Universitat de Girona

ANALYTICAL METHODOLOGIES BASED ON X-RAY FLUORESCENCE SPECTROMETRY (XRF) AND INDUCTIVELY COUPLED PLASMA SPECTROSCOPY (ICP) FOR THE ASSESSMENT OF METAL DISPERSAL AROUND MINING ENVIRONMENTS

Eva MARGUÍ GRABULOSA

ISBN: 84-689-9802-8
Dipòsit legal: GI-838-2006

University of Girona
Chemistry Department
Analytical Chemistry Unit

CSIC
Institute of Earth Sciences "Jaume Almera"
Laboratory of X-Ray Analytical Applications

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PhD Dissertation presented by

Eva Marguí Grabulosa

In candidacy for the degree of doctor in Chemistry
at the University of Girona

Girona, February 2006

Manuela Hidalgo Muñoz, Professora Titular de Química de la Universitat de Girona i **Ignasi Queralt Mitjans**, Científic Titular de l'Institut de Ciències de la Terra "Jaume Almera" (CSIC),

CERTIFIQUEM:

Que els estudis recollits en aquesta memòria sota el títol ***"Analytical methodologies based on X-Ray Fluorescence Spectrometry (XRF) and Inductively Coupled Plasma Spectroscopy (ICP) for the assessment of metal dispersal around mining environments"***, han estat realitzats sota la nostra direcció per **Eva Marguí Grabulosa**, llicenciada en Química, per optar el grau de Doctora en Ciències, especialitat Química.

I perquè així consti, signem la present certificació.

Girona, 1 de febrer del 2006

Dra. Manuela Hidalgo Muñoz Dr. Ignasi Queralt Mitjans

The development of this thesis has been funded by two research projects from the Spanish National Research Programme:

- "*Evaluación en el laboratorio de los procesos químicos que afectan al transporte de especies metálicas procedentes de la actividad minera"* (Ref. PPQ2001-2100-C04-04).
- *"Estudio integrado de la calidad del agua en zonas de abandono minero. Evaluación de los efectos sobre el medio hídrico superficial y subterráneo y de las posibles acciones para su atenuación"* (Ref. CGL2004-05963-C04-03/HID).

Eva Marguí gratefully acknowledges a PhD research grant from the Autonomous Government of Catalonia (Ref. 2002FI00577) as well as the CSIC-JNICT (Spain-Portugal) international co-operation agreement (2001PT0009) and a research mobility grant from the Autonomous Government of Catalonia (2005BE00711). Part of the experimental work of this thesis was performed in:

- Departamento de Física, Linha Experimental do Centro de Física Atómica, Universidade de Lisboa (Portugal).
- Micro and Trace Analysis Centre, Department of Chemistry, University of Antwerpen (Belgium).

LIST OF PAPERS RESULTING FROM THIS RESEARCH

- E.Marguí, V.Salvadó, I.Queralt, M.Hidalgo, *Comparison of three-stage sequential extraction and toxicity characteristic leaching tests to evaluate metal mobility in mining wastes.* Anal. Chim. Acta 524 (2004) 151-159.

- E.Marguí, I.Queralt, M.L.Carvalho, M.Hidalgo, *Comparison of EDXRF and ICP-OES after microwave digestion for element determination in plant specimens from an abandoned mining area.* Anal. Chim. Acta 549 (2005) 197-204.

- E.Marguí, M.Hidalgo, I.Queralt, *Multielemental fast analysis of vegetation samples by wavelength dispersive X-Ray fluorescence spectrometry: Possibilities and drawbacks.* Spectroc. Acta Pt.B-Atom. Spectr. 60 (2005) 1363-1372.

- E.Marguí, R.Padilla, M.Hidalgo, I.Queralt, R.Van Grieken, *High-energy polarised- beam EDXRF for trace metal analysis of vegetation samples in environmental studies.* X-Ray Spectrom. (In press).

- E.Marguí, M.Iglesias, I.Queralt, M.Hidalgo, *Lead isotope ratio measurements by ICP-QMS to identify metal accumulation in vegetation specimens growing in mining environments.* Sci. Total Environ. (In press).

- E.Marguí, I.Queralt, M.L.Carvalho, M.Hidalgo, *Assessment of metal availability to vegetation (Betula pendula) in Pb-Zn ore concentrates residues with different features.* Environ. Pollut. (In press).

- E.Marguí, M.Iglesias, I.Queralt, M.Hidalgo, *Precise and accurate determination of lead isotope ratios in mining wastes by ICP-QMS as a tool to identify their source.* Submitted to Talanta.

“To the hands in the laboratory and the heads seeking information…”

CONTENTS

SUMMARY

Metal pollution of the environment as a result of abandoned mining activities is an acute problem nowadays. Although mineral resource extraction has been carried out for centuries, until the last several decades relatively little attention has been given to minimise the metal dispersal around these areas coming from the indiscriminately dumped mining wastes. As a consequence, one of the challenges facing society today is the identification, evaluation and remediation of these old disused areas to protect public health and environment quality. These studies are heavily dependant on observation and quantitative measurements of the amounts and distribution of metals, leading to the necessity of appropriate analytical methodologies.

The research presented in this thesis is based on the application and improvement of analytical existing methodologies and the development of novel procedures that can be used to assess the environmental effects of metal dispersal around abandoned mining activities. To fulfil this purpose different kind of environmental samples including mining wastes, soils and vegetation specimens were collected in three disused Pb/Zn mining districts in Spain (located at Val d'Aran, Cartagena and Osor).

On the one hand, single and sequential extraction procedures were properly applied, in the analytical context, in order to study the potential mobility, risk and bioavailability of the metals contained in several mining wastes with different features. Information on particular physico-chemical characteristics (pH, organic carbon content, particle size distribution, total heavy metal content) and mineralogy of mining tailings was considered also necessary to understand metal mobility and bioavailability behaviour in each particular case. Mineralogical investigations of the samples indicate, in most cases, the oxidisation of parent ore material, mostly galena (PbS) and sphalerite (ZnS), to more mobile fractions such as anglesite ($PbSO_4$) and goslarite ($ZnSO_4$). It could be also pointed that the pH is one of the main parameters affecting the mobility of metals.

The results from the partitioning study (BCR sequential extraction procedure) indicate that more easily mobilised forms (acid exchangeable) were predominant for Cd and Zn whereas the largest amount of lead was associated with the iron and manganese oxide fractions. In addition, in order to gain additional information about

more easily mobilised metal forms in the mining wastes, two single extraction procedures (leaching tests) were applied: Toxicity characteristic leaching procedure (TCLP, US-EPA) and DIN-38414-S4 (German standard method applied by the Waste Council of Catalonia as acceptation criteria for waste disposal). Based on the preliminary data obtained from the lixiviation tests, all the mining wastes studied could be classified as hazardous according to specified regulations.

In order to provide an effective evaluation of the metal bioavailability (mining tailing-plant transfer), both studies based on single extractions and plant analysis (metal content in plant matrices) were conducted. Determination of metal contents in vegetation samples evidences a potential metal accumulation, particularly for lead and zinc, whose concentrations were, in some cases, over twenty times background values for these species. Regarding to single extraction procedures, the use of DTPA as extractant gave the best correlation with the metal content determined in the plant provided the pH of the mining tailing is higher than 5.

Circumstantial evidence pointed strongly to contamination of vegetation species from mining activities as the main source of lead but sources such as the past leaded-petrol emissions could not be excluded. Therefore, in an attempt to study the potential sources of the anomalous lead content in vegetation specimens growing on the waste and soils of abandoned mining areas, a methodology based on the use of stable lead isotope ratios ($^{208}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{204}Pb/^{207}Pb$) using ICP-MS instrumentation was evaluated.

The results of this investigation highlight that quadrupole based ICP-MS technique proposed for isotopic analysis provides sufficient precision for the given purpose (RSD~0.1% except in the case of isotope ratios involving ^{204}Pb, RSD~0.2-0.3%). Furthermore, instrumental bias factors including mass discrimination, dead time and spectral interferences were properly studied and corrected in order to obtain accurate analytical results. From isotopic analysis of vegetation samples and mining wastes it was shown that the lead in the leaves and flowering tops of *Buddleia davidii* specimens from the mining areas did not have the same isotopic composition as a specimen from an uncontaminated site (control sample) but was isotopically identical to the mine tailings collected. This trend suggests that the lead accumulation in plants is primarily derived from the mining operations rather than from other lead sources. Therefore, it could be concluded that the abandoned mining areas studied can

become important sources of lead contamination for the occurring vegetation growing in the soils and on the mining landfills located in these sites.

In view of the considerable number of vegetation specimen analysis necessary to assess the impact of mining activities, the study of high productivity analytical methods for metal determination in this kind of matrices was considered appropriate. Commonly, plant analysis has been carried out by means of atomic spectroscopic techniques (including FAAS, ETAAS, ICP-OES and ICP-MS). However, the use of these techniques involves sample preparation procedures for the total destruction of the matrix by mineralization, which may lead to problems of contamination or disturbances of the measured concentrations by element losses due to incomplete digestion, as it can be also derived from the analytical data obtained in the present research. Low recoveries were obtained in the quantitation of K, Mn, Fe and Cu in different vegetation species by using two routine microwave assisted digestions (HNO_3+H_2O_2, HNO_3+H_2O_2+HF) plus ICP-OES analysis. According to the results obtained by scanning electronic microscopy it was deducted that the discrepancies were caused by the presence of a silica residue. The addition of HF neither ensures complete matrix destruction due to the formation of a new solid residue mainly composed by calcium and fluoride. According to that, the study and use of a suitable digestion procedure for each specific matrix and analyte under study should be performed to obtain reliable analytical results when using classical destructive analytical methods. However, this becomes quite unfeasible in environmental studies in which several plant species are used as pollution indicators of different elements.

For this reason, the study of the suitability of other methodologies based on the direct analysis of vegetation matrices was pointed as one of the goals of this research. X-Ray fluorescence spectrometry comply with the desired features for the analysis of vegetation specimens including the multi-element capability, simple sample preparation, a wide dynamic range, high throughput and low cost per determination.

Despite that this technique has been successfully applied in different fields, its use for environmental purposes has been limited due to the poor sensitivity for some important pollutant elements (such as Cd and Pb) and a somewhat worst precision and accuracy compared to other atomic spectroscopic techniques.

In this sense, the implementation of suitable quantitative strategies and the recent improvements in the XRF instrumentation have been evaluated to achieve reliable analytical results for plant analysis. Besides, analytical figures of merit such as precision, accuracy and limits of detection have been carefully determined in the

diverse configurations of XRF spectrometers employed in the course of this work (EDXRF, WDXRF and EDPXRF) using different excitation sources in order to assess the capability of XRF for environmental analytical purposes.

Data obtained showed up the benefits of using polarised x-rays sources (primary beam scattered by a secondary target) to reduce the characteristic high degree of scattering of the x-ray source by organic matrices (including vegetation specimens) leading to an improvement of the limits of detection. The application of an EDXRF analytical methodology based on this configuration allowed the quantification of eight elements (K, Ca, Mn, Fe, Cu, Zn, Sr and Pb) at $mg \cdot kg^{-1}$ levels with a precision lower than 8% and in most cases lower than 5%, similar to those obtained by atomic spectroscopic techniques.

The suitability of WDXRF as analytical tool for multi-elemental analysis of vegetation samples was also evaluated. In this case, by using several synthetic calibrators made of cellulose together with a correction method based on the use of influence coefficients it was feasible the quantification of fourteen elements, including some major elements (Na, Mg, Al, P, S, K, Ca), trace elements (Mn, Fe, Co, Zn, As) and non-essential elements (Sr, Pb). In spite of achieving similar precisions in the obtained data, higher detection limits were obtained compared to the EDXRF methodology pointed previously, especially for heavier elements. Nevertheless, by means of WDXRF the determination of most of light elements was somewhat better.

Finally, improved instrumental sensitivity and detection limits (low $mg \cdot kg^{-1}$ range) for the determination of several trace elements (Cd, Pb, As, Cu, Fe and Zn) in vegetation matrices was achieved by using a new equipment based on high-energy polarised-beam energy-dispersive x-ray fluorescence analysis (EDPXRF). Using this configuration, Cd was determined by using its K_α line, while the mutual interference of As and Pb was solved by employing selective excitation conditions with targets of different materials. The use of a standard-less fundamental parameter approach (IAEA QXAS) allowed the determination of Pb, As, Cu, Fe and Zn in the absence of suitable certified reference materials and to accurately compensate for self-attenuation effects in the sample. The proposed methodology provides an alternative analytical tool to classical destructive analytical methods, commonly applied for the determination of these toxic elements in vegetation matrices, with accuracy and precision levels fulfilling the requirements for environmental studies.

RESUM

Actualment, la contaminació del medi per metalls, a causa de les activitats mineres abandonades, constitueix un greu problema. Malgrat que l'extracció dels minerals s'ha portat a terme durant segles, no ha estat fins a les últimes dècades que s'ha intentat minimitzar la dispersió dels metalls en aquestes zones, provinent de l'abocament incontrolat dels residus miners. Com a conseqüència, alguns dels reptes existents en l'actualitat són la identificació, avaluació i remediació d'aquestes àrees abandonades per a protegir la salut pública i la qualitat ambiental. Aquests estudis es basen en l'observació i determinació de la concentració i distribució dels metalls, la qual cosa fa necessari de disposar de metodologies analítiques adequades.

La investigació que es presenta en aquesta tesi es centra en l'aplicació i millora de metodologies analítiques existents i el desenvolupament de nous procediments que poden ser utilitzats per a l'estudi dels efectes ambientals de la dispersió dels metalls entorn a les zones mineres abandonades. Per aquest fi es van recol·lectar diferents mostres ambientals incloent residus miners, sòls i espècies vegetals en tres zones mineres Pb/Zn d'Espanya (situades a la Val d'Aran, Cartagena i Osor).

En primer lloc, es van aplicar diferents procediments d'extracció simple i seqüencial, en el context analític, per a estudiar la mobilitat, perillositat i bio-disponibilitat dels metalls continguts en residus miners de característiques diferents. L'estudi dels paràmetres físico-químics (pH, contingut de carboni orgànic, distribució de la mida de partícula, contingut de metall total) així com la mineralogia d'aquests van ser decisius per a entendre el comportament dels metalls en termes de mobilitat en cada cas particular. La composició mineralògica determinada va indicar, en la majoria dels casos, l'oxidació del mineral original (galena "PbS" i esfalerita "ZnS") a fraccions més mòbils com la anglesita ($PbSO_4$) i goslarita ($ZnSO_4$). Així mateix, el pH dels residus és un dels paràmetres que afecten de forma més important la mobilitat dels metalls.

Els resultats de l'estudi de fraccionament (procediment d'extracció seqüencial BCR) van mostrar que les fraccions més mòbils (fracció intercanviable) eren predominants per Zn i el Cd mentre que les quantitats majors de Pb estaven associades amb la fracció d'òxids de ferro i manganès. Per altra banda, per a obtenir

informació addicional sobre les fraccions de metall més mòbils en els residus miners, dos procediments d'extracció simple (tests de lixiviació) van ser aplicats: Toxicity characteristic leaching procedure (TCLP, US-EPA) i DIN 38414-S4 (Normativa alemanya aplicada actualment per la "Junta de residus de Catalunya"). Segons els resultats preliminars dels test de lixiviació, tots els residus estudiats poden ser classificats com perillosos tenint en compte les regulacions específiques.

Per a l'estudi de la biodisponibilitat es van portar a terme ambdós estudis centrats en procediments d'extracció simple i anàlisi d'espècies vegetals (contingut metàl·lic en les matrius vegetals). L'anàlisi del contingut metàl·lic en les espècies vegetals recol·lectades en els districtes miners va evidenciar un procés d'acumulació, particularment per Pb i Zn, les concentracions dels quals van ser en alguns casos fins a vint vegades superiors a les concentracions "normals" en les espècies vegetals estudiades. Pel que fa als processos d'extracció simple, les millors correlacions amb el metall determinat en les plantes es van obtenir utilitzant el DTPA com agent extractant, sempre que el pH del residu no fos inferior a 5.

Evidències fonamentades apuntaven a la contaminació de Pb de les espècies vegetals a causa de les activitats mineres però altres fonts de contaminació de Pb com les passades emissions de les gasolines amb plom, no podien ser excloses. Per tant, per a estudiar les fonts potencials de Pb en la vegetació de les zones mineres d'estudi, una metodologia basada en la utilització de les relacions isotòpiques de Pb ($^{208}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{204}Pb/^{207}Pb$) determinades mitjançant ICP-MS. Els resultats de la investigació permeten afirmar que la utilització de la tècnica d' ICP-MS amb analitzador de quadrupol proposada per a l'anàlisi isotòpic proporciona una precisió suficient per a discriminar entre les diferents fonts de plom (RSD=0.1%~ exceptuant les relacions on intervé l'isòtop ^{204}Pb, RSD=0.2-0.3%). A més, les principals derives instrumentals incloent la discriminació de masses, el temps mort del detector i les interferències van ser degudament estudiades i corregides a fi d'obtenir resultats analítics exactes. De l'estudi isotòpic de les mostres vegetals i els residus miners, es va apreciar que el plom de les fulles i flors de les mostres de *Buddleia davidii* col·lectades en les àrees mineres no presentava la mateixa composició isotòpica que la determinada en una espècie provinent d'una zona no contaminada (mostra control) però en canvi el plom era isotòpicament idèntic als residus miners de la zona. Aquest fet suggeria que la font primària de plom de les espècies vegetals era l'activitat

minera. Per tant, com a principal conclusió cal remarcar la potencial font de contaminació que poden suposar les àrees mineres estudiades per a la vegetació d'aquestes zones.

Tenint en compte l'elevat nombre de mostres analitzades per a avaluar l'impacte de les activitats mineres, es va considerar apropiat el desenvolupament de mètodes analítics d'elevada productivitat. Comunament, l'anàlisi de matrius vegetals s'ha portat a terme mitjançant la tècnica d'espectroscòpia atòmica (incloent FAAS, ETAAS, ICP-OES i ICP-MS). No obstant això, en aquestes tècniques es requereix d'una prèvia destrucció de la matriu de la mostra mitjançant una etapa de mineralització que pot comportar problemes de contaminació o alteracions en les concentracions determinades a causa d'una incompleta dissolució de la mostra, tal com es pot deduir a partir dels resultats analítics presentats també en aquesta tesi. Es van obtenir baixes recuperacions en la quantificació de K, Mn, Fe i Cu en diferents espècies vegetals utilitzant dos procediments de digestió àcida assistida amb microones (HNO_3+H_2O_2, HNO_3+H_2O_2+HF) amb una posterior anàlisi mitjançant ICP-OES. Segons els resultats obtinguts mitjançant microscopia electrònica d'escombrat les discrepàncies eren degudes a la presència d'un residu sòlid de sílice. L'addició de HF tampoc va assegurar una destrucció completa de la matriu de la mostra a causa de la formació d'un nou residu sòlid format principalment per calci i flúor. Per tant, aquests resultats van corroborar que el mètode utilitzat per a la destrucció de la matriu de la mostra depèn àmpliament de la composició química de la mostra i també de l'element a determinar. Així doncs, l'estudi i utilització d'un procediment de digestió adequat per a cada cas particular és necessari per a obtenir resultats analítics fiables quan s'utilitzen les tècniques destructives d'anàlisis clàssiques. No obstant això, tenint en compte que en la majoria d'estudis ambientals s'utilitzen diferents espècies vegetals com a indicadors de contaminació de diversos elements, aquests procediments són en alguns casos inviables. Per aquest motiu, es va plantejar l'estudi d'altres metodologies que ens permeten l'anàlisi directa sobre la mostra vegetal sòlida com un dels objectius d'aquesta tesi.

La fluorescència de raigs-X (XRF) compleix amb les característiques desitjades per a l'anàlisi de mostres vegetals incloent la seva capacitat multi-elemental, preparació simple de la mostra, ampli rang dinàmic de treball, elevat rendiment i baix cost per anàlisi. Tot i que aquesta tècnica ha estat àmpliament aplicada en diversos

àmbits industrials, la seva utilització per a fins ambientals s'ha vist limitada a causa de la seva insuficient sensibilitat per a la determinació d'alguns elements de gran rellevància ambiental com el Pb o el Cd i també a causa de una menor precisió i exactitud en els seus resultats en comparació a les tècniques d'espectroscòpia atòmica clàssiques.

En aquest sentit la implementació d'estratègies quantitatives així com l'aplicació de les millores instrumentals en els equips de XRF han estat avaluades per a aconseguir resultats analítics fiables en l'anàlisi de plantes. A més, alguns paràmetres de qualitat com la precisió, l'exactitud i els límits de detecció han estat curosament determinats en les diverses configuracions de espectròmetres de XRF utilitzats en el decurs d'aquest treball (EDXRF, WDXRF i EDPXRF) per a establir la capacitat real de la tècnica de XRF per a estudis ambientals.

Els resultats obtinguts van mostrar els beneficis de la utilització de les fonts de raigs-X polaritzades (radiació primària dispersada mitjançant un blanc secundari) per a reduir el fons espectral característic de les matrius orgàniques (incloent les mostres vegetals) conduint a una millora dels límits de detecció. L'aplicació d'una metodologia analítica basada en EDXRF va permetre la quantificació de vuit elements (K, Ca, Mn, Fe, Cu, Zn, Sr i Pb) a nivells de $mg \cdot kg^{-1}$ amb una precisió per a tots els elements inferior al 8% i en la majoria dels casos inferior al 5%, similar a la determinada en les tècniques d'espectroscòpia atòmica.

La utilitat de WDXRF com eina per a l'anàlisi multi-elemental de mostres vegetals també va ser avaluada. En aquest cas, la quantificació es va realitzar mitjançant la utilització de patrons sintètics de cel·lulosa juntament amb un mètode de coeficients d'influència. Es van determinar catorze elements incloent elements majoritaris (Na, Mg, AL P, S, K, Ca), elements traça (Mn, Fe, Co, Zn, As) i alguns elements no essencials (Pb, Sr) en diferents espècies vegetals. Encara que les precisions dels resultats obtinguts van ser similars als assolits amb la configuració instrumental anterior, els límits de detecció van ser superiors especialment per als elements pesats. No obstant això, mitjançant WDXRF la determinació de la majoria d'elements lleugers va ser lleugerament millor.

Finalment, es va aconseguir una millora de la sensibilitat instrumental i límits de detecció en la determinació de diversos elements traça (Cd, Pb, As, Cu, Fe i Zn) mitjançant la utilització d'un nou equip instrumental basat en radiació polaritzada d'alta energia (EDPXRF). Amb aquesta configuració, la determinació del Cd va ser possible mitjançant la seva línia K_α mentre que la interferència mútua d'As i Pb va

ser resolta utilitzant condicions específiques d'excitació mitjançant l'ús de blancs secundaris de diferents materials. La utilització d'un mètode de quantificació basat en paràmetres fonamentals (IAEA/QXAS) va permetre la determinació de Pb, As, Cu, Fe i Zn en absència de materials de referència certificats adequats i per a compensar els efectes d'auto-atenuació de la pròpia mostra. La metodologia proposada proporciona una alternativa analítica a la tècnica clàssica d'anàlisi, comunament aplicada per a l'anàlisi d'aquests elements en matrius vegetals, amb una exactitud i una precisió adequades per als requeriments propis dels estudis ambientals.

RESUMEN

Actualmente, la contaminación del medio por metales debido a actividades mineras abandonadas constituye un grave problema. A pesar de que la extracción de los minerales se ha llevado a cabo durante siglos, la preocupación por minimizar la dispersión de los metales fruto de la gestión inadecuada de los residuos mineros es relativamente reciente. Como consecuencia, algunos de los retos existentes en la actualidad son la identificación, evaluación y remediación de estas áreas abandonadas para proteger la salud pública y la calidad ambiental. Estos estudios se centran básicamente en la observación y determinación de la concentración y distribución de los metales, por lo que se requieren de metodologías analíticas adecuadas.

Los estudios que se presentan en esta tesis se centran en la aplicación y mejora de metodologías analíticas existentes y el desarrollo de nuevos procedimientos que pueden ser utilizados para el estudio de los efectos ambientales de la dispersión de los metales en zonas mineras abandonadas. Para este fin se recolectaron diferentes muestras ambientales incluyendo residuos mineros, suelos y especies vegetales en tres zonas mineras Pb/Zn de España (situadas en la Val d'Aran, Cartagena y Osor).

