extraction Procedure for Metal Speciation in Solids
J. Environ Qual 30,1195-1205 (2001)

210 PS. Fedetov, AG. Zavarzina, BYa. Spivakov, R.Wennrich, J. Mattusch, K. Titze, V. Demin
Accelerated fractionation of heavy metals in contaminated soils and sediments using rotating coiled columns
J Environ Monit 4:318–324 (2002)

211 P.S. Fedotov, W.J. Fitz, R. Wennrich, P. Morgenstern, W.W. Wenzel
Fractionation of arsenic in soil and sludge samples: continuous-flow extraction using rotating coiled columns versus batch sequential extraction
Anal Chim Acta 538(1-2), 93-98 (2005)

212 P. S. Fedotov, R. Wennrich, H.-J. Stärk, B. Ya. Spivakova
Continuous-flow fractionation of trace metals in environmental solids using rotating coiled columns.

Some kinetic aspects and applicability of three-step BCR leaching schemes
J Environ Monit 7, 22-28 (2005)

Lars Füchtjohann, Norbert Jakubowski, Dieter Gladtke, Dieter Klockow, Jose´ A. C. Broekaert
Speciation of nickel in airborne particulate matter by means of sequential extraction in a micro flow system and determination by graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry
J. Environ. Monit., 3, 681–687 (2001)

214 J. Shiowatana N, Tantidanai, S. Nookabkaew, D. Nacapricha
A flow system for the determination of metal speciation in soil by sequential extraction
Environ Int 26, 381–387 (2001)

215 P.O. Scokart, K. Meeus-Verdinne, R. de Borger
Speciation of heavy metals in polluted soils by sequential extraction and ICP spectrometry
Int. J Environ Anal Chem 29, 305-315 (1987)

216 D. Beauchemin, K. Kyser, D. Chipley
Inductively Coupled Plasma Mass Spectrometry with On-Line Leaching: A Method To Assess the Mobility and Fractionation of Elements
Anal. Chem. 74, 3924-3928 (2002)

H. Kurosaki, SM. L. Asbury, JD Navratil, Sb, Clark
Flow-through Sequential Extraction Approach Developed from a Batch Extraction Method
Environ. Sci. Technol. 36, 4880-4885 (2002)

218 R. Chomchoei, M. Miro, E. H. Hansen, J. Shiowatana
Sequential injection system incorporating a micro-extraction column for automatic fractionation of metal ions in solid samples Comparison of the extraction profiles when employing uni-, bi-, and multi-bi-directional flow plus stopped-flow sequential extraction modes
Anal Chim Acta 536, 183–190 (2005)

219 AN. Eaton, FR. Abu-Shakra
Future instrumental development for speciation
In Handbook of Elemental Speciation –Techniques and Methodology R Cornelis, H Crews, J Caruso and K Heumann (Eds) John Wiley & Sons 2003 ISBN: 0-471-49214-0

220 F. Vanhaecke, S. Saverwyns, G. de Wannemacker, L. Moens, R. Dams
Comparison of the application of higher mass resolution and cool plasma conditions to avoid spectral interferences in Cr(III)/Cr(VI) speciation by means of high-performance liquid chromatography –

inductively coupled plasma mass spectrometry
Anal Chim Acta 419, 55-64 (2000)

221 S.D. Tanner, V. I. Baranov, U. Vollkopf
A dynamic reaction cell for inductively coupled plasma mass spectrometry (ICP-DRC-MS) Part III. Optimization and analytical performance
J. Anal. At. Spectrom. 15(9), 1261 – 1269 (2000)

222 L. Rottmann , KG. Heumann
Determination of Heavy Metal Interactions with Dissolved Organic Materials in Natural Aquatic Systems by Coupling a High-Performance Liquid Chromatography System with an Inductively Coupled Plasma Mass Spectrometer
Anal Chem. 66, 3709-37 (1994)

223 JF. Tyson
Flow injection atomic spectrometry for speciation. In R. Cornelis, H. Crews, J. Caruso, K. Heumann (eds) Handbook of elemental speciation, techniques and methodology
John Wiley & Sons Ltd. 2000 pp 260