En primer lugar, fueron aplicados diferentes procedimientos de extracción simple y secuencial, en el contexto analítico, para estudiar la movilidad, peligrosidad y biodisponibilidad de los metales contenidos en residuos mineros de características diferentes. La información sobre los parámetros físico-químicos (pH, contenido de carbono orgánico, distribución del tamaño de partícula, contenido de metal total) así como la mineralogía de estos es imprescindible para entender el comportamiento de los metales en términos de movilidad en cada caso particular. La composición mineralógica determinada indicaba, en la mayoría de los casos, la oxidación del mineral original (galena "PbS" y esfalerita "ZnS") a fracciones más móviles como la anglesita ($PbSO_4$) y goslarita ($ZnSO_4$). Asimismo el pH de los residuos es uno de los parámetros que afectan de forma más importante a la movilidad de los metales.

Los resultados del estudio de fraccionamiento (procedimiento de extracción secuencial BCR) mostraron que las fracciones más móviles (fracción intercambiable) eran predominantes para el Zn y el Cd mientras que las cantidades mayores de Pb

estaban asociadas a los óxidos de hierro y manganeso. Por otra parte, para obtener información adicional sobre las fracciones de metal más móviles en los residuos mineros, dos procedimientos de extracción simple (tests de lixiviación) fueron aplicados: Toxicity characteristic leaching procedure (TCLP, US-EPA) y DIN 38414-S4 (Normativa alemana aplicada actualmente por la "Junta de residus de Catalunya"). Según los resultados preliminares de los tests de lixiviación, todos los residuos estudiados pueden ser clasificados como peligrosos teniendo en cuenta las regulaciones especificas.

Para el estudio de la biodisponibilidad se llevaron a cabo estudios centrados en procedimientos de extracción simple y en el análisis de especies vegetales (contenido metálico en las matrices vegetales). El análisis del contenido metálico en las especies vegetales recolectadas en los distritos mineros evidenció un proceso de acumulación, particularmente en el caso de Pb y Zn, las concentraciones de los cuales fueron en algunos casos hasta veinte veces superiores a las concentraciones "normales" en las especies vegetales estudiadas. Referente a los procesos de extracción simple, las mejores correlaciones con el metal determinado en las plantas se obtuvieron utilizando el DTPA como agente extractante, siempre que el pH del residuo no sea inferior a 5.

Evidencias fundamentadas apuntaban a la contaminación de Pb de las especies vegetales debido a las actividades mineras pero otras fuentes de contaminación de Pb como las pasadas emisiones consecuencia del uso de gasolinas conteniendo plomo, no podían ser excluidas. Por lo tanto, para estudiar las fuentes potenciales de Pb en la vegetación de las zonas mineras de estudio, se evaluó una metodología basada en la utilización de las relaciones isotópicas de Pb ($^{208}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{204}Pb/^{207}Pb$) determinadas mediante ICP-MS. Los resultados de la investigación indican que la utilización de la técnica de ICP-MS con analizador de quadrupolo propuesta para el análisis isotópico proporciona una precisión suficiente para discriminar entre las diferentes fuentes de plomo (RSD~0.1% exceptuando las relaciones en las que interviene el isótopo ^{204}Pb, RSD~0.2-0.3%). Además, las principales derivas instrumentales incluyendo la discriminación de masas, el tiempo muerto del detector y las interferencias fueron debidamente estudiadas y corregidas a fin de obtener resultados analíticos exactos. Del estudio isotópico de las muestras vegetales y los residuos mineros, se apreció que el plomo de las hojas y flores de las muestras de

Buddleia davidii colectadas en las áreas mineras no presentaba la misma composición isotópica que la determinada en una especie muestreada en una zona no contaminada (muestra control) pero en cambio el plomo era isotópicamente idéntico a los residuos mineros de la zona. Este hecho sugería que la fuente primaria de plomo de las especies vegetales provenía de las antiguas operaciones mineras.

Teniendo en cuenta el elevado número de muestras necesarias para evaluar el impacto de las actividades mineras, el desarrollo de métodos analíticos de elevada productividad se consideró apropiado. Tradicionalmente, el análisis de matrices vegetales se ha llevado a cabo mediante técnicas de espectroscopia atómica (incluyendo FAAS, ETAAS, ICP-OES e ICP-MS). No obstante, estas técnicas requieren una previa destrucción de la matriz de la muestra mediante una etapa de mineralización que puede conllevar a problemas de contaminación o alteraciones en las concentraciones medidas debido a una incompleta disolución de la muestra, tal como se puede deducir de los resultados analíticos presentados también en esta tesis. Se obtuvieron bajas recuperaciones en la cuantificación de K, Mn, Fe y Cu en diferentes especies vegetales utilizando dos procedimientos de digestión ácida asistida con microondas (HNO_3+H_2O_2, HNO_3+H_2O_2+HF) con un posterior análisis mediante ICP-OES. Los resultados obtenidos mediante el uso de la microscopia electrónica de barrido indicaron que las discrepancias eran debidas a la presencia de un residuo sólido de sílice. La adición de HF tampoco aseguró una destrucción completa de la matriz de la muestra debido a la formación de un nuevo residuo sólido formado principalmente por calcio y flúor. Por tanto, estos resultados corroboraron que el método utilizado para la destrucción de la matriz de la muestra depende ampliamente de la composición química de la muestra y también del elemento a determinar. Así pues, el estudio y utilización de un procedimiento de digestión adecuado para cada caso particular es necesario para obtener resultados analíticos fiables cuando se utilizan las técnicas destructivas de análisis clásicas. No obstante, teniendo en cuenta que en la mayoría de estudios ambientales se utilizan diferentes especies vegetales como indicadores de contaminación por varios elementos, estos procedimientos son en algunos casos inviables. Por este motivo, el estudio de otras metodologías que nos permiten el análisis directo sobre la muestra vegetal sólida fue planteado como uno de los objetivos de esta tesis.

La fluorescencia de rayos-X (XRF) posee las características deseables para el análisis de muestras vegetales incluyendo su capacidad multi-elemental, preparación

simple de la muestra, amplio rango dinámico de trabajo, elevado rendimiento y bajo coste. A pesar de que esta técnica ha sido ampliamente aplicada en diversos ámbitos industriales, su utilización para fines ambientales se ha visto limitada debido a su insuficiente sensibilidad para la determinación de algunos elementos de gran relevancia ambiental como el Pb o el Cd y también debido a una menor precisión y exactitud en sus resultados comparándolos con las técnicas de espectroscopia atómica clásicas.

En este sentido la implementación de estrategias cuantitativas así como la aplicación de las mejoras instrumentales en los equipos de XRF han sido evaluadas para conseguir resultados analíticos fiables en el análisis de plantas. Además, algunos parámetros de calidad como la precisión, la exactitud y los límites de detección han sido cuidadosamente determinados en las diversas configuraciones de espectrómetros de XRF utilizados en el curso de este trabajo (EDXRF, WDXRF y EDPXRF) para establecer la capacidad real de la técnica de XRF para propósitos ambientales.

Los resultados obtenidos mostraron los beneficios de la utilización de las fuentes de rayos-X polarizadas (radiación primaria dispersada mediante un blanco secundario) para reducir el fondo espectral característico de las matrices orgánicas (incluyendo las muestras vegetales) conduciendo a una mejora de los limites de detección. La aplicación de una metodología analítica basada en EDXRF permitió la cuantificación de ocho elementos (K, Ca, Mn, Fe, Cu, Zn, Sr y Pb) a niveles de $mg \cdot kg^{-1}$ con una precisión inferior al 8% y en la mayoría de los casos inferior al 5%, similar a la determinada en las técnicas de espectroscopia atómica.

La utilidad de WDXRF como herramienta analítica para el análisis multi-elemental de muestras vegetales también fue evaluada. En este caso, la cuantificación se realizó mediante la utilización de patrones sintéticos de celulosa juntamente con un método de coeficientes de influencia. Se determinaron catorce elementos incluyendo elementos mayoritarios (Na, Mg, Al, P, S, K, Ca), elementos traza (Mn, Fe, Co, Zn, As) y algunos elementos no esenciales (Pb, Sr) en diferentes especies vegetales. Aunque las precisiones de los resultados obtenidos fueron similares a los logrados con la configuración instrumental anterior, los límites de detección fueron superiores especialmente para los elementos pesados. No obstante, mediante WDXRF la determinación de la mayoría de elementos ligeros fue apreciablemente mejor.

Finalmente, se consiguió una mejora en la sensibilidad instrumental y límites de detección en la determinación de varios elementos traza (Cd, Pb, As, Cu, Fe y Zn)

mediante la utilización de un nuevo equipo instrumental basado en radiación polarizada de alta energía (EDPXRF). Con esta configuración, la determinación del Cd fue posible mediante su línea K_α, mientras que la interferencia mutua del As con el Pb fue resuelta utilizando condiciones específicas de excitación mediante el uso de blancos secundarios de diferentes materiales. La utilización de un método de cuantificación basado en parámetros fundamentales (IAEA QXAS) permitió la determinación de Pb, As, Cu, Fe y Zn en ausencia de materiales de referencia certificados adecuados y para compensar los efectos de auto-atenuación de la propia muestra. La metodología propuesta proporciona una alternativa analítica a las técnicas de análisis clásicas, comúnmente aplicada para el análisis de estos elementos en matrices vegetales, con una exactitud y una precisión adecuadas para los requerimientos propios de los estudios ambientales.

INTRODUCTION

The number of pollution sources is constantly increasing throughout the world mainly as a consequence of the growing industrial development. The consequent demand for energy and resources, required for a higher standard of living, are accompanied by a parallel increase in waste production and thus, environmental pollution.

Determining the nature and extent of environmental problems at a site involves a complete understanding of the study area both in terms of the natural state and the contamination problem. Major components of a site characterization include field investigations, laboratory analysis of field samples, data synthesis and analysis.

In order to bring a maximum contribution to the solution of environmental problems, the chemist must work toward an understanding of the nature, reactions, and transport of chemical species in the environment. The efforts carried out in such studies are often classified as a new discipline: Environmental Analytical Chemistry.

Some of the main objectives of environmental analytical chemistry are the development of novel procedures or the improvement of existing methodologies for the definition of environmental quality objectives and for the evaluation and prediction of environmental contaminants concentration. Together with statistical treatments of data and field investigations, chemical analysis is needed to assess the potential risk of contaminants for the environment and thus, to chose the best remediation process in each particular case [I.1, I.2, I.3].

I.1. ENVIRONMENTAL IMPLICATIONS OF ABANDONED METALLIC MINING AREAS: THE PROBLEM OF DEPOSITED MINING WASTES

The exploitation of our Earth resources has had a long and fascinating history since the search for stone tool-making materials to the sophisticated mining and extractive industries [I.4].

It is impossible to diminish the importance of mining for the development and sustainability of a modern society. The extraction and processing of minerals is a prerequisite for the lifestyle of all advanced societies [I.5].

The metals utilized in manufacturing are obtained from either the mining of ore bodies in the rocks of the earth's crust or from the recycling of scrap metal originally derived from geological sources.

Ore bodies are naturally occurring concentrations of minerals with sufficiently high concentrations of metals as to make them economically worthwhile exploited. However, it has been estimated that more than 70% of all the material excavated in mining operations world-wide is discarded. Therefore, apart from the direct disruption of the landscape, one of the more environmental troublesome by-products of mineral extraction consists of mining wastes.

Relatively little attention has been given until the last several decades to disposal of such types of wastes. In the past, most of the mining wastes were dumped indiscriminately, deposited in inadequate facilities or were simply released into the nearest water course [I.5,I.6]. As a result of these practices, a number of historic (meaning those operated prior to the last decades) mining and mineral processing sites that were abandoned once the profitable ores ran out, are now potential or ongoing sources of environmental contamination. [I.7, I.8, I.9].

In order to understand the adverse effects of mining operations it is important to study the types and the characteristics of the mining wastes present in such areas. Wastes can be generally classified into two major categories according to their origin sources in mining and mineral processing cycle (see Figure-I.1).

Generally, the greatest part of metals contained in the ores is extracted during the mineral processing to obtain the economically valuable product. However, due to the incomplete metal extraction from the original material, the mining wastes generated during this processing step could present also a substantially enriched metal content.

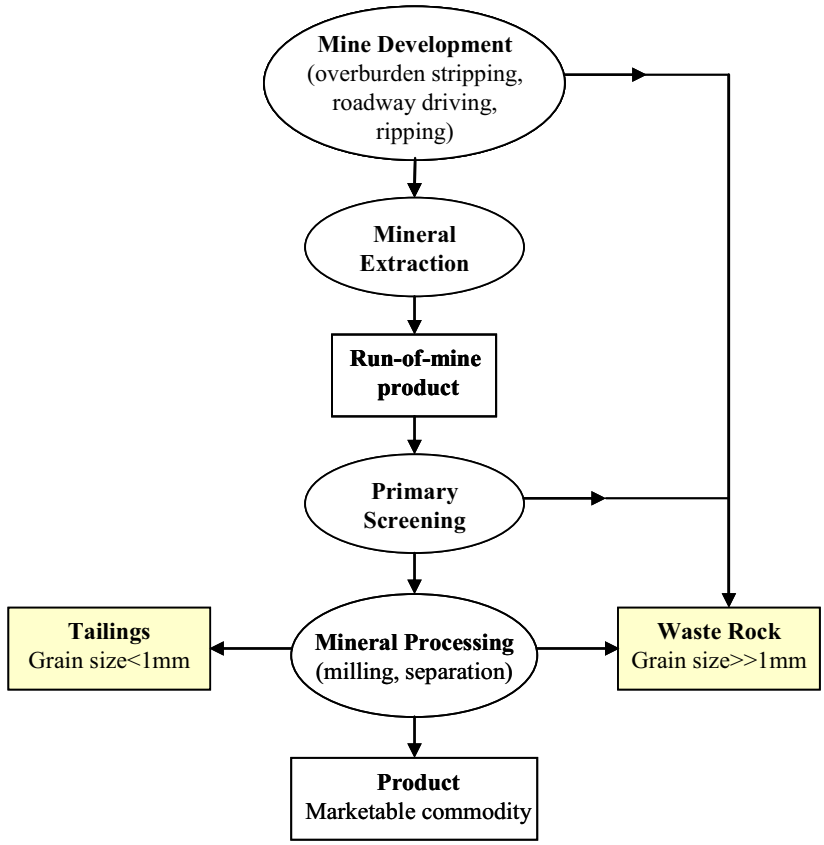

Figure-I.1.- Classification of mine wastes according to their source in the mining and mineral processing cycle.

The waste material plays and important role in controlling how readily metals are liberated from the wastes into the environment. The size of particles produced by mineral processing can dramatically influence their environmental impact. For instance, tailings solids, due to their fine grain size resulting from crushing and grinding, are likely to be more reactive than geologically similar waste rocks, and hence are likely to have greater potential metal mobility.

On the other hand, the techniques employed to enhance the mineral recovery (mineral processing) can also influence on the environmental impact, mainly due to the potentially polluting chemicals that are used. For example, sodium cyanide, cupper sulphate and different kind of surfactants were widely used to extract lead and zinc from ores [I.10]. Many of these processing solutions were recycled as much as possible in order to minimize water and reagent usage. However, disposal of this solutions is unavoidable once their extractive capacities have been exhausted, becoming an additional pollution source in mining operations.

Most of metallic mineral deposits beneath the Earth's surface contain sulphide minerals, which are largely chemically stable under in situ geological conditions. But when they are exposed by erosion or by mining activities to atmospheric oxygen and water, they can become quite unstable [I.11]. The chemical weathering of sulphide minerals represents a series of linked geochemical and microbiologically-mediated reactions through which metals are released from ores and mining wastes into the environment [I.4, I.5].

One of the most crucial properties of metals, which differentiate them from organic pollutants, is that they are not biodegradable in the environment [I.12]. As a result, they tend to persist in the various reservoirs of natural systems such as water, soils and sediments, or they accumulate in biological systems, leading to an important hazard to environment and human health.

Geologic features, such as the acid-generating or acid-consuming minerals in the deposit, host rocks, and wall rock alteration are examples of characteristics that influence the chemical response of the deposits to weathering.

For instance, the oxidation of iron sulphides (mostly pyrite, FeS_2) leads to the formation of acid mine waters due to the generation of sulphuric acid during the oxidation process. The overall reaction can be written as:

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_3 + 2\ H_2SO_4$$

Acid mine waters typically have pH values in the range 2-4 and high concentrations of the metals associated with the iron sulphides, and both these properties lead to an overall degradation of the water quality and the inability to support aquatic life. The release of these components into natural water is a widespread and persistent world-wide problem, and examples can be found in many countries [I.13, I.14, I.15].

Other sulphides such as sphalerite (ZnS) and galena (PbS) tend not to produce acid when oxygen is the oxidant. However, aqueous ferric iron is a very aggressive oxidant that, when it reacts with sulphides, generates significantly greater quantities of acid than those generated by oxygen-driven oxidation processes alone. In general, sulphide-rich mineral assemblages with high percentages of iron sulphides or sulphides having iron as a constituent will generate significantly more acidic waters than sphalerite and galena not containing iron sulphides [I.16].

In any case a typical feature of the weathering of mining wastes, apart from the possible acidic water formation, is the release of metals from the mineral matrix into

the environment. It must be kept in mind that not all the metal content in an earth material is usually susceptible to alteration and weathering reactions and thus, only a part of it could be problematic for the key compartments of the natural environment. Figure-I.2 illustrates the pathways and relationships between total metal content in mining wastes and potential toxicity to organisms.

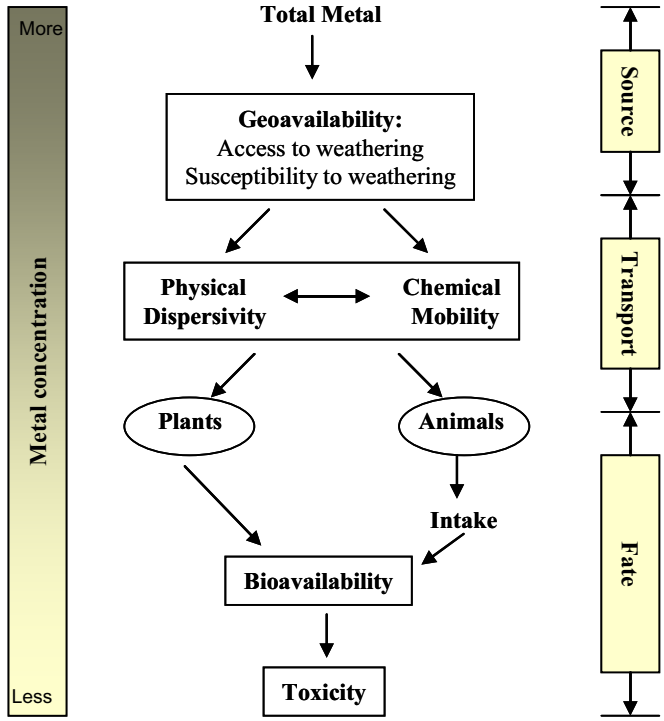

Figure-I.2.- Diagram showing the pathways and relationships between total metal in an earth material and toxicity. (Figure adapted from [I.16])

In general, the term "geo-availability" defines the portion of a chemical element's total content in an earth material that can be liberated to the surface or near-surface environment (or biosphere) through mechanical, chemical, or biological processes [I.16].

There are a lot of factors that can affect the concentration of geo-available metals, including the characteristics of the earth material itself (total metal content, mineral type, grain size, texture, structure, pH and redox conditions, impurities) and the weathering agents (climate and topographic relief).

Dispersivity refers to the ability to spread via non-chemical paths (physical processes), and mostly occur via transport through air (e.g., smelter emissions, wind erosion). Mobility of metals is based on chemical processes, which include chemical interactions with the surface or near-surface environment, and the capacity to migrate within fluids after dissolution. Mobility greatly depends on the physicochemical characteristics and binding forms of the elements in the solid matrix. Salomons [I.17] relates metal speciation to potential relative mobility:

Exchangeable cations ……	High mobility
Metals associated with Fe/Mn hydroxides ……	Medium mobility
Metals bound or fixed inside organic substances …..	Medium mobility
Metals bound or fixed inside mineral particles …….	Low mobility
Metals associated with a sulphide phase ……..	Dependent on environmental conditions

Commonly, the more labile interaction between metal-solid phases the higher potential metal mobility and thus, higher environmental impact.

According to Newman and Jagoe [I.18], bioavailability can be defined as the degree to which a contaminant in a potential source is free for uptake (movement into or onto an organism). It is a function of geo-availability, dispersivity and mobility but it also depends on the biological specificity and individual susceptibility of the organism. Generally, the bioavailability is a prerequisite for toxicity but does not necessarily results in toxicity; since toxicity implies and adverse effect on an organism and it varies widely with species and genotypes within species. Some individuals are genetically adapted to tolerating anomalous high concentrations of certain metals. Therefore, it is difficult to generalise about toxicity [I.19]. As it can be seen in Figure I.2, bioavailability is generally a requirement for uptake in plants, whereas animals may intake (ingest, inhale, etc.) toxicants that subsequently pass through their bodies without any systemic uptake.

Due to the above related reasons, the evaluation of the mobility and bioavailability of metals contained in the mining wastes is crucial to assess their behaviour and potential impact on the environment. Therefore, the implementation of analytical techniques suitable for the determination of metal mobility and bio-availability was outlined as one of the objectives of this research.

I.2. THE USE OF SPONTANEOUSLY OCCURRING VEGETATION AS ENVIRONMENTAL METAL POLLUTION INDICATORS

The ability of some plants to accumulate minor and trace elements from growth media has promoted their wide use as passive monitors in areas contaminated by heavy metals [I.8,I.20]. The incorporation of the heavy elements into plants is mainly achieved by uptake from the soil through the roots. However, uptake may also occur from deposition of the heavy elements on the leaves from soil or aerosol particles [I.21] (Figure I.3).

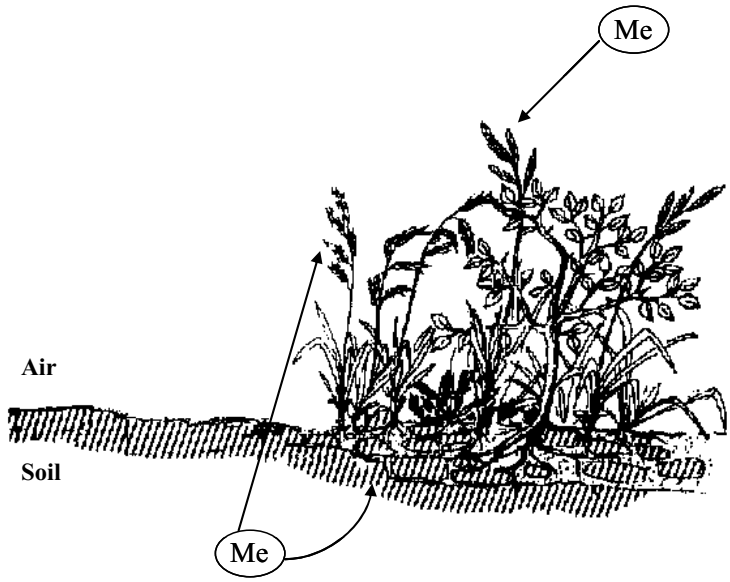

Figure-I.3.- Pathways of metal incorporation into plants.

Mosses and lichens are particularly effective as bio-monitors of aerial heavy metal contamination because of their lack of a cuticle and root system [I.22, I.23, I.24]. The analysis of leaves and roots of higher plants is a common technique in heavy metal pollution bio-monitoring studies of contaminated ground areas [I.25, I.26].

As it was mentioned in the previous section, the uptake of trace elements by plants is affected, in addition to plant-specific ability (different plant species take up metals in greatly different amounts), by the concentration and availability of the trace elements in the soil or waste where the plant is growing. Some of the most significant soil or waste-associated factors that influence element uptake are pH, speciation of metals, organic matter content, clay content, cation exchange capacity,

nutrient balance and concentration of other elements. Climatic conditions are also known to influence the rate of trace metal uptake, which may be partly an indirect impact due to the water flow phenomenon [I.21].

Once taken up, trace elements may be transported and stored in different concentrations through different parts of a plant (translocation). The distribution and accumulation patterns of trace elements vary considerably for each element, kind of plant, and growth season. The transport of elements among plant organs also depends on the electrochemical variables of the chemical elements. In general, easily transported form roots to above-earth parts are Ag, B, Li, Mo and Se; moderately mobile are Mn, Ni, Cd and Zn; and strongly bound in root cells are Co, Cu, Cr, Pb, Hg and Fe [I.21]. According to that, it is necessary to take note of the plant tissue when comparing the uptake of the heavy elements by plants.

Provided that other factors are constant, it is possible to compare the uptake of the heavy elements by different species. With respect to any metal, plants may be classified as either accumulators, indicators or excluders of the element [I.12,I.27] (Figure I.4).

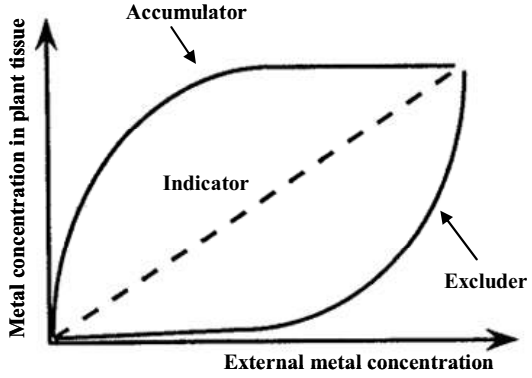

Figure-I.4.- Metal concentration in plant in relation to the external metal concentration.

The ‘excluder’ plants have a low metal uptake at quite high external metal concentrations. These plants have some kind of barrier limiting the uptake, but when the metal concentration in the medium becomes too high this barrier loses its function, probably due to the toxic action of the metal, and the uptake massively increases. The ‘accumulator’ plants have the ability to take up large concentrations of certain metals without showing toxic effects in the plant. These species usually have a low biomass because they use more energy in the mechanisms necessary to adapt

to high metal concentration. Plants that highly accumulate metals are promising in phyto-remediation programs [I.28, I.29] since bio-extraction methods using plants are likely to be less costly than those techniques based on conventional technology (pneumatic fracturing, vitrification, solidification/stabilization…). The use of accumulators has been a relevant topic in several recent conferences, and has been widely reviewed in recent publications [I.30, I.31]. The third type of uptake characteristic is found in the 'indicator' plants. These plants have a tissue concentration reflecting the external metal concentration, and an uptake linearly increasing with the metal concentration in the external medium. They can therefore be used as indicators of the source and intensity of metal contamination in a determined area. Several characteristics of an ideal bio-indicator have been related by various authors, and are summarized by Witting [I.32]:

- Suitable accumulation rate of some or selected elements
- Toxi-tolerance with no sensitivity to the accumulating element/substance
- Present in large amounts in the ecosystem under investigation
- Wide distribution in various environments, and wide geographic range
- Easy identification of the sample
- No seasonal differences in availability and applicability
- Existence of correlation between accumulation and input to the ecosystem

Not all the elements accumulated from the grow media are necessary for plant's development. The trace elementals "essential" for plants are those which cannot be substituted by others in their specific biochemical roles and that have a direct influence on the organism so that it can neither grow nor complete some metabolic cycle. In general, B, Co, Cu, Fe, Mn, Mo, Si and Zn are known to be essential for plants. These elements are involved in key metabolic processes such as respiration, photosynthesis, and fixation or assimilation of nutrients. In addition to essential elements, there are others which are always found in body tissues and fluids but for which no proof of essentiality has been established. These elements are often referred to as non-essential (Li, B, Ge, Rb, Sr). Some other elements, Cd, Hg and Pb are prominently classified as toxics, because of their detrimental effects even at low levels [I.33]. It should be noted, however, that all trace elements (including essential elements) could be predominantly toxic when their level exceed the limits of safe

exposure. Toxic concentrations of elements in plant tissues are very difficult to establish since greatly depend on each vegetation species. In a similar way, visible symptoms of toxicity also vary for each species and even for individual plants, but most common and non-specific symptoms of phyto-toxicity are brown spots in the leaves and leaf margins (chlorosis), and brown, stunted, coralloid roots [I.21].

A chemical balance in living organisms is a basic condition for their proper growth and development. Deficiency of one trace element essential to a plant can facilitate uptake of other micronutrients or trace elements from the grow media.

Competition between metals is also quite likely to occur and the competition may be either antagonistic, when the combined effect of the two elements is less than the sum of their separated effects, or synergistic where the combined effect is greater than the sum of the two element's individual effects. When a heavy toxic element is antagonistic to an essential element, the toxic effect could be due to a deficiency of the essential element. For instance, chlorosis of the plant leaves could be due to a deficiency of iron, and maybe produced by some heavy metals being taken up by the plant in preference to iron, or the heavy metal being involved in the plant biochemistry rather than iron [I.27].

As pointed out previously, there are some plants that can tolerate high concentrations of heavy metals in their tissues without showing evident symptoms of toxicity. When these plants are used in human or animal consumption, the accumulated trace elements can be highly toxic to humans or animals. Therefore, health-related limits for certain elements in food plants are carefully controlled by national and international legislation. For example, the World Health Organization (WHO) establishes maximum permissible levels in raw plant materials for cadmium (0.3 $mg \cdot Kg^{-1}$), arsenic (1 $mg \cdot Kg^{-1}$) and lead (10 $mg \cdot Kg^{-1}$) [I.34].

However, the tolerant plants, due to their ability to grow in contaminated substrates, and due to the accumulation of extremely high amounts of trace elements, could be valuable tools in the assessment and evaluation of metal pollution patterns in contaminated areas such as abandoned mining and mineral processing sites. Since the characterization of the contaminated areas requires the analysis of a considerable number of samples, the implementation of low cost and high productivity analytical methods for metal determination in vegetation specimens becomes an increasing need.

I.3. ANALYTICAL TOOLS TO EVALUATE THE POTENTIAL MOBILITY AND AVAILABILITY OF METALS

As mentioned in section I.1, the mobility and availability of heavy metals in the mining wastes strongly depends on their physicochemical characteristics and specific chemical forms of binding in the solid matrix. Consequently, toxic effects and biogeochemical pathways can only be assessed on the basis of the determination of these forms.

The interest in processes involving metal species has led to the emergence of a discipline of analytical chemistry called speciation analysis. According to the IUPAC guidelines, speciation is "the process yielding evidence of the atomic or molecular form and oxidation state of an analyte in a matrix" [I.35]. Whilst different elemental speciation methods are available for aqueous systems [I.33] implementations of methodologies for speciation studies in solids have been less well developed. Direct methods for determination of solid-state speciation (e.g., XRD, EXAFS, NRM spectroscopy) are generally of insufficient sensitivity for environmental trace analysis or require much specialised equipment. An alternative is the employment of methodologies involving an extraction, separation and pre-concentration step followed by a very sensitive detection. These demands are usually attainable by combining at least two analytical techniques: the first to separate the species and the second to identify and quantify them. The separation is usually achieved by the use of chromatographic techniques (GC, HPLC) whilst the detection is performed using highly sensitive and selective analytic methods (AAS, ICP-OES, ICP-MS). Such combinations have led to the emerging of modern hybrid techniques (i.e., GC-AAS, HPLC-ICP-MS, GC-ICP-MS). Although these techniques provide valuable information regarding the nature of the species present with high sensitivity, the high costs of the analysis and the required elevated degree of analytical expertise often constitute limiting factors.

As an alternative, the speciation studies involving soil and sediment analysis are often based on the use of extraction procedures (single or sequential) which enable broader forms or phases to be measured and which are, in most cases, sufficient for the purpose of environmental policy [I.36]. Measurements of fraction compositions in the laboratory extractions test require of accurate and rapid analytical techniques which allow the analysis of a large number of analytes in a reasonably short time.

For this reason, usually multi-elemental techniques as ICP-OES or ICP-MS are commonly applied.

I.3.1. Sequential Extraction Procedures

In sequential extraction procedures (EES), various extractants are applied successively (each extractant is chemically more active than the previous one) for a selective fractionation of the metals associated with the different constituents of the solid sample. The extracted metal fraction is thus depending on the chemical charcteristics of the extractant employed. Table I.1 summarises the most frequently used extracting agents and the extractable fraction with which they are associated. [I.37].

Released trace metal	Extractant agent
Water soluble	H_2O
Exchangeable (electro-statically weakly bound)	0.01-0.1M $CaCl_2$ 1M CH_3COONH_4 (pH=7) 0.1M NH_4NO_3 0.1M$Ca(NO_3)_2$ 0.1M $NaNO_3$ 0.3M $AlCl_3$ 0.1M $BaCl_2$ 1M $MgCl_2$ 1M $Mg(NO_3)_2$ (pH=7)
Sensitive to acidification processes (e.g. bound to carbonate)	0.11M-0.43M CH_3COOH 0.1M-1M HCl 1M CH_3COONa (pH=5, CH_3COOH)
Sensitive to complexation processes (e.g. non-silicate bound phases)	0.01-0.05M EDTA 0.005M DTPA CH_3COONH_4- CH_3COOH/EDTA
Easily or moderately reducible	0.04-0.5M $NH_2OH{\cdot}HCl$ (pH=1.5, HNO_3) $(NH_4)_2C_2O_4/H_2C_2O_4$
Bound to organic matter and sulphide	8.8M H_2O_2 + 1M CH_3COONH_4 (pH=2, HNO_3) 0.7M NaClO (pH=9.5) $K_4P_2O_7$ or $Na_4P_2O_7$
Residual phase	$HF+HClO_4$ HF+HCl HNO_3+HCl (1:3)

Table-I.1.- Most common extractants used in sequential extraction schemes to isolate metal fractions.

The results obtained following these procedures are “operationally defined”, which means that the “forms” of metals are defined by the determination of extractable elements using a given procedure, and therefore the significance of the analytical results is highly dependent on the selected extraction procedure.

On the other hand, the use of sequential extraction procedures is subject to an incomplete selectivity of the extractants for establishing the exact metal associated to a discrete geochemical phase [I.38]. A further difficulty arises from metal readsorption and redistribution during the extraction processes, meaning that an element released by one extractant could associate with other undissolved mineral components or freshly exposed surfaces within the time-scale of the extraction step [I.39]. However, and despite of these drawbacks, sequential extraction procedures are successfully applied as analytical tools in environmental studies.

The most widely used extraction scheme is the one proposed by Tessier [I.40] which has been modified by several authors [I.41, I.42]. Many of these modifications were made to achieve a more specific isolation of the iron and manganese oxide and hydroxide phases. The commonly used Tessier procedure is compared in Table I.2 with the modified procedures of Förstner [I.43] and Meguelatti [I.44]. During the recent decades, several other extraction procedures have been developed and applied to different kind of environmental samples [I.45, I.46, I.47, I.48].

	Tessier	**Förstner**	**Meguellati**
Step-1	$MgCl_2$ 1M (pH=7) *Exchangeable fraction*	NaOAc 1M (pH=5) *Exchangeable fraction* *Bound to carbonate*	$BaCl_2$ 1M (pH=7) *Exchangeable fraction*
Step-2	NaOAc 1M (pH=5) *Bound to carbonate*	$NH_2OH{\cdot}HCl$ 0.1M *Fraction easily reducible*	H_2O_2 8.8M+HNO_3 *Bound to organic matter and sulphide*
Step-3	$NH_2OH{\cdot}HCl$ 0.04M 25% HOAc *Bound to Fe/Mn Oxides*	NH_4Ox/HOx 0.1M (pH=3 in dark) *Fraction moderately reducible*	NaOAc 1M (pH=5) *Bound to carbonate*
Step-4	H_2O_2 8.8M HNO_3/NH_4OAc *Bound to organic matter and sulphide*	H_2O_2 8.8M NH_4OAc (pH=7) *Bound to organic matter and sulphide*	$NH_2OH{\cdot}HCl$ 0.1M 25%HOAc *Bound to Fe/Mn oxides*
Step-5	$HF/HClO_4$ *Residual+silicate phase*	HNO_3 *Residual*	Ashing+HF/HCl *Residual* *Silicate phase*

Table-I.2.- Sequential extraction schemes proposed by Tessier, Förstner and Meguellati.

The lack of uniformity among all the existing procedures usually does not allow the comparison of the results. The study performed by Sutherland and Tack [I.49] found order-of-magnitude fraction-specific concentration differences between four schemes in two contaminated certified reference soils. Therefore, the only possibility of performing a reasonable comparison among various studies is to refer to well-

defined schemes that have been collaboratively tested, harmonised and established as standardised methods [I.36].

With such purpose, the Community Bureau of Reference (BCR, now Standards, Measurements and Testing Programme) proposed in 1992 a three-step extraction procedure based on acetic acid extraction (Step-1: exchangeable and acid soluble fraction), hydroxilamine hydrochloride extraction (Step-2: reducible fraction) and hydrogen peroxide / ammonium acetate extraction (Step-3: oxidisable fraction). Once the scheme was designed, it was tested by different inter-comparison exercises and after some improvements of the original procedure (the adjustment of the pH and concentration of the hydroxilamine hydrochloride solution and the increase of centrifugation speed) it was established as a standard method [I.50]. Since then, the modified BCR sequential extraction has been successfully applied to a variety of matrices, including sediments of diverse origin, sewage sludge-amended soils and different industrially contaminated soils [I.37,I.51, I.52, I.53].

In recent years, the use of ultrasound and microwave-assisted extraction as alternatives to mechanical shaking in BCR procedure has risen up [I.54, I.55]. The main advantage of this sort of devices is a substantial reduction in time compared with traditional procedures. The applicability of the three-step BCR leaching scheme to the continuous flow fractionation of trace metals using rotating coiled columns has also been investigated to reduce the extraction time and improve reproducibility. For instance, the procedure proposed by Fedotov *et al*. [I.56] required 3.4 h instead of at least 50 h needed for the traditional sequential extraction.

I.3.2. Single Extraction Procedures

It is worth mentioning that not all the fractions obtained from applying sequential extraction procedures are equally important from the point of view of the environmental risk. The metals related to the residual fraction (obtained through extraction or digestion with mixtures of strong acids) are unlikely to be released under weathering conditions; whereas metals linked to the soluble and exchangeable fractions, and which are related to more labile metal species, are more mobile and hence more available. Therefore, in order to assess the environmental hazard, efforts should be applied only on the measurement of these fractions. The latter opens the possibility of using less laborious methods based on the extraction of the metal fraction of interest using a unique extracting agent (single extraction procedures). In

Table I.3 a summary of the most common single extraction procedures is given together with the corresponding purpose of application.

	Extracting agent	Use (Purpose)	References
Acid extraction	CH_3COOH 0.1M HCl 0.1-1M HNO_3 0.43-2M	Metal mobility and risk of metal leaching	[I.57] [I.38] [I.58]
Chelating agents	EDTA 0.01-0.05M DTPA 0.005M (+ TEA 0.1M)	Metal mobility within the system soil-plant (availability)	[I.25] [I.59]
Salt solution	$CaCl_2$ 0.01-0.1M $NaNO_3$ 0.1M NH_4NO_3 1M	Remediation and fertilization studies	[I.58] [I.60] [I.58]

Table-I.3.- Most common single extraction tests.

At present, several single extraction procedures (leaching tests) based on aqueous or acidic extractions are widely referred as approved analytical tools in national and international legislation organisms. Leaching tests are applied to waste materials to provide information about the release of specific components under reference conditions, or under conditions that may approximate more closely or simulate the actual field situation under consideration.

Subsequently, this information is usually used for the risk assessment of these wastes when they are deposited in a landfill or to characterise and classify them in terms of risk.

The common objectives of leaching tests applied to waste materials are presented in Table I.4, and Table I.5 provides an overview of the leaching procedures commonly used in various countries for regulatory testing [I.61].

Objective	Description
Environmental impact assessment	Estimate the potential impact of waste disposal or utilization on the environment
Quality control in waste treatment	Verify the efficiency of a treatment process using a simple pass/fail criterion
Waste classification	Compare wastes against performance criteria for classification e.g. as hazardous or non-hazardous
Identification of leachable constituents	Determine which constituents of a waste are subject to dissolution upon contact with a liquid
Evaluation of process modifications	Determine if modifications to a waste-generating process result in less leachable waste

Table-I.4.- Typical objectives of leaching tests applied to waste materials.

	Test procedure	Country/Region
Leaching tests	DIN 38414 S4	Germany
	AFNOR X31-210	France
	JST-13	Japan
	prEN 12457	Europe
	Nordtest Procedure: serial bach test	The Nordic Countries
	NEN 7349 Serial Bach Test	The Netherlands
	ENA Skaktest	Sweden
	TVA-Eluattest	Switzerland
	WRU Batch Extraction	United Kingdom
	EP Tox Method 1310	USA
	TCLP Method 1311	USA

Table-I.5.- Overview of selected leaching procedures for granular wastes (including mining wastes) and industrial wastes speciation studies.

Although in Spain there is not a characteristic procedure for regulatory testing, leaching tests commonly applied in other countries are used for this purpose. For instance, the German Standard Method (DIN 38414-S4) is, at present, used by the Waste Council of Catalonia as acceptation criteria for waste disposal in a municipal landfill.

Single extraction procedures are also widely used in soil sciences. These procedures are designed to dissolve phases that can be correlated with the availability of the element to the plants. This approach is well established for major elements and nutrients and it is commonly applied in studies of fertility and quality of crops, for predicting the uptake of essential elements, for diagnosis of deficiency or excess of one element and in studies of the physical-chemical behaviour of elements in soils [I.62]. For instance, solutions of neutral salts in various concentrations (frequently 0.01M) are used to simulate soil solution, which is of primary importance to assess the uptake of elements by plant roots. The suitability of using $CaCl_2$, which has more or less the same ionic strength (0.03M) that the average salt concentration in many soil solutions, has been discussed in some publications [I.63, I.64]. Complexation agents produced on an acetic acid basis (e.g., EDTA, DTPA) and their mixtures with other compounds are widely used for identifying problems of deficiency of micronutrients [I.65].

To a lesser extent, single extraction procedures have been applied also to elements considered as pollutants, such as heavy metals. The application of these procedures to polluted or naturally contaminated soils is manly focused to ascertain the potential availability and mobility of metals, in studies on the soil-plant transference and metal migration in a soil profile due to groundwater transport [I.61]. Some of the extraction

agents applied in contaminated soils and spoils studies are ammonium citrate [I.66], HCl [I.67], EDTA [I.25] and DTPA [I.59,I.68], as well as mixtures of different extracting agents, such as Mehlich 3 extractant (0.015M NH_4F, 0.25M NH_4NO_3, 0.2M CH_3COOH, 0.013M HNO_3, 0.001M EDTA) [I.8] or LMWOAs (acetic acid:formic acid:malic acid=2:2:1:1, molar ratio) [I.69].

However, to date no methods have been considered as universally applicable for the assessment of metal fractions in soils and plant bioavailability. In fact, the metal bioavailabilty depends not only on the chemical properties of the growing media but also on biological specificity and individual susceptibility of the plant. Therefore, knowing that a plant by itself can influence the uptake of a certain metal, one can only measure the bioavailable fraction as the fraction of a metal form that a plant can possibly take up, but never the total fraction that a plant actually takes up [I.70]. Therefore, to provide an effective evaluation of the set of bioavailable trace elements, both studies based on soil tests (metal content released by single extractions) and plant analyses (metal content in plant matrices) should be conducted together [I.21].

I.4. ANALYTICAL TOOLS TO STUDY SOURCES OF LEAD CONTAMINATION

Among the heavy metals extracted by mining operations, lead is one of the most frequently exploited since early times. The Romans used lead in ducting for water, for cooking vessels and also as a pigment [I.27]. During last century, the consumption of lead has risen dramatically due to its use in the automobile industry, in car batteries, petrol additives, steel products and solder. Therefore, lead pollution of the environment as a result of human activities is a significant problem nowadays.

As a result of the wide range of applications involving this metal, often lead source assessment is very difficult, but necessary to plan effective abatement strategies in areas contaminated by this metal.

I.4.1. Isotopic composition of lead

A powerful method for the identification of the source of lead is based on the variations in the terrestrial relative abundance of its stable isotopes. The isotopic composition of lead is variable in nature, as among the four stable isotopes of this

element (^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb) only ^{204}Pb is not the end product of the radioactive decay of thorium and uranium isotopes [I.71]:

$$^{238}U \rightarrow {}^{206}Pb + 8\ {}^{4}He + 6\beta^{-}$$
$$^{235}U \rightarrow {}^{207}Pb + 7\ {}^{4}He + 4\beta^{-}$$
$$^{232}Th \rightarrow {}^{208}Pb + 6\ {}^{4}He + 4\beta^{-}$$

So, although primordial lead had a fixed isotopic composition (^{204}Pb (1.4%), ^{206}Pb (24.1%), ^{207}Pb (22.1%) and ^{208}Pb (52.4%)), the amounts of ^{206}Pb, ^{207}Pb and ^{208}Pb have increased over time relative to ^{204}Pb. This fact produces small Pb isotope abundance variations depending on factors such as the concentration of primordial lead, thorium and uranium concentration in the sample, the half lives of the decay processes and the period of time over which the decay proceed [I.72] (See Table I.6)

	Minimum isotopic abundance (%)	**Maximum isotopic abundance (%)**
^{204}Pb	1.04	1.65
^{206}Pb	20.84	27.48
^{207}Pb	17.62	23.65
^{208}Pb	51.28	56.21

Table-I.6.- Isotopic abundance variations for lead in different earthly materials.

Since the pioneering works of Chow and Johnson [I.73] and Rabinowitz and Wetherill [I.74] which demonstrate that it was possible to distinguish between different sources of lead on the basis of isotopic composition, there have been numerous studies involving a wide range of materials. In Table I.7 a summary of the most commonly current applications in lead isotopic analysis is shown.

It is possible to use isotopic composition to identify the source of lead in individual samples and to trace the lead through the compartments of the environment such as water, soil, sediments, vegetation, and animals. Therefore, the application of isotopic analysis in environmental sciences has increased during last decades (see Table I.7).

Historical changes in the sources of atmospheric lead deposition may be resolved through the analysis of stable lead isotopes in various natural materials. For instance, vegetation samples such as mosses represented real time indicators of atmospheric deposition since they accumulate lead directly from the atmosphere. Similarly, a

number of studies have described the measurement of lead isotopes in sediments, peat bogs, aerosol samples and ice and snow cores. Some recent studies apply the combination of sequential extraction procedures and lead isotope analysis in soil and sediment depth profiles to distinguish between recent external (atmospheric deposition) and primary geogenic sources [I.75, I.76].

Field	Purpose	Sample	References
Environmental sciences	Atmospheric pollution studies	Moss	[I.77]
		Lichen	[I.78]
		Bark	[I.79]
		Annual rings	[I.79, I.80]
		Grass	[I.81]
		Soil	[I.81, I.82]
		Sediment	[I.83]
		Peat bogs	[I.84]
		Aerosol samples	[I.85, I.86]
		Ice, snow cores	[I.87, I.88]
	Fingerprinting the predominant sources of anthropogenic Pb in contaminated areas	Surface water	[I.89, I.90]
		Sediment	[I.89, I.91, I.92]
		Soils	[I.93, I.94]
		Peat	[I.91]
		Ore	[I.89]
		Mining wastes	[I.91]
		Blood (White storks)	[I.95]
		Mussels	[I.96]
		Fish	[I.90]
		Human hair	[I.97]
Archaeology	Provenance studies of ancient objects	Majolica shreds	[I.98]
		Bronze-age artefacts	[I.99, I.100]
		Geological glasses	[I.101, I.102]
Art	Authentication and origin assignment	Lead white pigments	[I.103, I.104]
Geochronology	Dating studies	Ore deposits	[I.105, I.106]
Food	Authentication and origin assignment	Wine	[I.107, I.108]
Health	Assessment of human health hazard	Tap water	[I.109]
		Bone, blood and soft tissues (liver, kidney, brain)	[I.110]

Table-I.7.- Summary of the most commonly current applications of lead isotopic analysis and samples related.

On the other hand, lead isotopic analysis has been also used for fingerprinting the predominant sources of anthropogenic Pb in contaminated areas. In this sense, biological samples present in these sites may be valuable tracers of lead pollution. For example, stork blood lead isotope ratios were related to ratios of the contaminated sludge and background sediments in a marshland ecosystem (Doñana, S.W.Spain) to ascertain the impact of Aznacollar's mine accident [I.95].

As shown in Table I.7, apart from environmental sciences, lead isotopic analysis is widely employed in other disciplines including archaeology, art, geochronology, food and health for a wide range of different purposes. Of particular interest are the studies related to model ages (dating studies) using U-Pb isotope ratios, which have even allowed the determination of the age of the Earth and meteorites [I.71].

The applications where findings may affect legal or political decision, for example when isotope ratio measurements are performed in food authentication or forensic analyses, it is not the value of an isotope ratio itself but the uncertainty of the measurement that decides what action should be taken. Likewise, in environmental studies, the uncertainty of the measured lead isotopes ratios is also a decisive factor; it has to be better than the expected spread in the isotope ratios for a group of samples due to inherent variations of lead source. Consequently, one of the most relevant features requested for a given analytical technique chosen for determining lead isotope ratios is the precision of the measurements. In addition, as environmental studies involve the analysis of a large number of specimens, the need of fast sample throughput techniques become a relevant need. Finally, but not less important, the cost or availability are often a decisive factor to chose the most appropriate analytical technique.