224 M. C. Herrera, M. D. Luque de Castro (2002)
Dynamic approach based on iterative change of the flow direction for microwave-assisted leaching of cadmium and lead from plant prior to GF-AAS
J. Anal. At. Spectrom. (11),1530-1533

225 M. Hoenig, H. Docekalova, H. Baeten
Study of matrix interferences in trace element analysis of environmental samples by inductively coupled plasma atomic emission spectrometry with ultrasonic nebulization
J. Anal. At. Spectrom 13, 195- (1998)

226 M. Grotti, E. Magi, R. Frache
Multivariate investigation of matrix effects in inductively couplet plasma atomic emission spectrometry using pneumatic or ultrasonic nebulization
J. Anal. At. Spectrom 15, 89-95 (2000)

227 M. Otto
Chemometrics Statistics and computer application in analytical chemistry
Wiley-VCH 1999

228 V. Müller, M. Jimoh, W. Frenzel
Neuartiges mikroanalytisches System für die In-line Extraktion von Schwermetallen in festen

Umweltproben
GIT-Labor 8, 821- 2003

229 M. Jimoh, W. Frenzel, V. Müller, H. Stephanowitz, E. Hoffmann
Development of a hyphenated microanalytical system for the investigation of leaching kinetics of heavy metals in environmental samples
Anal Chem 76:1197–1203 (2004)

230 E DIN 19738: 2000-05 (Norm Entwurf)
Bodenbeschaffenheit - Resorptionsverfügbarkeit von organischen und anorganischen Schadstoffen aus kontaminierten Bodenmaterial [Soil quality - Absorption availability of organic and inorganic pollutants from contaminated soil material]
Deutsches Institut für Normung, Beuth, Berlin

231 M. Jimoh, W. Frenzel, V. Müller
Microanalytical flow-through method for assessment of the bioavailability of toxic metals in environmental samples
Anal Bioanal Chem 381, 438–444 (2005)

232 P Matúš, J Kubová, M Bujdoš, V Streško, J Medveď
Chemical partitioning of aluminium in rocks, soils and sediments acidified by mining activity
Anal Bioanal Chem 379, 96–103 (2004)

233 M.D. Ho, G.J. Evans
Sequential extraction of metal contaminated soils with radiochemical assessment of readsorption effects
Environ. Sci. Technol. 34, 1030-1035 (2000)

234 E Dabek-Zlotorzynska, M Kelly, H Chen, C L. Chakrabarti
Evaluation of capillary electrophoresis combined with a BCR sequential extraction for determining distribution of Fe, Zn, Cu, Mn, and Cd in airborne particulate matter.
Anal Chim Acta 498, 175–187 (2003)

235 F.G. Huggins, G.P. Hauffman, J.D. Robertson
Speciation of elements in NIST particulate matter SRMs 1648 and 1650
J Haz Mat 74, 1–23 (2000)

236 A.G. Oomen, A. Hack, M. Minekus, E. Zeijdner, C. Cornelis, G. Schoeters, W. Verstraete, T. Van de Wiele, J. Wragg, C.J. Rompelberg, A.J. Sips, J.H. Van Wijnen
Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants.
Environ. Sci Technol 36, 3326–3334 (2002)

237 T. Graham
The Bakerian lecture—on osmotic force
Phil. Trans. R. Soc. London 144, 177–228 (1854)

238 J. A. Stenken, R. Chen and X. Yuan
Influence of geometry and equilibrium chemistry on relative recovery during enhanced microdialysis
Anal Chim Acta 436(1), 21-29 (2001)

239 J. A. Stenken, , C. E. Lunte, M. Z. Southard, L. Ståle
Factors that influence microdialysis recovery. Comparison of experimental and theoretical microdialysis recoveries in rat liver
J. Pharm. Sci. 86, 958-966 (1997**)**

Z. Fang
Flow Injection Separations and Preconcentration
VCH Wienheim 1993

241 M. Miró, W. Frenzel
A novel flow-through microdialysis separation unit with integrated differential potentiometric detection for the determination of chloride in soil samples
Analyst 128, 1291–1297 (2003)

242 P. Salaun, J. Buffle
Integrated Microanalytical System Coupling Permeation Liquid Membrane and Voltammetry for Trace Metal Speciation. Theory and Applications
Anal Chem 76, 31-39 (2004)