I.4.2. Determination of lead isotope ratios: "The use of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)"

Among the plethora of mass spectroscopic techniques developed in the 20th century, thermal ionization mass spectrometry (TIMS) has established itself as the reference technique for high-precision isotope ratio measurements of the heavier elements in solid samples [I.111]. This technique has been applied in a large variety of samples for lead isotope ratio analysis including peat bogs [I.84], ores [I.89, I.105,], grass samples [I.72], soils [I.72,I.89], mosses [I.77], surface water, sediments, and air samples [I.89].

The latest generation of these instruments is equipped with multiple detectors that allow isotopic measurements to an accuracy and precision of within 0.002%. However, despite the low uncertainty of the measurements, this technique has also some disadvantages. Analysis is complicated by labour intensive sample preparation, tedious handling protocols and long analysis periods (up to 24 h). Commonly, to produce a sample in a sufficiently pure form, considerable preparation and chemistry

separation methodologies, usually based on the isolation of lead by conventional anion-exchange chromatography, are required [I.112]. After that, a volume of this pure sample is deposited onto a metallic filament and evaporated to dryness, and each metallic filament containing a single sample is then loaded into the vacuum chamber (with a pressure of less than $1x10^{-7}$ mbar) of the mass spectrometer. A gradually increasing electric current is then passed through the filament to vaporize and ionize the sample, and the resulting ionic cloud is then accelerated into the mass spectrometer for isotope ratio analysis. Because there is a maximum temperature to which a metallic filament can be raised, the range of elements that can be analyzed by this technique is limited [I.71]. Therefore, the inflexibility of analytical routines has led to an unfeasible application of TIMS in some studies where results are drawn from a large number of analysed samples.

For this reason, the research of alternative techniques for isotopic analysis has been increased over the last few years, including inductively coupled plasma mass spectrometry (ICP-MS). The potential of this technique, developed as a multi-elemental trace analysis technique in the early 1980s [I.113], is based on the combination of the advantages of the inductively coupled plasma ion source (operates at atmospheric pressure, allows a high sample throughput, and it has the capability of efficiently ionise over 95% of the elements in the periodic table) with the possibility of measuring isotope ratios using a mass spectrometer as a detection system.

In general, a homogeneous solution is required to conduct ICP-MS. Therefore, isotopic analyses could be carried out directly in liquid samples or after a previous single digestion step for solid samples [I.114]. In some cases, due to the low analyte concentration in the studied sample, matrix separation and pre-concentration methods are also required. For example, Benkhedda *et al*. [I.115] applied an on-line flow injection (FI) pre-concentration method for the determination of lead isotope ratios in natural waters. In a similar way, a $Mg(OH)_2$ precipitation procedure was successfully applied in real seawater samples for the determination of lead isotope ratios [I.116].

On the other hand, isotopic ratio measurements on solid samples by using laser ablation systems (LA-ICP-MS) have been increased in the last few years. The possibility of using this sort of devices is particularly useful in earth sciences and

archaeology, since it allows the *in situ* determination of lead isotopic composition with a minimum damage to the sample [I.101, I.102].

Despite of the advantages of ICP sources compared to thermal ion sources, the analytical precision in these systems is limited by the inherent instability of the ion signal by the ICP source and the sample introduction devices. Instabilities may arise from either a change in energy transfer from the plasma to the sample, or variation efficiency of the nebulisation and transportation of sample. However, taking into account that in isotope ratio analysis these effects should influence both isotopes coherently, the contribution made by ion source to noise can be assumed to be negligible using appropriate data acquisition parameters [I.117]. Concerning to the fluctuations coming from liquid sample introduction systems, they can be reduced by choosing a pump speed in regard to the acquisition parameters [I.118] or by performing sample introduction by self-aspiration mode (concentric nebulisers). In this way, the discrete noise components caused by rotation of the peristaltic pump rollers could be effectively eliminated [I.119].

Different configurations of ICP-MS are currently employed in lead isotopic analysis. The most common ones involve the coupling of the ICP source to a quadrupole (ICP-QMS), to a single collector magnetic sector (ICP-SMS), to a multi collector magnetic sector (MC-ICP-SMS) or, to a lesser extent, to a time of flight mass analyser (ICP-TOFMS).

The precision of isotope ratio measurements available with the different types of instrumentation depends on inherent characteristics of the mass analyser. Therefore, it is helpful to know the "normal" range of the standard deviations that can be reached under good operating conditions in each particular case. The corresponding results, which are weighted averages of the literature data of the last few years, are summarised in Table I.7.

Type of mass spectrometer	**RSD (%)**
ICP-QMS	0.1-0.5
HR-ICP-MS (*)	0.05-0.2
ICP-TOFMS	0.05-0.1
MC-ICPSMS	0.002-0.02
TIMS	0.002-0.01

(*) HR-ICP-MS: Double-focusing ICP-SMS

Table-I.7.- Typical ranges of precision reported for isotope ratio measurements by different plasma source mass spectrometers. Representative RSD values for TIMS are also presented.

The use of quadrupole mass analysers in ICP-MS fundamentally limits the accuracy and precision which isotope ratio measurements may be done. They were specifically designed for, and hence perform optimally in, the scanning of relatively large mass ranges. As such, they are inherently less stable than magnetic sector mass analysers and less well suited to highly precise and accurate isotope ratio measurements [I.117]. However, the precision attained with this instrumentation is normally sufficient for isotope ratio measurements for many of environmental surveys. Applying HR-ICP-MS instead of quadrupole instruments, precision in isotope ratio measurements can normally be improved by a factor of 5-10. One of the advantages of magnetic sector analysers is the possibility of using the widths of the source slit and the collector slit to control the shape of the peaks. This arrangement is particularly useful for isotope ratio measurements. The shape of an isotope peak can influence the measured stability of an ion current even if there are no time-dependant fluctuations. Rounded peak tops (ICP-QMS) require an exact sequential set-up of the different isotope masses for precise isotope ratio measurements, whereas flat peak tops allow certain variations in this set-up. In contrast to quadrupole mass spectrometers, magnet sector field instruments can produce, working in appropriate conditions, a flat topped peak shape which is a better precondition for precise isotope ratio determinations [I.120].

However, in both cases (ICP-QMS and ICP-SMS) fluctuation ion currents are significant due to the jumping between peaks (sequential detection) limiting the precision of isotope measurements. Simultaneous detection of the ion current of the isotopes of interest is therefore the only correct way to eliminate such time-dependent variations [I.121]. This can be achieved by using multi-collector instruments (MC-ICPSMS). In general, this instrumentation comprises several Faraday detectors independently adjustable that allow a simultaneous isotopic detection eliminating the effects of signal intensity variations [I.71]. Therefore, the internal precision attained with MC-ICPSMS could be considered close to the counting statistics errors (theoretical limit). From Table I.7 it could be seen that MC-ICPSMS instruments are the only plasma source mass spectrometers, up to now, with similar precisions to those obtained by TIMS. Nevertheless, multi-collector instruments are expensive and not always available for routine applications.

Time-of-flight ICP-MS, in which ions of all masses are extracted from the plasma and then accelerated into the time-of-flight analyser simultaneously, can be

considered as quasi-simultaneous analyser provided there is no significant separation of measured analyte ions of different masses in time during their transport from the plasma to the ion beam modulating device [I.122]. Since the production of ions and not the time of detection is the real critical point, TOF has comparable capabilities in isotope ratio measurements as multi-collector magnetic sector field systems. The precision of the isotopic ratios obtained with ICP-TOFMS is similar to that attainable with quadrupole-based instruments or single-collector ICP-SMS (see Table I.7) with the important difference that the precision is unaffected by the number of isotopes monitored [I.123]. In any ICP-TOFMS instrument, the ion source must be operated in a pulse mode to define the starting point for the measurement of the flight time. This fact allows up to 30000 complete mass spectra per second to be registered, which will be particularly interesting for isotope ratio measurements of transient signals. This is the case of using a laser ablation as a sample introduction device in the determination of isotopic composition of solid samples. Several current studies are focused on the investigation of the capabilities of this coupling system LA-ICP-TOFMS [I.124, I.125]. Nevertheless, in comparison with other ICP-MS configurations, the ICP-TOFMS offers higher detection limits because of the need to pulse the plasma. Therefore, in some applications, it turned out to be necessary to separate the analyte from the matrix and to pre-concentrate it prior to determination of the isotope ratios [I.108].

In conclusion, the requirement of precise, rapid but inexpensive techniques has promoted the need of improving quadrupole-based instruments for isotopic analysis. Most application studies to date have focused on the applicability of a collision/reaction cell interface or twin-quadrupoles to increase the precision of isotope ratio measurements.

A collision/reaction cell is a powerful and versatile approach to overcome the problem of spectral interferences in quadrupole-based ICP-mass spectrometry [I.126]. By using an appropriate reaction gas, the intensity of interfering ions can be reduced by orders of magnitude. As an alternative, the target ion can also be converted into a condensation ion product, enabling interference-free determination at another mass-to-charge ratio. Of course, the possibility of avoiding spectral overlap also leads to an extension of the application range of ICP-MS for isotopic analysis, as it is now possible to accurately determine these isotope ratios for which otherwise, at least one of the signals involved is subject to overlapping [I.127]. For

instance, Moens *et al* [I.128] developed a strategy using CH_3F for selective SrF^+ ion formation, thereby offering a solution for the spectral overlap of the signals of the isobaric nuclides $^{87}Sr^+$ and $^{87}Rb^+$.

Collision or reaction cells have also been demonstrated to improve the precision of isotope ratio measurements. For example, using Ne (non-reactive collision gas) ions extracted from the ICP at slightly different moments in time are mixed in the cell an this mixing leads to an improved isotope ratio precision since short-term variations in the signal intensity are "dampened" [I.127]. Good precision in isotopic measruments has also been obtained using mixtures of He and H_2 in the collision cell using quadrupole-based instrumentation [I.126].

On the other hand, Houk and co-workers [I.129] constructed the first twin-quadrupole ICP-MS instrument, which allow the measurement of two different m/z values simultaneously (without scanning), to improve precision of isotopic analysis. However, the lack of sensitivity compared to a single-quadrupole device (ion signals are 100 times weaker) restricted its use.

I.4.2.1. Accuracy of lead isotope ratio measurements

So far, we have reviewed the main factors affecting precision of isotopic measurements attainable by ICP-MS (any of its configurations). However, the need of attaining also accurate isotope ratio determinations is a relevant factor that has to be taken into account.

The accuracy of an isotope ratio measurement by any mass spectrometry technique is limited by mass-dependent isotopic fractionation of the sample during the analysis. In general, TIMS is less susceptible to isotopic fractionation than ICP-MS. Fractionation effects in TIMS are usually in the order of parts per thousands, relatively stable and systematic, while isotopic fractionation in ICP-MS, commonly referred to mass bias, can be easily in the percent range and depend on instrument settings and time [I.121]. According to that, mass discrimination effects have to be studied and properly corrected to obtain reliable determination of isotope ratios when an ICP-MS instrument is used.

Mass bias is the deviation of measured isotope ratios from the "true value" due to the different transmission of ions according to their masses before the final detection. It has been postulated that several mechanisms are related to mass bias [I.121]:

- Differences in the efficiency of transmission through the mass analyser depending on the mass of the isotope measured.
- Space charge effects through the ion optics (interface region), which leads to a more extensive defocusing suffered by the light ions in the ion beam and, therefore, a preferential enrichment of the heavier ions of the ion beam.
- A nozzle separation effect (separation of gaseous isotopes) in the region between plasma torch and the skimmer cone, which leads to enrichment in the central ion beam of the heavier isotopes because lighter ones are preferably pumped down when the supersonic jet is produced.

It is worth mentioning that the space charge effect is assumed to be the strongest influence on the total mass discrimination in any inductively coupled plasma ion source mass spectrometer. Taking into account that commercial ICP-MS are different in their optics and extraction potentials, the mass discrimination can be fairly different depending on the instrument used. The potential of the different lenses in the ion optics play an important role in mass discrimination. For instance, Xie and Kerrich [I.130] observed significant variations of the isotope ratio as a function of ion lens settings when zirconium and hafnium were measured with the same type of quadrupole ICP-MS.

As commented previously, the introduction of a collision/reaction cell in an ICP-QMS improves the precision of isotope ratio measurements. However, with its addition, some concerns have arisen as to whether this introduces unpredictable mass bias effects. Boulyga and Becker [I.131] stated that since the use of He in a collision reaction cell results in collisional focusing of the heavier ions, while the light ions suffer from collisional scattering, there should be no doubt that on pressurising such a cell, mass discrimination is altered.

As a consequence, mass bias should be measured and hence corrected by use of an external standard of known isotopic composition (External correction) or using a fixed constant isotope ratio of an element either present or added to the sample (Internal correction).

- External mass bias correction: consists on measuring the isotope ratio of interest in an external standard of known isotopic composition and then the bias is used to correct the same ratio in the sample. Estimation of mass bias by use of an external

standard is advantageous since the mass bias is measured at the same masses of analyte and at approximately the same abundances. However, the magnitude of the mass bias may change in the presence of the sample matrix and/or in the time lapse between measurement of the standard and sample.

- Internal mass bias correction: the mass bias is determined in the sample solution either using a known isotope ratio of an element added to the sample for that purpose, or using a pair of isotopes of the analyte element that are invariant in the nature; then the bias is applied as part of a mathematical model to correct the analyte ratio. In the first case, usually, the element with known isotopic composition is in the same mass range of the analyte. For instance, Roehl *et al*. [I.132] determined the $^{69}Ga/^{71}Ga$ ratio of an internal gallium standard to correct for instrumental mass bias of zinc isotope ratio measurements. An example of the second case is the use of $^{88}Sr/^{86}Sr$ isotopic ratio (which is constant in nature) for internal standardisation of the ratio $^{87}Sr/^{86}Sr$ [I.133].
  Internal standardisation provides near continuous monitoring of the mass bias and can be used to correct matrix effects. The work from Ehrlich *et al.* [I.134] demonstrates that by the use of thallium as an internal standard, the detrimental effect of the matrix at total dissolved solid levels of up to 700 $mg \cdot L^{-1}$ can be corrected when lead isotope ratios are measured.
  However, it has to be kept in mind that using internal correction inaccuracies may be introduced as the isotopes used to calculate the bias have masses, ionisation characteristics and isotopic abundances that are different to the analyte isotopes.
  There are three mathematical functions which are commonly applied for the correction of mass bias depending on whether the mass bias is assumed to be a linear (I.1), power (I.2) or exponential (I.3) law function of the mass difference:

$$R_{corrected} = R_{measured} / (1 + MD\ \Delta m) \quad \text{(I.1)}$$

$$R_{corrected} = R_{measured} / (1 + MD)^{\Delta m} \quad \text{(I.2)}$$

$$R_{corrected} = R_{measured} / \exp^{(MD\ \Delta m)} \quad \text{(I.3)}$$

Where $R_{corrected}$ is the mass bias corrected ratio, $R_{measured}$ is the observed ratio, MD is the bias factor per unit mass and Δm is the mass difference between the isotopes involved in the ratio.

It is relevant to note that the use of an inappropriate algorithm in estimation of mass bias may lead to a certain degree of inaccuracy. For this reason, the study of the suitability of the different functions for each particular case is strongly recommended.

Some researchers have hypothesised that a single internal reference standard is insufficient for absolute mass bias correction. Differences in mass between the analyte and internal reference, even as small as one mass unit, are sufficient to introduce some error in the correction. Although very small, this error is nevertheless, unacceptable for isotope ratio measurements requiring extreme accuracy. Al-Amar and Barnes [I.135] proposed the Common Analyte Internal Standardization (CAIS) chemometric technique as an alternative. It requires the measurement of at least three or four isotope ratios from one or more internal reference elements (close in m/z to the analyte) to establish an isotope ratio curve and calculate the correction factor needed to transform the measured analyte isotope ratio to the true isotope ratio. The CAIS approach seems to be especially useful for the determination of isotope ratios in samples containing high matrix element concentrations or for the determination of isotope ratios for which no certified isotopic standard reference materials are available. In similar way, Ingle *et al*. [I.136] put forward another approach to the estimation and correction of mass bias based on modelling the underlying instrument response function which is then employed to define the expression that should be used to correct isotope ratio data.

Dead-time of the detector system is another effect causing deviations of measured isotopic ratios from given ratios (accuracy). At high count rates, different effects can cause pulse counting systems to register fewer events than actually occurring. After an ion generates an electron pulse in the multiplier, there is a finite time during which the system is unable of recording another event; this interval lacking resolution of successive pulses is termed “deadtime” [I.133]. As a consequence, signal responses become non-linear above certain higher count rates (typically $>10^6$ Counts$\cdot s^{-1}$). This fact becomes significant when dealing with isotopes in strongly different abundances (e.g., ^{208}Pb and ^{204}Pb). In general, the correction of this effect is performed using the following equation:

$$I_{real} = \frac{I_{\exp}}{(1 - \tau I_{\exp})} \qquad \text{(I.4)}$$

Where I_{real} is the count rate if there were no dead time errors, I_{exp} is the measured count rate and τ is the dead time. Commonly, to evaluate the dead time, standard solutions containing increasing concentration of the analyte are measured and the "normalised" isotope ratios (experimental isotope ratio divided by the theoretical value) for each concentration are plotted as a function of the dead time used for the correction. The intersection of the curves gives an estimation of the dead time [I.137] (see Figure I.5). The typical deadtime values for ICP-MS instruments are in the range 15-100 ns.

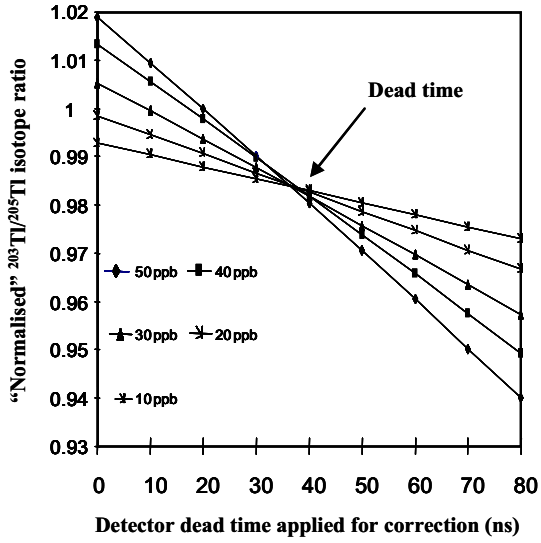

Figure-I.5.- Estimation of the dead time, as the intersection of curves containing increasing concentrations of thallium [I.137].

According to the definition of dead-time, only the detectors based on pulse counting systems (pulse mode) will be affected by this outcome. Other detectors, such as Faraday cups, will be not dependent on analyte concentration since the detection is derived from the measurement of a continuum current (analogic mode).

At has been pointed previously, mass bias and dead-time corrections are a prerequisite for accurate isotopic analysis. However, the uncertainty propagations due to the mass bias and detector dead time have to be taken into consideration when global precision of the isotope ratio data is calculated. Always, the uncertainty on the

corrected isotope ratio will be higher than the uncertainty on the experimental value. Consequently, an improvement of the accuracy of isotope ratio measurement leads to a worse precision of global results.

Finally, the interference effects must also be considered in isotope ratio measurements carried out by ICP-MS as they can negatively affect the accuracy of the obtained data. The spectral interferences in ICP-MS are classified into two major categories: Polyatomic interferences and isobaric interferences.

Polyatomic interferences are defined as molecular species, including basic background species from argon, water and air; molecular species derived from the sought-for analytes in the sample; and molecular species derived from the sample matrix, which can interfere in the measure of the mass of our analyte. However, in isotopic analysis of heavier elements (such as lead) this kind of interferences are not especially problematic since polyatomic interferences commonly present a total mass not higher than 82. On the contrary, overlaps between analyte ions (Isobaric interferences) could be quite serious in isotopic analysis and must be carefully studied and corrected to obtain accurate isotope ratio data. Because isobaric overlaps are predictable and natural abundances are tabulated, mathematical correction schemes can be developed to correct for the occurrence of isobaric spectral overlaps. For example, an isobaric overlap occurs between ^{48}Ti and ^{48}Ca, but this overlap can be corrected for titanium determination by measuring another isotope of calcium and using the natural abundance information. That is, measuring ^{44}Ca and substracting 0.089645 of this measured count from the ^{48}Ti signal serves to correct the ^{48}Ca overlap [I.138]. In some particular cases, the application of mathematical functions to correct isobaric overlapping is not possible and then the analyte of interest have to be separated from the interference before analysis. This is the case of the determination of the ^{87}Sr in the presence of ^{87}Rb. Since ^{87}Rb is a radioactive ion and thus the natural abundance of its isotopes (^{87}Rb and ^{85}Rb) is not stable, the use of mathematic algorithms has to be rejected. As an alternative, in such a case, the rubidium and strontium could be separated by means of a chromatographic procedure before ICP-MS analysis [I.133, I.139].

To summarise, plasma source mass spectrometry plays an important role today in isotope ratio measurements because of the large requirements of fast sample throughput, low sample analysis cost, instrument robustness, and simplified sample

preparation and measurement processes. Moreover, provided that instrument bias factors commented previously are properly studied and corrected, in most cases, the precision and accuracy of isotopic measurements attainable with ICP-MS instrumentation fit the needs of environmental studies.

I.5. ANALYTICAL TOOLS TO DETERMINE CHEMICAL COMPOSITION OF VEGETATION MATRICES

As pointed out in section I.2, the use of vegetation as bio-monitors for environmental studies is growing in acceptance and popularity. Therefore, the implementation and improvement of suitable analytical tools to determine chemical composition of vegetation matrices has become an increasing need.

Once the aims of the analysis are defined it is possible to proceed to the selection of an appropriate methodology. The fitness for purpose of a given analytical technique chosen for the analysis of vegetation samples in environmental studies is related to the compliance to some "desired" features:

- Low detection limits taking into account that some elements may be present in vegetation matrices at very low concentration levels. (See, Table I.8)
- A wide dynamic range is required to compare element composition of vegetation samples collected at non-polluted sites (low metal content) with those from polluted areas (higher metal content). (See, Table I.8)
- The achieved accuracy and precision shall be sufficient for the given purpose.
- Multi-element capability, allowing gathering as much information as possible, since there is not always a-priori knowledge of the kind of information that can be of relevance.
- Simple sample preparation taking into consideration the high number of samples involved in sampling campaigns.
- High throughput and low cost per determination in view of the considerable number of analysis required in environmental studies.

Trace element	Site	Elemental range ($mg \cdot Kg^{-1}$, dry weight)	
		Grass (leaves)	Grass (roots)
Pb	Non-contaminated	0.8-3.4	1.7-6.8
	Contaminated:		
	Mining	360-870	78-160
	Metal processing	350-3845	63-280
	Sewage sludge	43-121	78-310
Cd	Non-contaminated	0.05-0.14	0.08-0.24
	Contaminated:		
	Mining	0.7-5.6	1.3-9.8
	Metal processing	1.7-20.3	1.8-5.6
	Sewage sludge	0.5-8.7	5.6-48.3
Cu	Non-contaminated	3.5-14.5	4.1-18.2
	Contaminated:		
	Mining	78-290	73-320
	Metal processing	35-78	21-75
	Sewage sludge	31-68	48-107
As	Non-contaminated	0.004-0.009	0.006-0.007
	Contaminated:		
	Fertiliser	0.07-0.23	0.11-0.37
Zn	Non-contaminated	15-56	21-72
	Contaminated:		
	Mining	80-274	94-460
	Metal processing	130-380	180-690
	Sewage sludge	120-265	118-380

Table-I.8.- Typical metal content in grasses' tissues growing in non-contaminated areas and in contaminated sites. (Data obtained from [I.33]).

I.5.1. Classical analytical methods (wet/dry ashing procedures plus atomic spectroscopic analysis)

In fact, there is no universal analytical technique fulfilling all these requirements but usually, atomic spectroscopic techniques, including FAAS (Flame Atomic Absorption Spectrometry), ETAAS (Electrothermal Atomic Absorption Spectrometry), ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) and ICP-MS (Inductively Coupled Plasma Mass Spectrometry) are the techniques used for determination of major, minor and trace elements in vegetation specimens.

Each of these techniques has its advantages and drawbacks and thus, few laboratories relay only on one of these analytical methods, but often use compatible combinations of them. Table I.9 summarises the comparative benefits of these techniques and in Table I.10 the typical detection limits for elements commonly determined in environmental studies are presented.

	FAAS	ETAAS	ICP-OES	ICP-MS
Single/Multi-element	S	S	M	M
Linear range	10^2	10^3	10^3	10^8
Precision (%)	>10	3-5	1-10	<5
Accuracy	Moderate	Good	Moderate	Good
Cost	$	$$	$$	$$$

Table-I.9.- Comparison of analytical performance characteristics of the main techniques used in vegetation element analysis. (S-single element, M-Multiple element)

	FAAS	ETAAS	ICP-OES	ICP-MS
Al	<1	<0.05	<1	<0.1
Cr	<1	<0.05	<1	<0.1
Mn	<1	<0.05	<0.1	<0.05
Co	<1	<0.05	<1	<0.05
Ni	<1	<0.05	<1	<0.1
Cu	<1	<0.05	<0.1	<0.05
Zn	<0.1	<0.05	<0.1	<0.1
As[(1)]	>1	<0.1	<1	<0.1
Se[(1)]	>1	<0.1	<1	<0.1
Cd	<0.1	<0.05	<1	<0.05
Sn[(1)]	>1	<0.1	<1	<0.1
Hg[(2)]	>1	<0.1	<1	<0.1
Pb	<1	<0.05	<1	<0.05

Detection limits: $mg \cdot L^{-1}$ ($mg \cdot Kg^{-1}$)
[(1)] Improved by hydride generation (HG), [(2)] Improved by cold vapour (CV)

Table-I.10.- Comparison of the typical detection limits for the main techniques used in environmental trace element analysis [I.33].

Atomic absorption spectrometry (AAS) became the backbone of many of the environmental laboratories by the mid 1970s and continues today to be a very popular, thoroughly tested and well established technique for the analysis of vegetation specimens. The reasons for AAS popularity are its relatively low purchase price and maintenance costs, acceptance by many regulatory agencies (i.e., US-EPA 7000 analytical method series [I.140]), capability of determining about 70 elements, sensitivity and detection limits which satisfy the needs of many studies, reasonable precision and accuracy, and simplicity. But AAS has also limitations, such as the sequential character of the determination of the elements, which increases dramatically the analysis time, as well as the impossibility of determining non metals such as sulphur.

Depending on the sample introduction and atomization system several versions of AAS are distinguished. In FAAS, a flame made of different mixtures of gases (depending on the temperature required to desolvate and atomize the analyte of

concern) is used as atomization device. This configuration has seen great application over the years as the method of choice for element analysis in vegetation samples [I.141, I.142, I.143]. However, FAAS is not always sensitive enough for the levels of some elements present in plants. In such cases, ETAAS is the technique of choice. In this configuration the flame is replaced with a heated graphite tube and the sample injected into this tube for a more complete and efficient atomization of the sample, leading to an improvement of the detection limits in about two orders of magnitude. ETAAS is widely used for the determination of heavy metals such as Pb and Cd which are usually present at low concentration levels in vegetation samples [I.144, I.145, I.146]. However, ETAAS tends to be a very tedious, slow technique which also suffers from significant chemical and spectral interferences that can lead to systematic errors. To minimize the interferences, usually chemical modifiers such as Pd, $(NH_4)_2HPO_3$, among others, are used [I.147].

Hydride generation AAS (HGAAS) and cold vapour AAS (CVAAS) are special combinations of chemical separation and enrichment with AAS which allow the specific determination of some elements such as As, Se, Bi, Sb, Sn (HGAAS) or Hg (CVAAS) [I.148].

The introduction of ICP as ion source increased the possibilities of atomic emission spectrometry in the field of environmental analysis. In this technique, element excitation and atomization is achieved in an argon plasma sustained by the interaction of ionized argon gas inductively coupled to a radio-frequency energy field. An inductively coupled plasma is an attractive spectral source because of its high temperature (up to 10 000ºK) which lead to a high degree of excitation and ionisation and thus, good selectivity and sensitivity for elemental analysis are achieved. At present, about three-fourths of the common elements can be determined by this technique with sensitivity between FAAS and ETAAS. One of the most attractive features of ICP-OES instruments is the possibility of performing analysis in a simultaneous mode. In this way, results for a large number of elements may be obtained in a minute or less. Therefore, ICP-OES is the preferable technique when several analytes have to be determined in a substantial number of vegetation samples [I.149, I.150].

Inductively coupled plasma-mass spectrometry (ICP-MS) has recently become an important tool in the environmental laboratories despite its relative youth in terms of commercial instrumentation [I.151]. ICP-MS has gained the U.S EPA approval for

trace metal determinations, both in water and solids [I.152]. It has many of the attributes and capabilities of ICP-OES, including the ability to simultaneously determine a variety of elements over a wide dynamic concentration range. However, the primary reasons for the popularity of ICP-MS are its great sensitivity (due to the use of a mass spectrometer as detection system) and general lack of interferences for elements with masses higher than 70. But, in contrast to ICP-OES, ICP-MS instruments are expensive and not always available for routine applications.

The main drawback of all atomic spectroscopic techniques commented previously is that the market of this type of instruments offers principally instrumentation initially dedicated to the analysis of liquid samples. Therefore, solid samples (including vegetation specimens) have to be brought into solution before analysis by means of a previous chemical treatment. Sample dissolution is usually a tedious and time-consuming step in the analytical procedure that limits sometimes its application in environmental studies where conclusions are drawn on the basis of the results of a large number of analysed samples.

Commonly, ashing procedures are used to prepare plant samples for elemental analysis. Both dry ashing, involving combustion of the sample, and wet ashing, involving digestion with strong acids, have been used to destroy the organic matter and dissolve the analytes in this kind of matrices [I.153].

Dry ashing consists generally in calcination of the sample in a muffle furnace which ensures the decomposition of organic matter at temperatures between 450ºC and 550ºC. The resulted inorganic residue is then dissolved by an appropriate acid (usually nitric acid or hydrochloric acid) prior to the analysis. The biggest advantage of dry ashing procedures is the possibility of treating large sample amounts and dissolving the resulting ash in a small volume of acid. This procedure allows the pre-concentration of trace elements in the final solution, which is useful when very low analyte concentrations must be determined. Besides, using this procedure the resulting ash is completely free of organic matter which is a prerequisite for some analytical techniques. The French “Comité Inter-Instituts d’Etudes des Techniques Analytiques (CII)”, founded at the end of the fifties, proposed in 1975 a dry oxidation procedure as a reference analytical methodology for plant samples dissolution [I.154]. However, despite of the good recoveries obtained for most elements (N, P, K, Ca, Mg, Na, Cl, Al, B, Cd, Co, Cu, Fe, Mn, Mo, Pb and Zn) the

dry ashing method does not permit values to be proposed for elements such as Hg, As and Se which due to the high temperatures applied during the ashing step could be lost by volatilization. Some works dealing with the study of an appropriate methodology for plant sample pre-treatment also highlight the inherent problem of volatilization losses of some elements in dry ashing procedures, depending on the composition of the sample [I.143, I.155]. For this reason, wet digestion procedures conducted at lower temperatures may ensure better recoveries for most elements and are increasingly used for the mineralization of vegetation samples.

Compared to dry ashing methods, wet mineralization procedures using acid digestions present a wide range of options depending on the choice of reagents and their mixtures as well as on the devices used for the procedure application. In the sample dissolution for the determination of total contents of elements, the majority of wet digestion procedures require the use of one or several reagents to achieve the destruction of the organic matter (nitric, sulphuric and perchloric acids and hydrogen peroxide) and/or to ensure the dissolution of inorganic compounds (hydrochloric and hydrofluoric acid) present in the sample.

At first sight, a plant matrix seems to be very similar to other biological matrices in the environment which are well known to be relatively easy to decompose using mixtures of strong acids and oxidants. There is however, a fundamental difference between plant material and animal organic matrices that is the higher content of silicon in most of vegetation samples (up to 10%) [I.156]. The latter results are of prime importance when an efficient mineralization procedure has to be considered since in such a case an hydrofluoric acid attack is mandatory to achieve the total destruction of the matrix and thus, the determination of the total concentration of analytes. For instance, Wieteska *et al*. [I.157] stated that for a quantitative determination of aluminium and iron in pine needles the use of HF is indispensable. In a similar way, according to Baffi *et al.*[I.151], a digestion procedure using $HF/HNO_3/H_2O_2$ proved to be more suitable, in terms of accuracy and precision, than a procedure based on digestion with HNO_3/H_2O_2, for the determination of Al, Cr, Cu, Fe, Mn, Ti, V, and Zn in lichen samples.

The large number of acid mixtures in wet ashing procedures that may be found in the literature (see Table I.11) illustrates the fact that a lack of a consensus exists for plant specimen dissolution.

Acid Mixture	Samples	References
HNO_3	Vegetables, bark, mosses, pine	[I.142] [I.144]
HNO_3+HF	(22 different vegetation species)	[I.141]
HNO_3+H_2O_2	Grasses, weeds, reed plants	[I.162] [I.155]
HNO_3+ H_2O_2+HF	Lichens, spinach, orchard, tobacco	[I.151] [I.158]
HNO_3+$HClO_4$	Tea leaves	[I.150]
HNO_3+$HClO_4$+HF	Pine needles, rye grass, beech	[I.161]
HNO_3+ $HClO_4$+ HCl+HF	Pepperbush, tea leaves	[I.159]
HNO_3+ H_2SO_4 + H_2O_2+HF	Pine needles, rye grass, beech	[I.161]

Table-I.11.- Acid mixtures commonly applied to vegetation samples decomposition using wet ashing procedures.

The choice of a procedure has to be oriented as a function of type and nature of the matrix. For example, the use of sulphuric acid is not recommended in samples rich in alkaline earth elements (i.e., some plant species) due to the possible precipitation of insoluble sulphates (i.e., $CaSO_4$) [I.160]. The possibility of low recoveries due to potassium precipitation as perchlorate has also been discussed by some authors who use a mixture of HNO_3-$HClO_4$ for medicinal plant decomposition [I.143]. Sometimes, it has to be kept in mind also the analytical technique used afterwards for element determination. It is known that ETAAS instrumentation does not tolerate the presence of perchloric and hydrofluoric acids in the digests [I.148]. Similarly, hydrochloric acid must be avoided if determination of ^{75}As is required using ICP-MS technique due to the polyatomic interference of ^{40}Ar-^{35}Cl.

Classically, open systems (digestions at atmospheric pressure) have been applied using conventional sources of heating such as sand baths, heating plates, etc. However, some works showed up the problem of losses of volatile compounds for some plant materials using this kind of devices [I.161]. Besides, in most cases long periods of time are needed to ensure a complete sample digestion (up to 10 hours) [I.162]. These problems can be minimized if wet ashing is carried out in closed systems (pressurized acid digestion). Digestions performed following these procedures benefit of synergic effects of temperature and pressure leading to a more efficient mineralization. Over the last 15 years, microwave oven has been employed extensively in closed systems for shortening the time required for sample dissolution and the amount of reagents employed as well as to avoid analyte losses and contamination from other samples or from the surroundings [I.163]. The use of microwave-assisted digestion is also accepted as a standard method (Method 3052)

for the digestion of siliceous and organically based matrices (such as plant matrices) by the US-EPA [I.164].

Ultrasound-assisted extraction as an alternative for classical vegetation sample digestions has been also the aim of some recent works, [I.165, I.166]. The main advantages of the proposed methodologies are higher sample throughput, lower analytical costs (when comparing to microwave ovens), lower consumption of reagents and improved safety since neither pressure nor high temperature are present during the extraction procedure. However, it has to be kept in mind, that they are based on an acid extraction and thus, the vegetation matrix is not decomposed. This fact could cause poor recoveries of some elements depending on the type of sample of concern [I.142].

To sum up, it can be said that the choice of the best decomposition procedure for vegetation samples should be preceded by verification of the procedure for each specific matix and analyte under study. This becomes unfeasible in environmental studies where several plant species are used as pollution indicators of different heavy metals. In such cases, the application of techniques which allow obviating the matrix destruction stage is a tempting desire.

I.5.2. Techniques based on direct analysis of solid samples: "The use of X-Ray Fluorescence Spectrometry (XRF)"

Neutron activation analysis in its instrumental non-destructive form (INAA) is one of the potential candidate techniques that not require a previous destruction stage of the sample. It is based on the measuring of radioactive activity produced by nuclear reactions on naturally occurring isotopes. In addition to the possibility to perform the analysis directly on a solid, the INAA present some more advantages that make this technique appropriate for plant analysis, including high selectivity and sensitivity (detection limits are in the ng/g range), good precision and accuracy (1-10%), large dynamic range, and high sensitivity for most heavy metals. For instance, INAA has been successfully applied in the analysis of trace amounts of Co in different plant materials [I.158]. The most serious shortcomings of INAA are its high costs, the availability of a nuclear reactor for irradiation as well as the rather long time of analysis imposed by the long waiting (cooling) periods for the decay of short-lived radioisotopes. Moreover, INAA does not allow determining some environmentally important elements like Pb and Cd.

Most of the X-Ray Fluorescence (XRF) techniques comply with the desired features for the analysis of vegetation specimens indicated previously, including the multi-element capability, the possibility to perform qualitative, semi-quantitative and quantitative determinations, simple sample preparation, a wide dynamic range, high throughput and low cost per determination. The main drawbacks of XRF instrumentation, restricting a more frequent use for environmental purposes, have been the insufficient sensitivity for some important pollutant elements (such as Cd, Pb, among others) and a somewhat worse precision and accuracy compared to the other atomic absorption spectroscopic techniques. Nevertheless, the recent improvements in the XRF instrumentation such as the development of digital signal processing based spectrometers in combination with enlarged X-ray production using better designs for excitation-detection has added the advantage of increasing instrumental sensitivity, thus allowing the improvement of both precision and productivity. This fact has promoted an increasing interest of using X-ray fluorescence spectroscopy as an alternative to destructive analytical methods in the environmental field. Particularly, the implementation of different quantitation strategies using diverse configurations of XRF spectrometers as analytical tools to determine chemical composition of vegetation matrices was outlined as one of the goals of this thesis.

I.5.2.1. Theoretical principles of X-Ray Fluorescence Spectrometry

The principle of XRF methodologies is based in the use of some excitation radiation to produce the ionization of the atoms present in the sample due to photoelectric absorption. The process of atom ionization and X-ray characteristic emission is illustrated in Figure I.6. If a photon with energy E_0 larger than the binding energy of an inner electron orbital ($E^{ab}_{k,l,m}$) impinges into the atom (a), part of its energy can be absorbed with certain probability $\tau(E_0)$ (photoelectric absorption) and the atom is ionized (b). The atom can return to its rest energy by one of two possible processes (c,d), being ω the probability of X-ray characteristic emission to occur (fluorescence yield). It is important to note that the fluorescent yield takes a value of around unity for the higher atomic numbers to less than 0.01 for the low atomic elements. It is mainly for this reason that the sensitivity of the X-ray spectrometric techniques is rather poor for light elements.

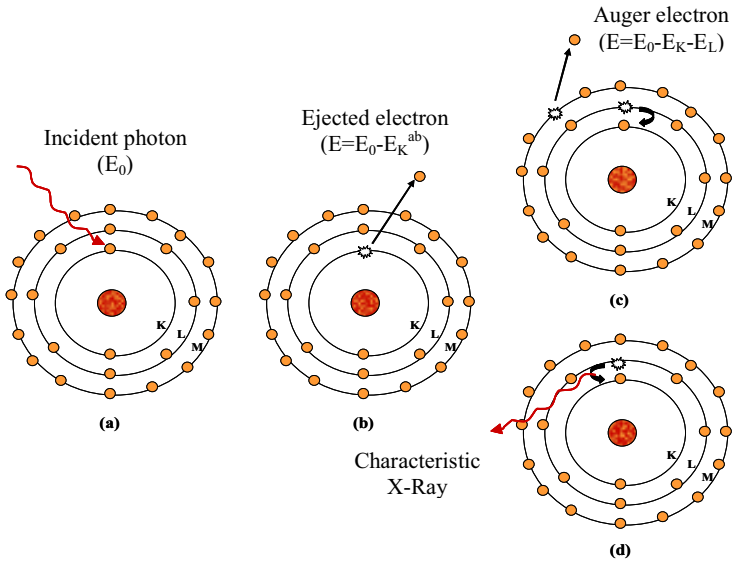

Figure-I.6.- Process of atom ionization and X-ray characteristic emission

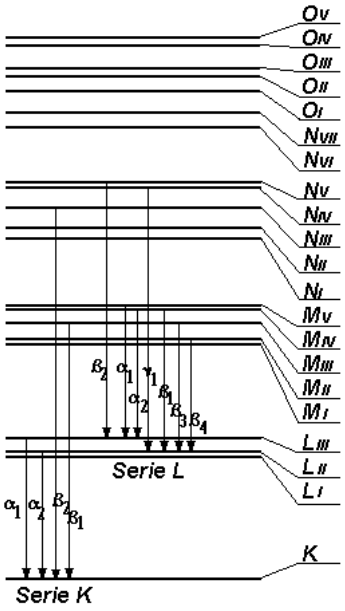

Figure-I.7.- Allowed transitions originating the X-ray characteristic emission

The law of Moseley relates the wavelength of the characteristic emission with the atomic number as:

$$\nu = K(Z-\sigma)^2 \qquad \text{(I.5)}$$

where K and σ are constants which values are specific for the energy distribution levels and sub-levels involved in a given transition (Figure I.7).

The wavelength and the energy of a photon can be related using the Plank constant $\hbar$ and the speed of light c:

$$E = \hbar c\nu = \frac{\hbar c}{\lambda} \qquad \text{(I.6)}$$

and when photon energy is expressed in kilo electron volts:

$$E(keV) = \frac{12{,}398}{\lambda(\text{Å})} \qquad \text{(I.7)}$$

As far as the characteristic emission corresponds to transitions ruled by quantum mechanics principles, the method constitutes a unique technique for the identification of the atoms present in the sample (qualitative analysis). A straightforward

identification of any element present in the sample is possible by detecting the presence of several of its characteristic emission energies. Since the X-ray production does not depend of the chemical association of the elements (transitions arise from inner orbitals), the method has also a multi-elemental capability.

To achieve X-ray production from a given element the only requirement is using an excitation radiation with energy larger than the absorption edge for the corresponding group of lines. Most commercially available X-ray spectrometers have a range from about 0.4 to 20 Å (40 to 0.6 keV), and this range allow measurement of the K series from fluorine (Z=9) to lutetium (Z=71), and the L lines form manganese (Z=25) to uranium (Z=92) [I.167]. Several different types of sources have been employed for the excitation of characteristic X-ray radiation. Despite that the most common source today is the X-ray photon source (sealed X-ray tubes) the use of radio-isotopes as a γ-source is also well-established mostly in low cost and portable systems.

I.5.2.2. Sample preparation procedures

As commented before, another of the advantageous features that make XRF techniques an attractive alternative to destructive analytical methods is the possibility of performing the analysis directly on solid samples. This fact usually implies a simpler sample preparation with a considerable reduction of reagents and risk of contamination.

Determination of the composition of solid samples, without any sample preparation, is possible for samples that are homogeneous in all three dimensions, with a flat surface. This is the case of the direct analysis of metals and alloys which has been one of the main applications of XRF. However, the majority of solid materials require a previous sample pre-treatment step to meet the conditions for homogeneity and to ensure the quality and reproducibility in measurements [I.168].

Commonly, this procedure is based on the crushing and/or grinding of the materials into fine powder followed by a pelletization at high pressure. Specifically, this is the most frequent sample preparation of vegetation specimen analysis by XRF techniques [I.169]. At present, in the literature, there are a wide variety of plant preparation methodologies which make use of different sample amounts (from a few hundred of mg to several g), grain sample sizes, pressures and time of pelletization, etc [I.170, I.171, I.172]. The addition of a blinder or backing material such as boric

acid [I.173] or wax [I.174] is often used to make vegetation pellets more stable. However, it has to be kept in mind that the introduction of a binder during the sample preparation leads to dilution of the sample. This fact could be of particular interest if trace element determination is required.

If the amount of plant sample available is scarce the possibility of making a pellet is unpractical. In such cases, the sample preparation for XRF analysis is usually based on the distribution of the plant powder on the adhesive side of a tape [I.175] or on an X-ray foil [I.176, I.177] to obtain a plant layer ranging from 0.8-2.5 $mg \cdot cm^{-2}$. Other authors propose to ground the powdered vegetation samples in a ceramic mortar with alcohol before being drizzled on to foils [I.178].

Finally, in order to improve the detection limits of some trace elements other sample preparation methodologies have been applied including the use of wet/dry ashing procedures followed by a pre-concentration step [I.179]. However, these procedures imply a similar sample pre-treatment to those involved in atomic spectroscopic techniques pointed out previously.

I.5.2.3. Physical and chemical matrix effects

In XRF the analytical signal is the intensity of measured characteristic radiation (in counts/second), which is proportional to the mass fraction of the element originating it in the analysed sample. However, this relationship is not linear in the more general case, since it depends on physical and chemical matrix effects.

Physical matrix effects are the result of variations in the physical characteristics of the sample, including particle size, uniformity, homogeneity and surface condition. To minimize these effects, a thorough and consistent sample preparation is crucial.

Chemical matrix effects result from differences in concentrations of interfering elements present in the sample. These inter-element effects fall into two broad categories, absorption effects (primary and secondary) and enhancement effects [I.167].

- Absorption effects: Both the excitation radiation and the X-ray emission are attenuated while traveling through the sample. Primary absorption occurs because all atoms of the specimen matrix will absorb or scatter photons from the primary source. Thus, the portion of the spectrum most effective in the excitation of a given analyte will be modified depending on the other matrix elements. Secondary

absorption refers to the effect of the absorption of the characteristic analyte radiation by the specimen matrix. As characteristic radiation passes out from the specimen in which it was generated, it will be absorbed by all matrix elements, in amounts relative to the mass absorption coefficients of these elements.

- Enhancement effects: Occur when a non-analyte matrix emits a characteristic line that has energy just in excess of the absorption edge of the analyte element. This means that the non-analyte element in question is able to excite the analyte, giving the characteristic photons over and above those produced by the primary continuum. This gives an increased, or enhanced, signal from the analyte.

It is interesting to note that the intensity of an analyte line is subject not only to the influence of matrix effects, but also to random and systematic errors due to the spectrometer and counting procedure employed. Provided that a sufficient number of counts is taken, and provided that the spectrometer source is adequately calibrated, random errors from these sources are generally insignificant, relative to other errors. However, systematic errors from these sources are not irrelevant and effects such as counting dead time, background and line overlap can all contribute to the total experimental uncertainty in the measured intensity. Therefore, it is essential to identify and correct them properly to obtain reliable analyte intensities.

Plant materials consist mainly of C, N, H and O (~ 98%) which serve as natural diluent for the other elements. As a result, the absorption of the measured X-rays by the matrix is relatively small compared to rock or soil samples. Nevertheless, due to the widely variable range of concentrations of K, Ca, Cl and other elements in plant samples, in most cases, corrections for the matrix effects have to be done in order to obtain quantitative results.

Several methods have been described for matrix effect correction. Each of these methods has its own advantages and disadvantages and the choice of it is determined by the particular application.

If the objective of the quantitative analysis is the determination of a single or several elements in an unknown but relatively constant matrix, four basic methods are commonly applied [I.167, I.180]:

- Scattered radiation: Besides the already described photoelectric absorption, X-rays can also undergo scattering through elastic interaction (coherent or Rayleigh scatter) or inelastic interaction (incoherent or Compton scatter) with the outer electrons in the atoms. The intensity of the scatter is dependent on the energy of the incident ray and the average atomic number of the matrix. According to that, if the variation in matrix effects is mainly due to absorption, scattered X-rays can be used to correct the intensity of the analyte. As the scatter increases with decreasing mean atomic number, it is particularly suitable for matrix correction of elements in light matrices such as plant material. For instance, Compton scatter correction was used successfully to correct for matrix effects on the shorter wavelength fluorescent radiation from the heavy elements present in different vegetation specimens [I.181]. In the same way, Garivait *et al*. [I.182] show up the suitability of scatter correction for the determination of essential elements (Na, Mg, P, S, Cl, K, Ca, Mn and Fe) in plants. These authors also stated that Compton correction compensates also for different disturbances of the fluorescent intensity due to the physical characteristics of the prepared specimens.

- Internal standard: In this method an element is added to each sample in a fixed proportion to standards and unknowns. The element added (internal standard) should be similar to the analyte in terms of absorption and enhancement properties. As the internal standard is affected in much the same way as the intensity of the analyte, provided there are no absorption edges, it could be used to correct for absorption and enhancement. However, the addition of reagents and the requirement of homogeneity of the sample limit the practical application of the method to the analysis of liquids and fused specimens.