[243] N. Torto, B. Lobelo, L. Gorton
Determination of saccharides in wastewater from the beverage industry by microdialysis sampling, microbore high performance anion exchange chromatography and integrated pulsed electrochemical detection
Analyst 125, 1379–1381 (2000)

244 N. Torto, J. Mwatseteza, G. Sawula
A study of microdialysis sampling of metal ions
Anal Chim Acta 2002, 456, 253-261 (2002)

245 Wei-Chang Tseng Yuh-Chang Sun, Mo-Hsiung Yang Tyen-Po Chen, Te-Hsien Line, Yeou-Lih Huang
On-line microdialysis sampling coupled with flow injection electrothermal atomic absorption

spectrometry for in vivo monitoring of extracellular manganese in brains of living rats
J. Anal. At. Spectrom 18, 38–43 (2003)

246 R. J. Olson, J. B. Justice, Jr
Quantitative Microdialysis under Transient Conditions
Anal Chem 65, 1017-1022 (1993)

247 J. A. Stenken, , D. M. Holunga, S. A. Decker, L. Sun
Experimental and Theoretical Microdialysis Studies of in Situ Metabolism
Anal. Biochem 290, 314-323 (2001)

248 R. Chen, J.A. Stenken
An in Vitro Hydroxyl Radical Generation Assay for Microdialysis Sampling Calibration
Anal Biochem 306, 40-49 (2002)

249 M. Miro, M. Jimoh, W. Frenzel
A novel dynamic approach for automatic micro-sampling and continuous monitoring of metal ion release from soils exploiting a dedicated flow-through microdialyser
Anal Bioanal Chem submitted (2005)

250 W. Frenzel, M. Jimoh, M. Miro
Development and application of hyphenated microanalytical systems for on-line and in-situ fractionation studies and investigations of leaching kinetics of heavy metals in solid samples of environmental interest using atomic spectrometric detection
Chem Anal 50, 279- 299 (2005)

251 P.M. Bungay, P.F. Morrison, R.L. Dedrick
Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro
Life Sci 46, 105-119 (1990)

252 J L. Burguera, M. Burguera
On-line sample pre-treatment systems interfaced to electrothermal atomic absorption spectrometry
Analyst 123, 561-569 (1998)

N. Torto, J. Mwatseteza, T. Laurell
Microdialysis sampling- Challenges and new frontiers
LC GC North America 19, 462-467 (2001)

254 Schombara S
Experimentelle Untersuchungen zur analytischen Anwendung der Dialyse im Mikrofließsystem bei der

Bestimmung von Anionen in Umweltproben
Diplomarbeit an der TU Berlin (1996)

255 MS. Martinez-Martínez, B. Gutiérrez-Hurtado, CI. Colino-Gandarillas, J. Martínez-Lanao, J. Sánchez-Navarro
In vitro study of experimental factors affecting the microdialysis results
Anal Chim Acta 459, 143–150 (2002)

256 Zhao Y-P, Liang X-Z, Lunte CE
Comparison of recovery and delivery in vitro for calibration of microdialysis probes
Anal Chim Acta 316, 403–410 (1995)

257 B. Nowack, F.G. Kari, H.G. Krüger
The Remobilization of Metals from Iron Oxides and Sediments by Metal-EDTA Complexes
Water Air Soil Pollut 125:243–257 (2001)

258 A. Sahuquillo, A. Rigol, G. Rauret
Overview of the use of leaching/ extraction tests for risk assessment of trace metals in contaminated soils and sediments
Trends Anal Chem 22, 152–159 (2003)

259 A. Sauquillo, A. Rigol, G. Rauret
Comparison of leaching tests for the study of trace metals remobilisation in soils and sediments
J Environ Monit 4, 1003–1009 (2002)

260 Q.J. Song, G.M. Greenway
A study of the elemental leachability and retention capability of compost
J Environ Monit 6, 31–37 (2004)

261 D. Mogopodi, N. Torto
Maximising metal ions flux across a microdialysis membrane by incorporating poly-l-aspartic acid, poly-l-histidine, 8-hydroxyquinoline and ethylenediaminetetraacetic acid in the perfusion liquid
Anal Chim Acta 534, 239–246 (2005)