- Standard addition: Sometimes an appropriate internal standard cannot be found and, in this instance, it may be possible to use the analyte itself as an internal standard. The technique of standard addition is especially useful for the determination of analyte concentrations below 5%. However, care must be taken in the application of the method whenever the relationship between analyte concentration and analyte intensity is not linear, since a linear relationship between concentration and intensity is assumed. To ensure the linearity, it is a

common practice to repeat the standard addition step at least twice, so that a minimum of three data sets (zero concentration plus two additions) are available to work with. Standard addition has been used in the quantitative analysis of several elements in different vegetation matrices such as lichens [I.183] and cabbage [I.184].

- Dilution methods: Dilution methods can also eliminate or reduce the variation of the matrix effect, rather than compensating for such variations. Since dilution requires the addition of reagents to the sample its use in powdered samples is limited due to inherent problems of homogeneity. In addition, the application of this methodology to trace element determination is critical taking into account that with the dilution procedure the analyte intensity is also reduced.

On the other hand, if the matrix effect is more variable from one sample to another and/or multi-element determination is required the use of the so called "mathematical methods" is mandatory. In this respect, these methods are more flexible than the others methods described above but they require more knowledge of the complete matrix. All elements contributing significantly to the matrix effect must be quantified (either by X-ray measurement of by another technique), even if the determination of their levels is not required for a given purpose. Included in this category are influence coefficient correction methods and the fundamental parameter method.

- Influence coefficient correction methods: These methods are based on the matrix effect correction by means of the "Influence coefficients" which can be determined theoretically or empirically. In the first case, depending on if a single coefficient is considered per interfering element, if more than one is used or whether the influence coefficient is a constant for a given application or the value of the coefficient varies with composition, different algorithms have been developed to calculate them [I.180]. Once the coefficients are calculated theoretically, it is possible to estimate the matrix correction term by the measurement of several standards with known element weight fractions. Then, a regression line could be determined by plotting the measured intensity of the

analyte multiplied by the corresponding matrix correction term against analyte weight fraction, as it is shown in the following expression:

$$W_i = a + b\, I_i\, [1+ (model)] \qquad (I.8)$$

Where W_i is the analyte weight fraction, $a + b\, I_i$ is related directly to relative intensity of the analyte and [1+ (model)] represents the matrix correction term that depends on the mathematic model used to calculate the influence coefficients. The weight fraction of the analytes in the unknown samples is determined by solving the set of equations with the general form showed in (I.8) either algebraically or by means of an iterative method.

On the other hand, influence coefficients can also be determined empirically using regression analysis. They were the earliest correction methods but are now largely superseded by more theoretical methods. Such coefficients tend to mix the matrix correction with the sensitivity of the spectrometer. Therefore, the coefficients determined are instrument specific and are not transferable to other instruments.

The study performed by Chuparina and Gunicheva [I.173] on different plant materials showed up that adequate accuracy of analytical results could be achieved using theoretical influence coefficients as matrix correction.

- Fundamental parameter method: One of the characteristics of X-Ray fluorescence analysis is that, once the sample composition is known, the results allow theoretical calculation of the intensities of generated fluorescent X-rays by using the measuring conditions and physical constants (fundamental parameters). The fundamental parameter (FP) method utilises these characteristics in a reverse manner, that is, it tries to obtain the composition from the actually measured intensities. The summarised following steps should be taken in order to calculate the sample composition following the FP methodology.

  Step-1: The measured intensity is converted to a theoretical intensity scale by using the instrumental sensitivity and thereby an initial estimation of the composition is made.

Step-2: Then, the theoretical intensity is calculated on the basis of the estimated composition and then compared with the measured intensity. The concentrations are subsequently adapted.

Step-3: A convergence check for the concentration is made. If the difference between the previous and the present concentration is reduced to a certain value, the resultant concentration is considered to be the final one. If not, the operation returns to Step 2 so as to repeat the calculation until the required convergence has been attained.

It is interesting to remark that before the application of these steps an instrumental calibration procedure is necessary in order to calculate the sensitivity factors needed in step-1. Commonly, the instrumental sensitivity is determined by means of the analysis of pure elements considering the appropriate geometry of the spectrometer used: incidence and takeoff angles and the parameters of tube spectrum (the anode material, voltage, thickness of beryllium window and so on). Taking into account that only pure elements are needed for instrumental sensitivity determination, this methodology is specifically useful if insufficient standards are available for calibration purposes.

In a general way, the relative precision and accuracy of the results using this quantitation method are usually better than 5-10% which is sufficient for most environmental purposes. For instance, there are a wide variety of studies involving multi-elemental determination in vegetation specimens that use FP as quantitation method [I.170, I.181, I.185].

The choice of the method used for matrix effect corrections is more often made on considerations like availability of the programs and computers than on differences in analytical capabilities.

I.5.2.4. XRF instrumentation

There are many types of X-ray fluorescence spectrometers available on the market today but most of them fall roughly in two categories, wavelength dispersive X-ray fluorescence (WDXRF) and energy dispersive X-ray fluorescence (EDXRF).

In WDXRF the characteristic radiation emitted from the sample is separated by wavelengths using a diffraction device (see Figure I.8).

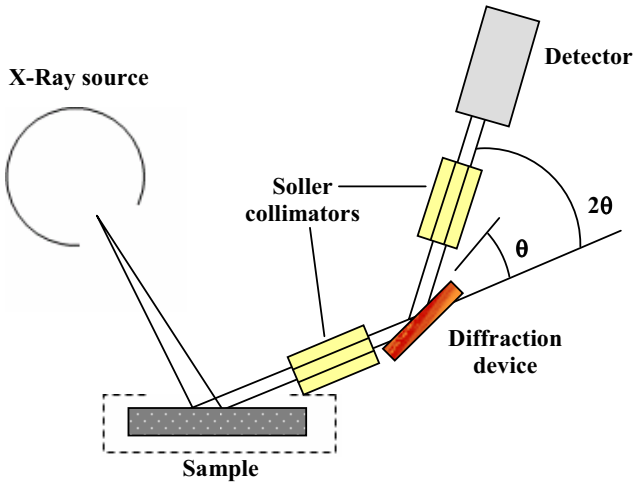

Figure-I.8.- Configuration of a conventional WDXRF spectrometer

Due to the high resolution power of single crystals or multilayered structures used for this purpose and the use of additional Soller collimators, photons corresponding to characteristic lines with close energies can be detected without interfering each other, thus providing a high specificity in the analysis. Since spectral interference is avoided, the detection can be performed using proportional counters (usually gas flow or scintillation counters), with large efficiency at even low energies.

In WDXRF spectrometers the analysis of different elements is usually carried out in a sequential way by changing synchronously the orientation of the monochromator device and the detector (2θ). However, in multi-channel spectrometers, the use of several diffraction devices/detectors sets allows measuring several elements simultaneously (the number of channels is limited).

Unlike the wavelength dispersive X-ray fluorescence systems, the conventional EDXRF spectrometers consists of only two basic units, the excitation source and the spectrometer / detection system (see Figure I.9). In this case, since the resolution of the energy dispersive system is equated directly to the resolution of the detector, typically a semiconductor detector of high intrinsic resolution is employed (Si (Li)). The use of this type of detectors allows obtaining an electronic signal which current is proportional to the energy of the detected photon. Then a multi-channel analyzer is used to collect, integrate and display the resolved pulses.

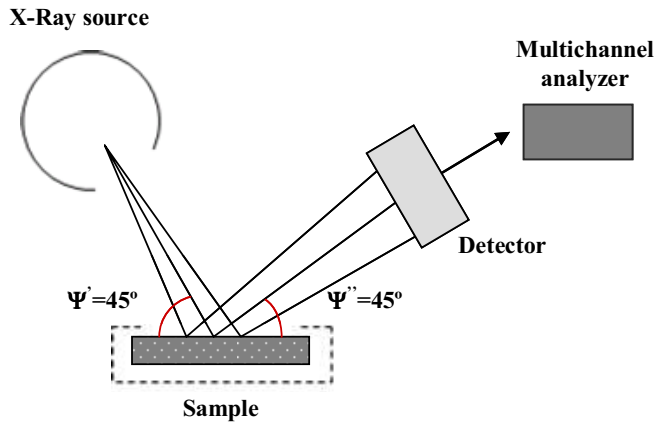

Figure-I.9.- Configuration of a conventional EDXRF spectrometer

It is interesting to remark that using this configuration all of the X-rays emitted by the sample are collected at the same time (simultaneously measured), giving great speed in the acquisition and display of data. In practice, however, there is a limit to the maximum count rate that the spectrometer con handle and this led, in the mid-1970s to the development of the called *secondary* mode of operation. In the secondary mode, a carefully selected pure element standard is interposed between the primary source and the sample in such a way that a selectable energy range of secondary photons is incident upon the sample. This allows selective excitation of certain portions of the energy range, thus increasing the fraction of useful and unwanted photons entering the detector. While this configuration does not completely eliminate the count rate and resolution limitations of the primary system, it certainly does reduce them [I.167].

Both configurations (WDXRF and EDXRF) have been widely used in a large number of applications. The selection of the most suitable configuration is based on the requirements for a given purpose. For instance, multi-channel wavelength dispersive instruments are used almost exclusively for routine, high throughput analyses where the great need is for fast accurate analysis, but where the flexibility is of no importance. WDXRF is extremely popular in the geological field and it has been used for measuring raw materials and finished products composed of minerals [I.186]. Perring and Andrey [I.187] also show up the potentiality of WDXRF for quality control analysis of several elements (Na, Mg, P, Cl, K, Ca, Fe and Zn) in commercial milk-based products. Its application in vegetation samples analysis is not widespread. However, some works highlight the possibilities of the technique for the

quantitative analysis of macro and micro-nutrients in vegetation matrices [I.182, I.188, I.189, I.190]. The possibility of determining some elements that can hardly be determined by other spectroscopic techniques (such as sulphur) is also emphasised by some authors [I.191].

Energy dispersive spectrometers have the great advantage of being able to display information on all elements at the same time. They lack somewhat in resolution over the wavelength dispersive spectrometer, but the ability to reveal elements absent as well as elements present make the energy dispersive spectrometer ideal for general troubleshooting problems. They have been particularly effective, among others, in the fields of scrap alloy sorting, forensic science and the provision of elemental data to supplement X-ray powder diffraction data [I.167]. The application of portable EDXRF have also found special usefulness in art-archeometry studies [I.192] due to the possibility of analyse objects that can not be brought into the laboratory. The US-EPA also presents a Standard Operation Procedure (SOP) for elemental analysis of metals in soils and sediments using a portable XRF analyzer [I.193].

An area where these instruments are finding increasing application is in the analysis of pollutants. More recent developments have allowed sensitivities that are quite sufficient for the sample masses typically encountered in the analysis of trace metals in environmental samples. A good example of this is the analysis of chemical composition of atmospheric aerosols [I.194, I.195]. As far vegetation samples, the determination of major and minor elements at $g \cdot Kg^{-1}$-$mg \cdot Kg^{-1}$ levels using conventional EDXRF has been the goal of some analytical researches [I.177,I.184, I.185,I.196]. However, a comparative evaluation of the possibilities of INAA, EDXRF, ICPAES and AAS in the analysis of plants [I.197] showed up that conventional EDXRF technique allows the acceptable determination of most nutrient and essential elements in plant samples but its application to determine toxic elements (Al, As, Br, Cd, Cr, Hg, Pb, Ni, Sb) is unfeasible due to the lack of sensibility of the technique. The use of EDXRF-secondary mode of operation has been also applied in the analysis of different vegetation matrices [I.172,I.178]. Commonly in these configurations the primary, the secondary and the characteristic radiation from the sample formed mutually orthogonal angles (tri-axial geometry). This geometry reduced the background radiation relative to the characteristic radiation from different element in the sample, allowing an improvement of the sensibility and limits of detection of minor and trace elements. For instance, the

combination of a tri-axial EDXRF geometry with an appropriate sample preparation allowed the determination of Hg at sub-mg·Kg^{-1} levels in different environmental samples [I.198]. With novel instrumentation (Polarised-beam EDXRF), the combination of polarisation (tri-axial geometry) with the possibility of using different secondary sources allows specific element excitation and thus, a further improvement of the limits of detection of analytes (see Figuref I.10).

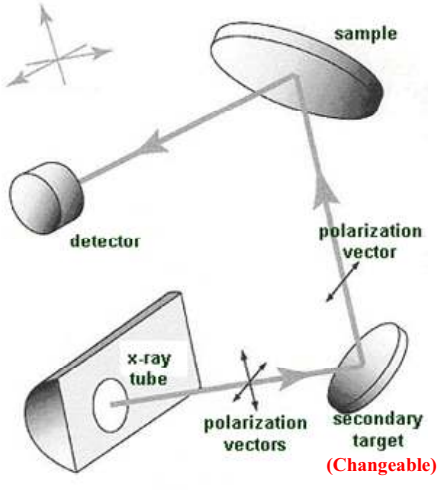

Figure-I.10.- Configuration of a polarised-beam EDXRF spectrometer [I.181]

Another advantage of scattering primary X-rays with targets is that it is much more practical to vary targets than X-Ray tube anodes, and targets may be used to produce fluorescence lines to act as near-monochromatic secondary sources at slightly higher energies than the absorption edges of the analytical lines to be excited. By combining polarisation with the possibility of different secondary sources, an instrument can be designed to generate a range of excitation and polarisation conditions optimised for different groups of analytes. A recent study developed by Stephens and Calder [I.181] highlight the suitability of a P-EDXRF system to the analysis of various toxic elements in plant leaves and tobacco samples.

In addition to WDXRF and EDXRF systems, there are other X-ray spectrometers which may not be generally available to the general user community but they have important roles to play in special areas of application. Included in this category are total reflection spectrometers (TXRF) and synchrotron source spectrometers (SSXRF). Both systems have in common a very high sensitivity and ability to work with extremely low concentrations and/or small specimens.

The TXRF system makes use of the fact that at very low glancing angles, primary X-ray photons are almost completely absorbed within thin specimens, and the high background that would generally occur due to scatter from the sample is absent. Because the background is so low, concentrations in the range of a few tenths of $\mu g \cdot L^{-1}$ can be measured in aqueous solutions without recourse to pre-concentration [I.167]. For this reason, one area in which the TXRF techniques has found great application is in the analysis of natural waters. TXRF has also been used in the analysis of different vegetation specimens with a previous acid digestion of the samples [I.199, I.200]. In some plant matrices such as xylem fluid the sample pre-treatment is not necessary and the analysis by TXRF could be carried out directly, as reported by Varga *et al* [I.201].

The high intensity of the synchrotron sources (four or five orders of magnitude greater than the conventional sealed X-ray tubes) in combination with the polarisation in the plane of synchrotron ring, allows very rapid bulk analysis to be obtained on small areas with very low detection limits. This factor is especially attractive for the analysis of archeometric samples, for example, ancient ceramics [I.167]. Despite that this type of instrumentation is hardly available some environmental studies which required the analysis of vegetation samples have been performed using SSXRF. For instance, Sokolovskaya *et al*. reported the determination of 14 toxic metals in different aquatic vascular plants at the low $mg \cdot Kg^{-1}$ levels using this instrumentation [I.202].

In view of the above considerations, it could be concluded that XRF techniques, at a cursory glance, could be a tentative approach for the determination of chemical composition in vegetation specimens, as an alternative to the classical destructive analytical methods. Nevertheless, the study of suitable quantitative methodologies as well as analytical figures of merit (precision, accuracy, limits of detection…) of the proposed techniques must be carefully determined in order to state the capability of XRF for environmental analytical purposes.

I.6. OUTLINE OF THE THESIS

This thesis reports on the research performed to the improvement of analytical existing methodologies and the development of novel procedures that can be applied to assess environmental effects of abandoned mining activities. The combination of the implementation of analytical tools to predict potential mobility and bioavailability of metals released from abandoned mining wastes with the development of low cost and high productive analytical methods to study metal bioaccumulation and chemical composition of spontaneously occurring vegetation collected at these areas could be summarised as the main goal of this study.

A review of the past and present of mining activities in Spain and the description of the mining districts particularly studied in the present research are provided in **Chapter 1**. In addition, a depiction of the types of samples collected at each area and the importance of "what to look for" is emphasised.

In **Chapter 2** an evaluation of the suitability of a standardised sequential extraction procedure (BCR scheme) and two representative leaching tests (TCLP and DIN-38414-S4) to study potential mobility and hazardousness of metals from dumped mining wastes is presented. Some modifications of the original BCR scheme are reported so as to improve its analytical applicability in the laboratory.

Some of the main factors affecting mobility and bioavailability of metals in different kind of mining wastes are discussed in **Chapter 3**. Furthermore, to provide an effective evaluation of the metal bioavailability (mining tailing-plant transfer), both studies based on soil tests (metal content released by single extractions) and plant analyses (metal content in plant matrices) have been conducted together.

Chapter 4 highlights the usefulness of using stable lead isotope ratios to study the potential sources of anomalous lead content in vegetation specimens growing on the wastes and soils of abandoned mining areas. To fit to this purpose, the development of an ICP-QMS methodology for lead isotope ratio measurements with a sufficient

precision and accuracy has been developed, and the main instrumental bias factors has been properly studied and corrected to obtain reliable analytical results.

In view of the considerable number of vegetation specimen analysis conducted to assess the impact of mining activities, the study of high productivity analytical methods for metal determination in this kind of matrices was considered appropriate. In this sense, different X-ray fluorescence methodologies and quantitation strategies have been applied to the analysis of major, minor and trace elements in different kind of vegetation species collected at the mining areas of study. Results obtained are summarised in Chapters 5-7.

The main difficulties arising of using classical destructive analytical methods (digestion procedures plus atomic spectrometry) for the analysis of different plant materials are emphasised in **Chapter 5**, as well as the benefits of using XRF as alternative technique.

In **Chapter 6** the development and suitability of a quantitative WDXRF methodology for the determination of some major (Na, Mg, Al, P, S, K, Ca), trace (Mn, Fe, Co, Zn, As) and non-essential elements (Sr, Pb) in vegetation matrices is reported. The main drawback of the proposed methodology is that the determination of some environmentally important elements such as Cd could not be achieved using this kind of instrumentation.

For this reason in **Chapter 7** the study of the applicability of a polarised-beam EDXRF system with improved detection limits due to the combination of selective excitation and the reduction of the spectrum is presented. With this improvements the determination of cadmium and other toxic elements is feasible at the $mg \cdot Kg^{-1}$ levels. Furthermore in this chapter an evaluation of using a standard-less fundamental parameter quantitative analytical procedure in a EDPXRF system is performed.

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CHAPTER 1

Mining districts involved in the present study

1.1. PAST AND PRESENT OF MINING ACTIVITIES

As pointed out in the introduction section, one of the more troublesome by-products of mining activities consists of mining wastes. Depending on the nature of the mineral processing operation employed, tailings are usually finely divided and, as a result, subjected more easily to chemical weathering processes if they are not properly disposed of. Consequently, heavy metals associated with metal ores can be leached from mining tailings and become an important source of environmental metal pollution.

Although mineral resource extraction has been carried out for several millennia, relatively little attention has been given until the last several decades to minimizing the effects of such extraction on the environment. In the past, much waste was dumped indiscriminately or disposed of in inadequate facilities. Older landfills worked on the principle of waste attenuation where it was accepted that leachate involved would not cause any acute environmental problem. Later, it was realised that this type of waste disposal was responsible for many aquifers being significantly polluted with a wide range of chemicals, including metals and products used in mining operations [1.1], and an official law was established in Spain concerning to mining activity development ("Ley de Minas" Ley 22/1973 de 21 de Julio) [1.2]. This legislation addresses preventive measures to protect the environment and the corresponding sanctions to the exploiter if the regulations are violated. For instance, modern landfill sites need to be located in impermeable strata or sealed with an appropriate barrier before the wastes are deposited and should have a system for managing the leachate. However, it is interesting to mention that at present the possible environmental impacts of mining have grown increasingly, in contradiction to the fact that environmental objections are often the principal obstacle to the development of new mines.

One of the most significant environmental problems that society faces today is the challenge of identifying, containing and remediating (cleaning up) these old abandoned mining areas, which were not subject to appropriate legislation during the activity period, in order to protect the public health and the environmental quality. The best action that can be undertaken in such cases is to return the mined area as nearly as possible to its pre-mining state or to revert the desolation that may have resulted from mining works by reshaping the areas or finding alternative uses for the waste, such as turning an opencast into a sailing lake or abandoned mines into a

tourist resort, or converting waste heaps into material for the construction industry [I.3].

As mentioned in the outline of the thesis, the main goal of this research is the improvement of existing analytical methods and the development of novel procedures that can be applied to assess environmental effects of abandoned mining activities. Specifically, the studied samples were collected in three abandoned mining areas of Spain (see Figure 1.1).

Figure-1.1.- Location of the mining districts related with the present work : Val d'Aran (a), Osor (b) and Cartagena (c)

In all of these areas, Galena (PbS) and Sphalerite (ZnS) were the principal minerals extracted to recover lead and zinc as valuable products. Therefore Pb and Zn were supposed to be the most abundant metals present in the mining wastes and thus, the metals of environmental importance. However, it has to be kept in mind that other minor metals are associated with sulphide ores such as copper, iron, arsenic, cadmium, silver, nickel and small quantities of gold. Hence, in the present research,

in addition to Pb and Zn, some other trace elements such as Cd will also be of significance due to its high toxicity even at low concentrations.

In the following sections, detailed descriptions of each of the studied mining districts is provided, including the history of mining operations and the actual state of the art of these abandoned areas.

1.1.1. Val d'Aran mining district

Val d'Aran is situated in the northern part of Spain, specifically in the Catalan Pyrenean Range (see Figure 1.2).

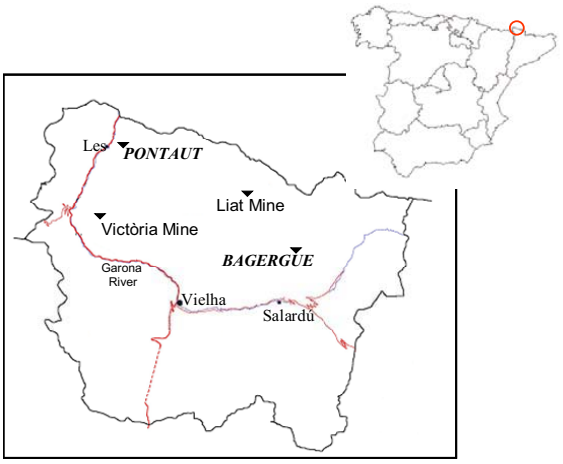

Figure-1.2.- Mining areas and ore treatment plants related with the present study (Val d'Aran region)

Along all over the Aran Valley many old mines and mineral treatment plants can be found. During the period of mining activity, the mineral was extracted from two main mines (Victoria Mine and Liat Mine). The ore vein was mainly composed of galena (PbS) and sphalerite (ZnS) although other minor sulphides such as calcopyrite ($CuFeS_2$) and pyrite (FeS) were also found. A typical feature of the mineralization is also a high content of minor elements including Mn, Cd, Ni, Cu and As [1.4].

Mine works were located at 1500m in altitude. The exploitation system was a complicated network of underground galleries having a recognized length of, at least, 15 km. Nowadays, many of the galleries are flooded and the lower gallery (called Sincay gallery) exhibits a continuous output of running waters, coming from the whole mine, during the entire year (Figure 1.3).

Figure-1.3.- Entrance to a partially flooded gallery (Victoria Mine)

Figure-1.4.- Characteristic precipitate found inside a partially flooded gallery (Victoria Mine)

It is interesting to note that in old abandoned mines usually the acidic pH is buffered by the host rocks and the atmosphere, originating waters near neutrality. This fact produces an increase of pH from the vein to the mine outlet, promoting the progressive precipitation of metals such as Mn, Pb and Fe (see Figure 1.4).

At the mining outlets, water is enriched in metals having high solubility at pH near neutrality such as Zn, Cd, Ni and Co. This phenomenon is reflected by the chemical analysis of mining waters (from Victoria mine) carried out by Marques *et al*. [I.5]. In this study, the concentrations of Pb and Fe were found to be 5 $\mu g \cdot L^{-1}$ and 53 $\mu g \cdot L^{-1}$, respectively.

On the contrary, the contents of Cd, Co and Ni in the waters were several times higher than those determined for Pb and Fe, although its content in ore vein is substantially lower.

Once the mineral was extracted from the mines it was transported by mean of overhead cables and trucks to treatment factories where minerals were treated by flotation techniques in order to enrich lead and zinc. During the period of mining operations, the concentrates and mining wastes were accumulated at open air. Exploitation ended in the 1950's and ever since, mines and areas affected by mining operations have not been reclaimed. Therefore, the surroundings of the factories are at present still covered by remains of ore concentrates and wastes (Figures 1.5, Figure 1.6).

Nowadays, in order to provide some uses for the abandoned mining works, a project to habilitate the mines and treatment factories for tourist visits is carried out. For instance, some arrangements have been done in a few galleries in order to

improve safety conditions for tourists (Figure 1.7) or the abandoned railway has been arranged in some places (Figure 1.8) [I.6].

Figure-1.5.- View of Pontaut treatment factory

Figure-1.6.- Ore concentrates remains (Pontaut)

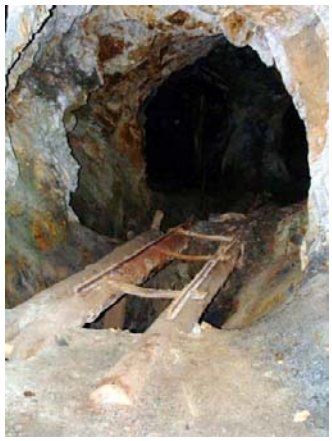

Figure-1.7.- Arrangement of a gallery to improve safety conditions for tourists.

Figure-1.8.- Rehabilitation of the abandoned mining railway

To asses the impact of mining activities, different kind of samples were collected in the Val d'Aran mining district. In order to study the metal mobility and bioavailability, mining wastes with different features were sampled from the treatment factory of Pontaut. Spontaneously occurring vegetation growing at different sites of the mining area was collected to study the potential bioaccumulation of metals. Different vegetation species were chosen for this purpose including some higher plants, grasses and mosses (see Figure 1.9). A more detailed description of the samples collected and the sampling sites will be presented in more details in the relating chapter.

Figure-1.9.- Vegetation species collected at the Val d'Aran mining district.
(a): *Betula pendula* [1.7]; (b): *Buddleia davidii* [1.8]; (c):*Bromus sp.* [1.9]; (d):*Quercus robur* [1.10]; (e): *Pinus sylvestris* [1.11]; (f): *Leucobryum sp.* [1.12]; (g): *Pleurocarpus sp.* [1.13].

1.1.2. Osor mining district

Osor is situated in the north-east of Spain, specifically in the Catalonian Region of "La Selva" (see Figure 1.10).

In the same way as in Val d'Aran mining district, the mineral economically valuable was extracted by deep mining methods. The ore vein of Osor mine was mainly composed of different sulphides (mostly PbS, ZnS) and also of large amounts of fluorite (CaF_2).

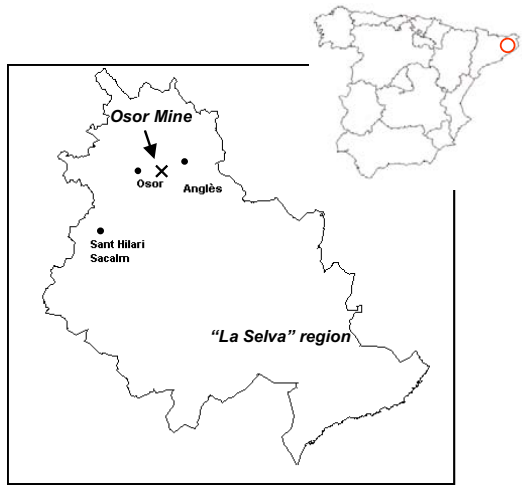

Figure-1.10.- Location of Osor mining district.

The first mining activities are dated in the beginning of the last century. During this period the main extracted minerals were galena and sphalerite which were transported from the mining shaft to the treatment factory by means of trucks. Once there, the minerals were milled and further beneficiated by flotation techniques. In the 1940's the extraction of Pb and Zn ended due to economic problems. A few years later, the mining district was re-opened but at that time the mineral of interest was fluorite. This mineral was exported to Europe and United States of America so as to get hydrofluoric acid for inorganic chemistry use. However, at the end of the 1980's, the extraction of fluorite became worthless and thus, the mining operations finally stopped [I.14].

Since the mine closure, the areas affected by mining activities have not been remediated and at present the impact of mining operations are still evident, as can be seen in Figure 1.11.

In the present study different kind of mining wastes were collected in the mineral treatment factory together with some *Buddleia davidii* specimens growing at the mining area. A more detailed description of sampling sites is given in Chapter 4.

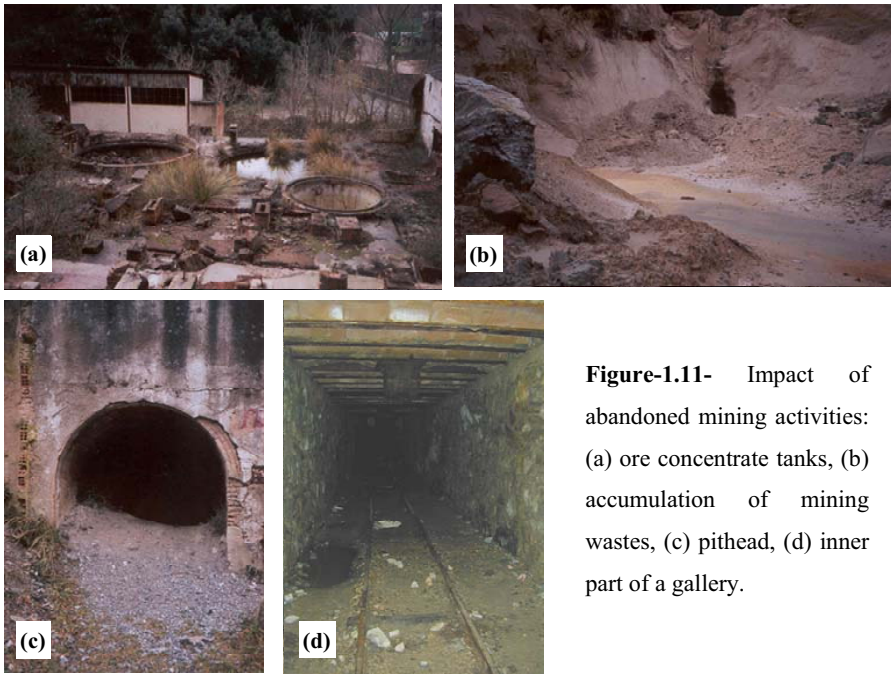

Figure-1.11- Impact of abandoned mining activities: (a) ore concentrate tanks, (b) accumulation of mining wastes, (c) pithead, (d) inner part of a gallery.

1.1.3. Sierra de Cartagena-La Unión mining district

The mining district of "Sierra de Cartagena-La Unión" is located in the south-east of Spain (see Figure 1.12) and is considered one of the most representative open cast mining areas (surface mining) of the country.

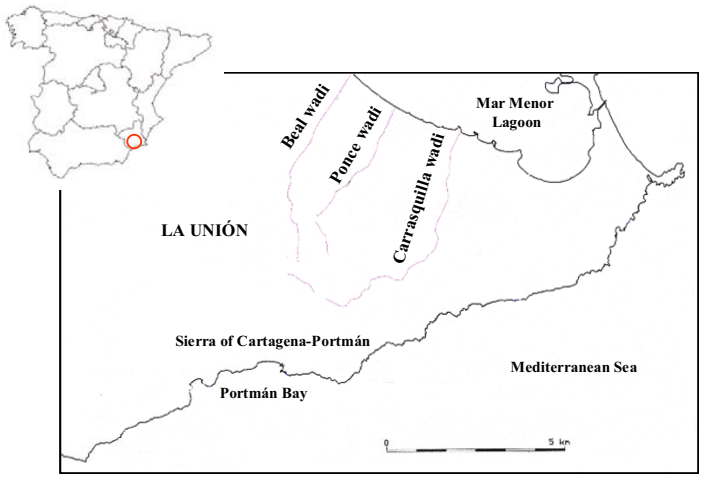

Figure-1.12.- Location of Sierra de Cartagena-La Unión mining district

Historically, the Sierra of Cartagena-Portmán was exploited to extract pyrite, galena and sphalerite (Fe-Pb-Zn). During the 20th century, the mineral laundries used floating techniques to extract metal, producing large quantities of mining wastes [I.15]. These tailings were discharged into the Portmán Bay (Mediterranean Sea) and the adjacent Mar Menor coastal lagoon, producing a high degree of sediment metal contamination. Until the 1950's wastes from the mining activities were discharged into the coastal lagoon through the temporary streams that exist in the area. Even now, when torrential rains occur, the remaining tailings continue to enter the lagoon (see Figure1.12). Since 1958 and up to the closure of the mineral extraction (1991) the mining operations pumped 3000-10000 tons of tailings per day into Portmán Bay [I.16]. Consequently, the bay is completely filled up with tailings remains, as can be seen in Figure 1.13.

Figure-1.13.- View of the Portmán Bay (Sierra of Cartagena-Portmán).

The mining area comprises a total extension of about 100 Km^2 and because of its extensive mining history it has been the target of most current environmental and geological studies [I.17, I.18]. Nowadays, 89 ponds (containing the fine wastes from the cleaning and concentration of minerals) and 358 slag heaps (formed by rocky material from the mineral cover) have been identified with a total amount of 174000000 m^3 of wastes, which have been classified in eight categories according to the mineral treatment process [I.17] (see Figure 1.14).This large volume of tailings has become a serious unsolved environmental problem. A small part of the tailings has been used in the construction of highways and sporting harbours although no remediation processes or environmental regeneration methods have been applied for the time being.

In 2004 the "Consejería de Turismo y Ordenación del Territorio de la Región de Murcia" issued a document to encourage the development of tourism in the Sierra

Minera de La Unión y Cartagena. The aim of this project was to present to the population the costs and benefits of developing the tourism activities in the abandoned mining area [I.19].

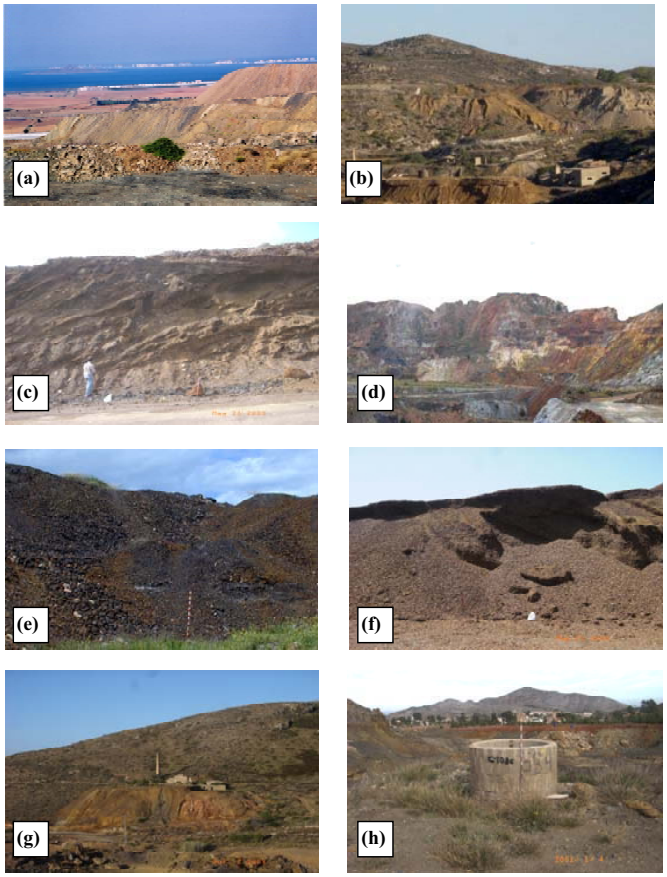

Figure-1.14.-Views of catalogued mining wastes: (a)open-pit spoils; (b)post-flotation wastes; (c)gravity concentration spoils; (d)"false gossan"; (e)molten slag; (f) pre-concentration wastes; (g)mine spoils; (h) well borings [I.17].

In the present research different types of mining wastes as well as two vegetation species growing in the areas affected by mining operations (Figure 1.15) were collected. A more detailed description of samples will be provided in the related chapter.

Figure-1.15.- Vegetation species collected at the "Sierra de Cartagena-La unión" mining district: (a) *Citrus limonium* [I.20]; (b) *Salicornia sp*. [I.21].

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CHAPTER 2

Evaluation of metal mobility in mining wastes

The combined use of sequential extraction procedures (BCR scheme "Community Bureau of Reference") and leaching tests (TCLP "US-EPA" and DIN 38414-S4) provides an interesting basis to evaluate potential mobility and hazardousness of metals from dumped mining wastes.

While sequential extraction procedures give information about the fractionation of metals in the different lattices of the waste samples, which is advantageous for assessing the degree of metal mobility, leaching tests are a complementary tool for characterising and classifying waste samples in terms of environmental hazard.

These methodologies were applied to four types of mining waste samples with different features. The information obtained served as a good starting point for further evaluation of bioavailability studies.

The contents of this chapter are published in:

E.Marguí, V.Salvadó, I.Queralt, M.Hidalgo, *Comparison of three-stage sequential extraction and toxicity characteristic leaching tests to evaluate metal mobility in ming wastes.* Anal. Chim. Acta 524 (2004) 151-159.

2.1. INTRODUCTION

As pointed out in the introduction section, measurement of total metal concentrations in mining waste samples is useful to evaluate the heavy metal burden but their mobility depends strongly on their specific chemical forms or ways of binding. However, the determination of specific chemical species or binding forms is difficult and often hardly possible. For this reason, sequential extraction procedures are commonly applied because they provide information about the fractionation of metals into the different lattices of the solid sample which is a good compromise to give information on environmental contamination risk [2.1, 2.2, 2.3,2.4].

The data obtained from different sequential extraction schemes are difficult to compare, since the results depend on the extraction procedure used. With the aim to harmonise different extraction schemes, the Community Bureau of Reference (BCR, now Standards, Measurements and Testing Programme) proposed a three-step extraction procedure which was based on acetic acid extraction (Step-1: exchangeable and acid soluble fraction), hydroxilamine hydrochloride extraction (Step-2: reducible fraction) and hydrogen peroxide / ammonium acetate extraction (Step-3: oxidisable fraction). However, due to the lack of reproducibility of this extraction scheme it was necessary the improvement of some operational conditions of the original procedure, such as the pH and concentration of the hydroxilamine hydrochloride solution and the increase of centrifugation speed [2.5].

The modified BCR sequential extraction has been successfully applied to a variety of matrices, including sediments with distinct origins, sewage sludge-amended soils and different industrially contaminated soils [2.6, 2.7]. The aim of the present work is to study the applicability of the modified BCR scheme to mining wastes so as to evaluate the mobility of Zn, Pb, Cd and Ni and their possible environmental implication. In order to improve its applicability in the laboratory some modifications of the original BCR scheme were carried out and they were properly evaluated by analysing a suitable reference material, BCR-701.

On the other hand, in order to gain additional information about more easily mobilised metal forms in the mining wastes and thus the potential environmental implications, two leaching tests were applied. The first one (TCLP: Toxicity Characteristic Leaching Procedure) was developed by the United States Environmental Protection Agency (US-EPA) to determine if a waste had the characteristic of toxicity and is therefore hazardous [2.8]. The other test, which is

based on a German Standard Method (DIN 38414-S4), was developed to assess leaching of sludge and sediments form water and wastewater treatment [2.9] and it is used by the Waste Council of Catalonia as acceptation criteria for waste disposal in a municipal landfill.

Moreover, the information obtained with the different extraction procedures applied to the study of metal mobility in mining wastes has been discussed.

2.2. EXPERIMENTAL

2.2.1. Samples studied

Sampling of the mining wastes was performed at three sites in the Aran Valley and one in Cartagena (descriptions of the mining areas is provided in Chapter 1). From the first mining district three samples were collected, one located at Bagergue (mining dump) and the others in Pontaut. From there, two sampling sites were chosen, an old landfill with the remains of the Pb-Zn concentrates (sample called Pontaut) and another place where an old foundry was located (sample called Pontaut-Fe). In Cartagena, a sample was taken from a pond (fine wastes accumulation) situated in "La Unión" mining district.

Samples were collected using a polypropylene shovel, and subsequently transferred to clean polypropylene bags. In the laboratory, the wastes were oven-dried (50ºC), sieved (<500 μm to 63 μm and <63 μm) and stored in polypropylene containers until analysis.

2.2.2. Apparatus and reagents

A Sequential Inductively Coupled Plasma Emission Spectrometer (ICP-AES) (Liberty RL, Varian) with V-groove nebuliser was used for the analysis of zinc, lead, cadmium and nickel in digests and extracts. The wavelengths selected were: Zn (202.551 nm), Pb (220.553 nm), Cd (214.438 nm) and Ni (352.454 nm).

Metal concentrations below the detection limit of ICP-AES were determined by an Atomic Absorption Spectrometer (SpectrAA-300) coupled with a graphite tube atomizer (GTA-96). Samples were injected into GF system by an auto-sampler. Pyrolytic graphite coated tubes and wall atomization were used in all cases. Instrumental parameters are summarised in Table 2.1.

	Pb	Cd	Ni
Wavelenght (nm)	283.3	228.8	232.0
Lamp intensity (mA)	10.0	4.0	4.0
Slit-width (nm)	0.5	0.5	0.2
Type of signal	Peak Area	Peak Area	Peak Area
Temperature Programme [a]			
Drying	120 / 45 / 10	120 / 45 / 10	120 / 45 / 10
Pyrolysis	500 / 5 / 3.2	350 / 5 / 5	800 / 5 / 3.2
Atomisation	2100 / 1 / 2.0	1800 / 0.8 / 2.0	2400 / 1.1 / 2.0
Cleaning	2400 / 2 / 2.0	2000 / 2.0 / 2.0	2500 / 2.0 / 2.0

[a] Temperature (º C) / Ramp time (s) / Hold time (s)

Table-2.1.- Measurement conditions for ETAAS

A CEM MDS-81D (Gomensoro, S.A) closed-vessel microwave digestion system (630W full power) with pressure control and teflon PFA reactors was used for acid digestion of samples. A Mixtasel P-Selecta centrifuge was employed for the separation of the extracts. A rotatory shaker was used so as to keep samples in suspension during extraction. A Crison GLP22 pHmeter was used for pH measurement and adjustment when required.

All reagents used were analytical grade o suprapur quality: acetic acid, hydrofluoric acid and hydrogen peroxide (Trace Select, Fluka, Gillingham-Dorset); amonium acetate (Extrapure, Riedel-deHaën) and nitric acid (Suprapur, Merck, Germany). Standard solutions were prepared by serial dilution of 1000 $\mu g \cdot L^{-1}$ Spectroscan solutions of the appropriate element. Ultrapure water obtained form a Milli-Q purifier system was used throughout the work.

2.2.3. Extraction procedures

2.2.3.1. Sequential extraction

The optimised BCR three-step sequential extraction procedure was slightly modified. For practical reasons the weight of sample and volume of extractant were reduced by half, so that the weight per volume ratio was the same as in the original BCR scheme. Details of the experimental protocol are available in Figure 2.1.

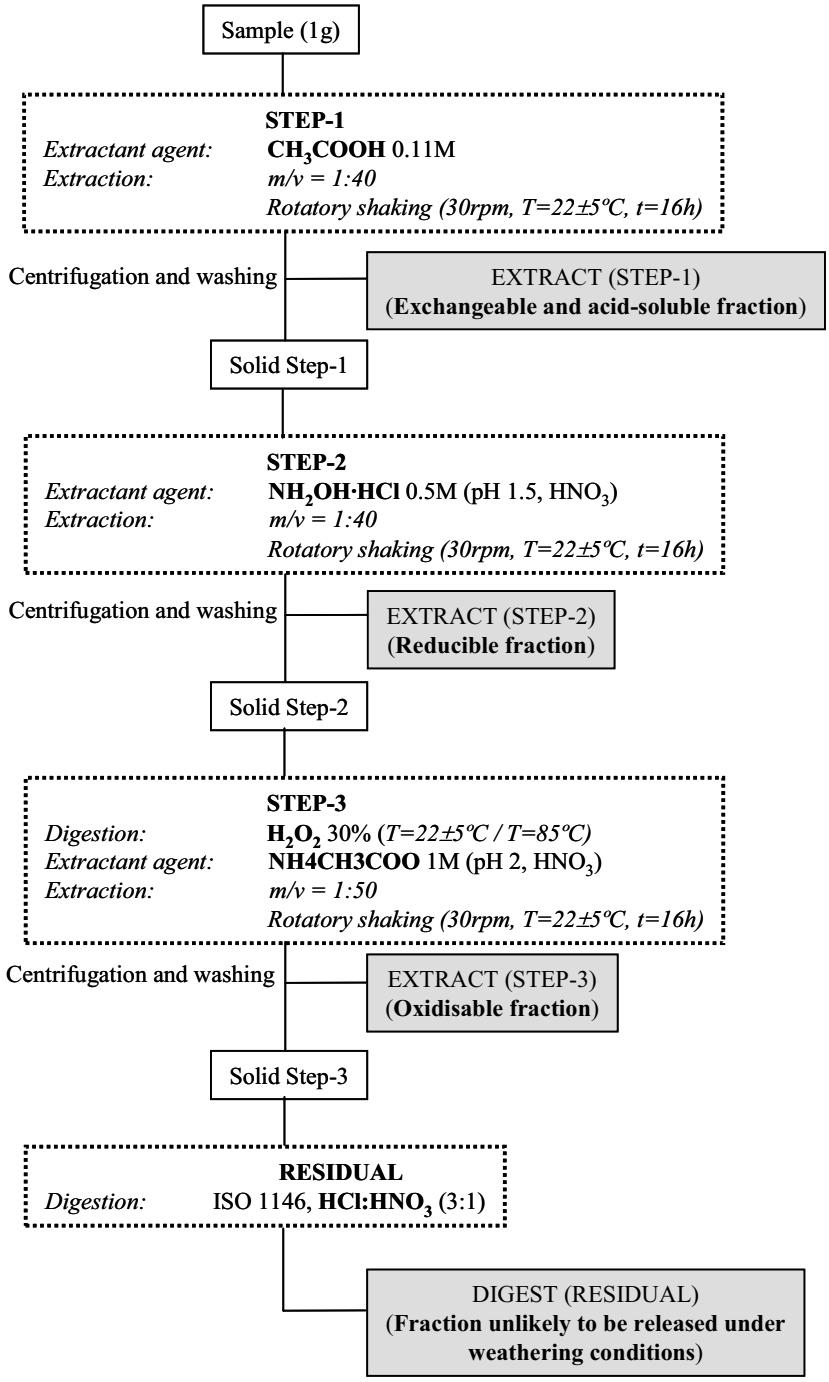

Figure-2.1.- Scheme of the optimised BCR three-step sequential extraction procedure [2.5].

For an internal check on the procedure, the residue from Step 3 was digested in aqua regia (Residual Step) and element concentration from the three steps plus residual was compared with concentration after *pseudototal* content (aqua regia) of

the original sample. Instead of following the ISO 11466 protocol used in the original BCR scheme, in this work a microwave-assisted digestion procedure was applied so as to achieve a shorter digestion time. About 500 mg of sample was weighted and placed in a PTFE reactor. Four millilitre of 65% HNO_3 Suprapur and 12 ml of 37% HCl were added. The reactor was sealed and let contact for five hours. Then it was heated following a three-stage digestion programme: Stage-1 (250W, 2min); Stage-2 (400W, 2min); Stage-3 (500W, 8min). After cooling, sample digests were filtered with a Whatman 42 filter paper, transferred into a 25 ml flask and brought to volume with MilliQ water.

The sequential extraction procedure, once the modifications were done, was evaluated by using a certified reference material, BCR-701 [2.10] which certifies the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn and gives indicative values for the aqua regia extractable contents of the six elements in the sediment and the residue obtained after sequential extraction.

Blank extractions (without sample) were carried through the complete procedure for each set of analysis. For corrections to dry mass, a separate 1g sample was dried in an oven at 105 ± 5 º C for 2 h or until constant mass was achieved (successive weightings differed by less than 1 mg).

2.2.3.2. Leaching tests

- Toxicity Characteristic Leaching Procedure (TCLP)

TCLP test was performed as specified in the EPA method [2.8]. First, if the sample particle size was more than 10mm a size reduction of the sample was carried out. Then, it was extracted for 18 h with an amount of extraction fluid equal to 20 times the weight of the solid phase. In the present work, for practical reasons, the weight of sample and extractant was reduced ten times respect the original procedure. The extraction fluid employed was chosen on the basis of waste alkalinity (Fluid-1: CH_3COOH pH=4.93 ± 0.05 for sample pH<5 and Fluid-2: CH_3COOH pH=2.88 ± 0.05 for sample pH>5). Following the extraction, the liquid extract was separated form the solid phase by filtration through a 0.8 μm glass fibre filter. The extract obtained was then analysed to determine if the metal concentrations (Zn, Pb and Cd) would exceed the thresholds established by the EPA (100 times National Interim Primary Drinking Water Standards for metals, NIPDWS).

Quality assurance of the leaching procedure was achieved using US-EPA QA/QC protocols which include the analysis of a method blank for each batch of samples run and a matrix spike for each waste type (after filtration of the TCLP extract and before preservation) so as to determine whether matrix interference exist.

- DIN 38414-S4.

This method provides information about the behaviour of a solid waste when it comes into contact with water. So then, in this leaching test, Milli-Q water is used as an extractant. Details of the experimental protocol were followed from the German Standard Method [2.9]. First of all, if the sample particle size was more than 10 mm a size reduction of the sample was carried out. Then, leaching at room temperature for a period of 24 h was achieved by using a ratio 1:10 (solid:liquid). In this study we used a smaller sample weight due to practical reasons like in the TCLP test. After the leaching period had elapsed, the undissolved residue was separated by filtration using a membrane filter of pore size 0.45μm, pre-washed with water. Finally, pH value, electrical conductivity and concentrations of Zn, Pb and Cd from the extract were analysed.

Since the materials, when they are dumped, repeatedly come into contact with fresh rain -water or ground water, the leaching procedure was repeated three times so as to better simulate real conditions.

2.3. RESULTS AND DISCUSSION

The portion of the total metal content in a solid sample (mining waste) that can be liberated to the environment depends mostly on the characteristics of the sample itself. Therefore, determination of pH, water content and organic matter of mining wastes sieved through two particle size (<500μm to 63μm and <63μm) was carried out. pH values were determined from the original sample using a ratio 1:2.5 (waste: Milli-Q water) and organic matter content was obtained by titration (with Fe^{2+}) of the $K_2Cr_2O_7$ that had not been consumed for the oxidation of the organic compounds of the sample in strongly acidic conditions [2.11].

As it can be seen from Table 2.2, pH values of wastes were different depending on their origin. Samples from Aran Valley (Pontaut, Pontaut-Fe and Bagergue) presented a slightly acidic pH (4<pH<6) while the waste collected in Cartagena had a basic pH (7.83). In all cases, the <63μm fraction had the most significant association

with organics, specially in Pontaut-Fe and Bagergue samples, which show the highest percentages of organic matter. The values obtained for water content were used for correction to dry mass in the following extraction procedures.

	Pontaut (pH=4.05)		**Pontaut-Fe** (pH=5.89)		**Bagergue** (pH=6.18)		**Cartagena** (pH=7.83)	
	<500μm	**<63μm**	**<500μm**	**<63μm**	**<500μm**	**<63μm**	**<500μm**	**<63μm**
W (%)	1.35	1.1	2.2	2.9	6.2	4.3	5.12	4.82
OM (%)	0.9	2.68	7.2	12.5	3.44	5.2	0.75	1.51

W: water content, OM: Organic matter content. All results are referred to n=2, except organic matter content (n=3).

Table-2.2.- pH values, water content and organic matter content of the mining wastes studied.

2.3.1. Sequential extraction

2.3.1.1. Analytical performance

In order to determine the matrix effects of the different extractant reagents used in the BCR scheme, a comparison between standard solution prepared in 2% v/v HNO_3 with those obtained from solutions prepared in each of the extractants was performed. Standards (with a metal concentration within the calibration range) prepared with the suitable extractant agent (CH_3COOH, $NH_2OH{\cdot}HCl$, CH_3COONH_4 and aqua regia) were analysed several times by using a direct calibration with standard solutions in 2% HNO_3 and with a reagent-matched standard calibrate. Then, two statistics tests (F-test and t-test) were applied to evaluate if there were significant differences between standard deviation and averages of the two procedures described. The obtained results are summarised in Table 2.3. As may be noted from this table, in most cases, there are not significant differences between standard deviation using the two procedures (F-test). Nevertheless, taking into account the t-critical values, a significant difference can be observed between the average concentrations obtained with and without reagent-matched standards. Analytical data reveals the presence of a clear matrix effect of ammonium acetate (Step-3) in metal determinations by ICP-AES. Whereas in metal analysis by ETAAS matrix effects depend on the metal studied rather than the extractant used.

Quantisation of metals in extracts was not therefore possible by direct calibration with standard solutions in 2% HNO_3 and reagent matched standards were used for metal analysis in each step of BCR sequential extraction procedure.

	Zn [a]		Pb				Cd				Ni			
	ICP-AES		ICP-AES		ETAAS		ICP-AES		ETAAS		ICP-AES		ETAAS	
	F	t	F	t	F	t	F	t	F	t	F	t	F	t
CH_3COOH	1.19	8.27	1.08	5.76	1.53	1.24	1.14	12.10	4.00	19.5	1.20	27.49	1.00	5.61
$NH_2OH \cdot HCl$	1.11	3.98	1.04	1.93	2.00	20.03	1.09	4.12	9.00	*	1.02	1.09	1.44	6.44
CH_3COONH_4	1.50	35.88	1.33	28.56	1.23	10.04	1.42	51.34	4.00	10.65	1.42	52.56	1.96	7.67
Aqua Regia	1.17	11.28	1.35	14.24	1.36	10.65	1.14	11.25	1.00	2.01	1.09	8.43	2.01	9.03
Critical Values [b]	3.79	2.14	3.79	2.14	4.28	2.18	3.79	2.14	4.28	2.18	3.79	2.14	4.28	2.18

[a] Zn concentrations were analysed in all cases by ICP-AES.
[b] ICP-AES: F, t (one-tailed test, n =8, 95% confidence level); ETAAS: F, t (one-tailed test, n =7, 95% confidence level).

Table-2.3.- Matrix effects of extractant agents (BCR sequential extraction procedure) in Zn, Pb, Cd and Ni determination by ICP-AES and ETAAS.

As described in the experimental section, the analytical performance of the laboratory and the analytical variability were evaluated by applying the three-stage procedure on BCR-701. The results obtained are given in Table 2.4. In order to evaluate these data, a study of precision and accuracy was performed.

	Zn		Pb		Cd		Ni	
	Determined [a]	**Certified** [b]	**Determined**	**Certified**	**Determined**	**Certified**	**Determined**	**Certified**
Step-1	170 ± 2	*205 ± 6*	3.1 ± 0.1	*3.18 ± 0.2*	8.0 ± 0.4	*7.34 ± 0.35*	18.0 ± 0.8	*15.4 ± 0.9*
Step-2	111 ± 5	*114 ± 5*	116 ± 5	*126 ± 3*	2.8 ± 0.2	*3.77 ± 0.28*	27 ± 2	*26.6 ± 1.3*
Step-3	40.5 ± 0.7	*45.7 ± 4*	7.9 ± 0.7	*9.3 ± 2*	0.28± 0.03	*0.27± 0.06*	16.3 ± 0.6	*15.3 ± 0.9*
Residual	106 ± 2	*95 ± 13*	12.6 ± 0.6	*11 ± 5.2*	0.15 ± 0.01	*0.13± 0.08*	45 ± 3	*41.4 ± 4*
∑Steps + R	428 ± 6		140 ± 5		11.3 ± 0.4		106 ± 5	
Pseudototal	446 ± 4	*454 ± 19*	129 ± 2	*143 ± 6*	10.9 ± 0.1	*11.7 ± 1.0*	100 ± 3	*103 ± 4*
Relative error (%)	4.0		8.5		3.7		6.0	

[a] Results are expressed as the mean of four parallel sample determinations ± standard deviation.
[b] Certified (Step-1+ Step-2 + Step-3) and indicative values (residual and pseudototal)

Table-2.4.- Determined, certified and indicative values (mg kg-1) for BCR-701 and comparison between aqua regia results (*pseudototal*) and the values from three steps plus residual.

The analytical precision is the short term variability of the measurement and it was calculated as: (standard deviation / mean) x100. Fraction-specific experimental precisions were very satisfactory with values for all elements <12%, and most <8% (Figure 2.2).

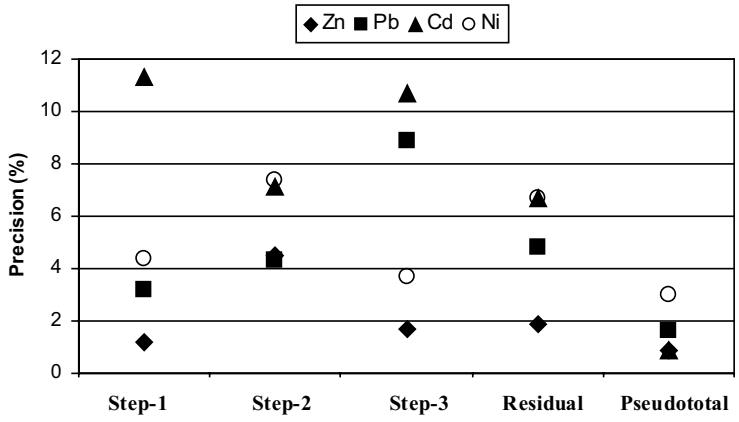

Figure-2.2.- Fraction-specific precision for the optimised BCR sequential extraction procedure for BCR-701.

Accuracy corresponds to the closeness of the agreement between the measured concentration and the agreed upon value and it was computed as: [(measured concentration – indicative concentration) / indicative concentration] x100. Fraction-specific accuracy determined, in general, was acceptable with values typically ±15% of certified or indicative values (Figure 2.3). In this study, the Cd concentration in Step-2 of BCR-701 (3.77 ± 0.28 mg·Kg^{-1}) was the most different from the certified values (2.8 ± 0.2 mg·Kg^{-1}).

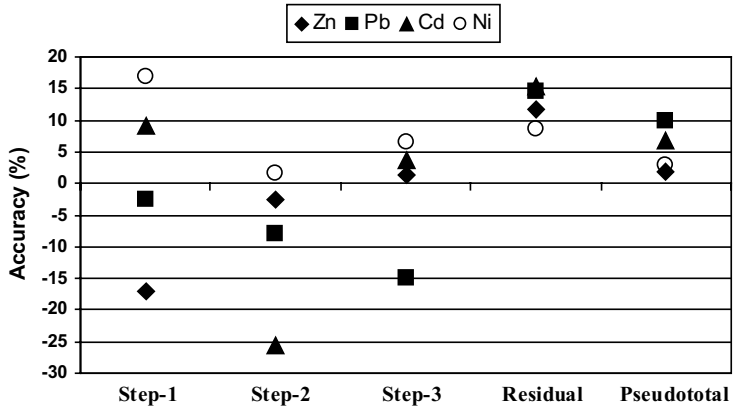

Figure-2.3.- Fraction-specific accuracy for the optimised BCR sequential extraction procedure for BCR-701.

In addition, a comparison between aqua regia results form the original sample (*pseudototal*) and the values from the three steps plus residual (Σ Steps + aqua regia extractable from residual) was carried out (Table 2.4). As it can be seen, no

significant differences were observed between the pseudototal metal content following the aqua regia digestion and the sum of extracted metals following the sequential extraction procedure. Low relative errors (<10%) indicate the good quality of the results obtained.

2.3.1.2. Application of BCR scheme to mining wastes

Duplicate mining waste samples (both <500μm to <63μm and <63μm) were subjected to the BCR protocol and a microwave digestion with aqua regia was also applied to the residue from Step-3 (Residual) and original sample (Pseudototal). In almost two thirds of the cases, the results for equivalent extracts were within 6% each, and discrepancies greater than 11% were not found. Comparison between the sum of the extracted metals form the three steps plus residual and the pseudototal content of the original samples was acceptable for each metal and waste in almost all cases (Table 2.5).

A preliminary study, showed that Ni pseudototal concentration in the sample wastes was much lower than others metals and also there was a very strong association (more than 80%) with the residual phase. For this reason, the study of the fractionation of this metal in the mining wastes was not finally carried out.

As can be seen in Table 2.5, in most samples, the <63μm size fraction contains a higher metal concentration than the <500μm particle size. Notable differences were observed for metal burden in the different mining wastes due to the different nature of the materials. The waste with the highest lead content was Pontaut (up to 20.000 $mg \cdot Kg^{-1}$) whereas in the case of zinc and cadmium higher values were found in Bagergue and Cartagena wastes, specially in the first one (Zn up to 145.000 $mg \cdot Kg^{-1}$ and Cd up to 250 $mg \cdot Kg^{-1}$).

	Zn		Pb		Cd	
	<500µm	**<63µm**	**<500µm**	**<63µm**	**<500µm**	**<63µm**
Pontaut						
Σ Steps + Residual	3240 ± 50	4060 ± 50	21000 ± 300	23000 ± 1000	4.2 ± 0.2	2.12 ± 0.04
PseudoTotal	3700 ± 300	3800 ± 90	20000 ± 1000	23200 ± 600	3.9 ± 0.2	2.3 ± 0.1
Pontaut-Fe						
Σ Steps + Residual	7600 ± 100	9720 ± 70	6000 ± 100	9170 ± 90	28.7 ± 0.3	33 ± 1
PseudoTotal	8600 ± 50	10900 ± 200	5500 ± 90	8300 ± 300	22.3 ± 0.3	28 ± 2
Bagergue						
Σ Steps + Residual	148000 ± 1000	175000 ± 2000	470 ± 10	880 ± 40	320 ± 2	410 ± 10
PseudoTotal	145000 ± 7000	183000 ± 3000	480 ± 40	750 ± 30	260 ± 20	350 ± 20
Cartagena						
Σ Steps + Residual	11900 ± 100	12700 ± 200	7500 ± 100	9600 ± 100	33 ± 1	36.9 ± 0.2
PseudoTotal	13000 ± 100	14000 ± 100	7500 ± 30	10000 ± 200	31 ± 2	37.0 ± 0.4

Table-2.5.- Comparison between the sum of the extracted metals from the tree steps (BCR scheme) plus residual and the pseudototal content in mining wastes (results are given as mean ± SD in mg kg^{-1}).

The results of the fractionation study (Figure 2.4) indicate that, in general, the behaviour of metal distribution between the two size fractions it is similar, except for metal content extracted in Step 3 that increase with particle size reduction. Particularly in Pontaut-Fe and Bagergue samples, because of their greater organic matter content.

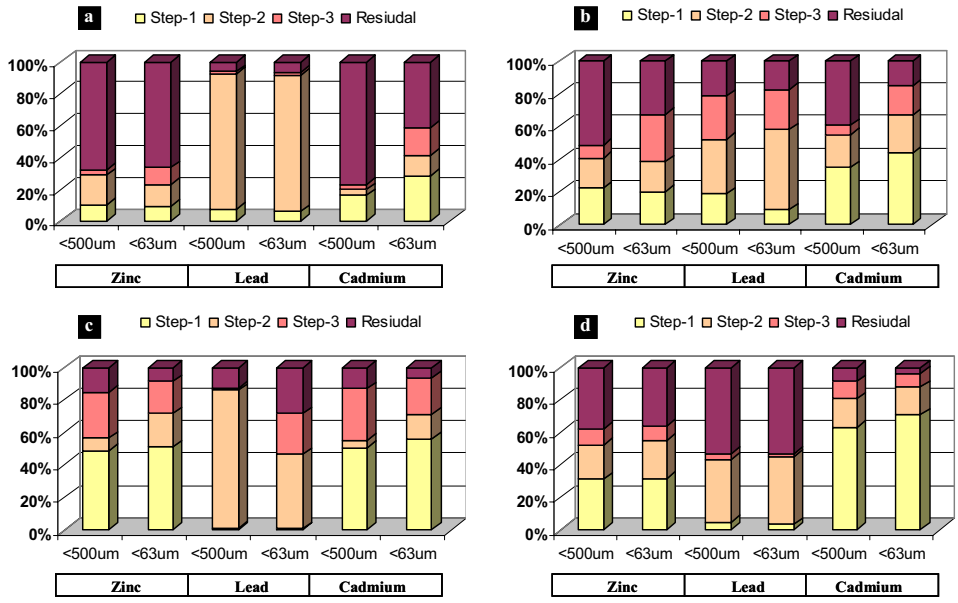

Figure-2.4.- Fraction-specific percentages (Zn, Pb and Cd) for the optimised BCR sequential extraction procedure for the mining wastes: (a) Pontaut; (b) Pontaut-Fe; (c) Bagergue and (d) Cartagena.

Among the metals studied the highest values of exchangeable fraction (Step-1) were observed for Zn and Cd, especially for Bagergue and Cartagena wastes where metal percentage in both cases were higher than 70% for cadmium and 25% for zinc, respectively. Taking into consideration the high mobility and potential bioavailability of heavy metals in this fraction and their *pseudototal* concentration (Table 2.5) can be concluded that these wastes could have potentially hazardous effects on environment. On the other hand, only a small fraction (<0.2%) of lead was present as acid exchangeable species in all cases and instead, a predominance of reducible lead (Step-2) had been found with values from 36 to 90% of pseudototal content. As for the distribution of metal concentrations in the residual phase it depends on the metal content released in the previous steps.

So as to study metal retention of metals in each waste, individual contamination factors (C_f) were calculated. These factors are defined as the sum of heavy metal concentration in the mobile phases (non-residual phases) of the sample divided by the residual phase content. Then, the lower C_f value the higher the relative metal retention [2.12]. The obtained results (Table 2.6) showed that the behaviour of relative metal retention was not the same for all the samples. Cadmium presented high values of individual contamination factors in Bagergue and Cartagena wastes

which meant a large mobility of this metal. In a general way, lead was quite retained in the wastes, except in the case of Pontaut sample, which presented (for the two size fractions studied), elevated C_f values (up to 14). With regard to zinc, the highest mobility was found in Bagergue waste, particularly, for the <63 μm fraction.

	Zn		Pb		Cd	
	<500μm	**<63μm**	**<500μm**	**<63μm**	**<500μm**	**<63μm**
Pontaut	0.5	0.5	15.4	14.6	0.1	1.3
Ponaut-Fe	1.0	2.1	3.7	4.8	0.3	5.6
Bagergue	5.4	10.5	6.9	2.5	6.4	14.6
Cartagena	1.6	1.8	0.9	0.9	10.5	25.4

Table-2.6.- Individual contamination factors (C_f) values for the mining wastes

From the data of the partitioning study and pseudototal metal content can be deduced that wastes from Bagergue and Cartagena presented a similar behaviour to each other and quite different from samples collected in the mill situated in Pontaut. This fact can be associated with different mineralogical origin and also due to the ore processing carried out in each case.

2.3.2. Leaching tests

2.3.2.1. Toxicity Characteristic Leaching Procedure (TCLP)

As described in the experimental section, the extractant employed in this leaching test depends on waste alkalinity. In this study, two fluids have been used. Fluid-1: CH_3COOH pH = 4.93 ± 0.05 and Fluid-2: CH_3COOH pH = 2.88 ± 0.05. The first one was employed in Pontaut and Pontaut-Fe samples because their pH was lower than 5, and Fluid-2 was used to test the samples from Bagergue and Cartagena (pH>5). In all cases, metal contents were determined by ICP-AES using reagent-matched standard calibrates with the appropriate extractant fluid. As for the quality control, metals were not detected above the detection limit for any of the method blanks done. Furthermore, the recoveries obtained from matrix spikes of each waste sample varied from 85 to 107%, which are acceptable according to EPA.

The results of TCLP extraction applied to the mining wastes are given in Table 2.7.

	Zn	Pb	Cd
Pontaut	10.2 ± 0.6	36 ± 1	<0.09
Pontaut-Fe	31 ± 2	13.5 ± 0.3	0.144 ± 0.009
Bagergue	2344 ± 37	<0.50	4.7 ± 0.2
Cartagena	127 ± 3	2.6 ± 0.3	0.79 ± 0.02
NIPDWS	5 [a]	0.015	0.005
Regulatory Level	500	1.5	0.5

[a] Zinc is regulated under a secondary water quality standard

Table-2.7.- TCLP extracts from mining wastes (expressed as the mean of two replicates ± S.D., mg l^{-1}) with the thresholds established by the US-EPA.

Data obtained reveal that extracts exceeded in all cases the criteria of 100 times the NIPDWS (National Interim Primary Drinking Water Standards for metals) [2.13] for at least one of the metals. Taking into account that fact, all the mining wastes studied could be classified as hazardous according to EPA criteria.

2.3.2.2. DIN 38414-S4 (German Standard Method)

The extraction procedure with Milli-Q water based on the German Standard Method DIN 38414-S4 was repeated three times so as to better simulates real conditions. In each extract, pH and conductivity were measured and also metal concentrations were determined by ICP-AES. The results of leachability with water are shown in Table 2.8.

In a general way, conductivity and metal concentration decrease from the first to the third extraction, whereas pH values increase. Pontaut and Bagergue wastes present metal concentrations higher than the regulatory levels, it should be noted that Zn concentration in Bagergue extracts was 60 times higher than the permissible value. On the other hand, for the mining waste from Cartagena, which presents a basic pH value, concentrations of all the metals analysed were bellow the detection limits. Therefore, it can be concluded that metal leachability by water strongly depends on the waste pH.

		pH	Conductivity (mS cm^{-1})	Zn (mg l^{-1})	Pb (mg l^{-1})	Cd (mg l^{-1})
Pontaut	1st	3.98 ± 0.01	159 ± 3	15.6 ± 0.8	11.02 ± 0.06	<0.05
	2nd	3.93 ± 0.01	110.6 ± 0.8	7.4 ± 0.2	12 ± 2	<0.05
	3rd	4.115 ± 0.007	87.3 ± 0.7	3.2 ± 0.1	17.3 ± 0.4	<0.007
Pontaut-Fe	1st	6.71 ± 0.01	45 ± 7	2.0 ± 0.4	<0.2	<0.007
	2nd	6.51 ± 0.07	24.9 ± 0.6	0.89 ± 0.07	<0.2	<0.007
	3rd	6.86 ± 0.02	19.2 ± 0.4	0.77 ± 0.06	<0.2	<0.007
Bagergue	1st	6.76 ± 0.08	2375 ± 7	307 ± 4	<0.408	2.79 ± 0.05
	2nd	7.11 ± 0.03	2135 ± 7	67 ± 5	<0.408	0.59 ± 0.03
	3rd	7.20 ± 0.01	2049 ± 58	40.2 ± 0.6	<0.408	0.34 ± 0.03
Cartagena	1st	8.2 ± 0.1	4255 ± 7	<0.4	<0.02	<0.007
	2nd	8.30 ± 0.05	2310 ± 0	<0.4	<0.02	<0.007
	3rd	8.33 ± 0.01	2110 ± 0	<0.4	<0.02	<0.007
Regulatory Level [a]		4 - 13	$50x10^3$	5	1	0.2

[a] Regulatory levels established by the Waste Council of Catalonia as acceptation criteria for waste disposal in a municipal landfill.

Table-2.8.- Leachability by water (DIN 38414 - S4) for the mining wastes (expressed as mean of two replicates ± SD, $mg \cdot L^{-1}$).

2.3.3. Usefulness of the studied extraction procedures to assess the metal mobility.

To evaluate the usefulness of the studied extraction procedures to assess metal mobility, a comparison study was carried out between the information provided by the three-stage sequential extraction scheme and that assessed by the leaching tests.

Sequential extraction procedures provides useful information for risk assessment since the amounts of metals mobilisable under different change in environmental conditions can be estimated (weak acidic conditions: Step-1, reducible conditions: Step-2 and oxidisable conditions: Step-3). Mobility and bioavailability of the metals decrease approximately in the order of the extraction sequence. Hence the exchangeable fraction (Step-1:CH_3COOH 0.11M) may indicate which metals are more mobile and therefore most available for plant uptake and other releases into environment. On the other hand, the use of leaching tests such as TCLP or DIN 38414-S4 results to evaluate the potential environmental risks associated with pollutants lixiviation under a weak natural organic acid or water.

Each of these mentioned extraction procedures address a specific aspect of the integral leaching behaviour of a material. Consequently, each of the tests gives a part of the answer needed to obtain an evaluation of the material properties relevant for an assessment of material behaviour under field conditions. However, there is a need

for understanding the differences and similarities between tests methods and to decide on what type of information should be derived from each test [2.14].

Figure 2.5 includes, for comparative purposes, the data from the three extraction procedures that have been applied in this study: the "mobilisable" fraction of three-stage sequential extraction as estimated by the amount of metal which was acetic acid extractable, the TCLP leaching fraction as acetic acid extraction at different pH chosen on the basis of waste alkalinity and DIN 38414-S4 leaching fraction estimated by water lixiviation.

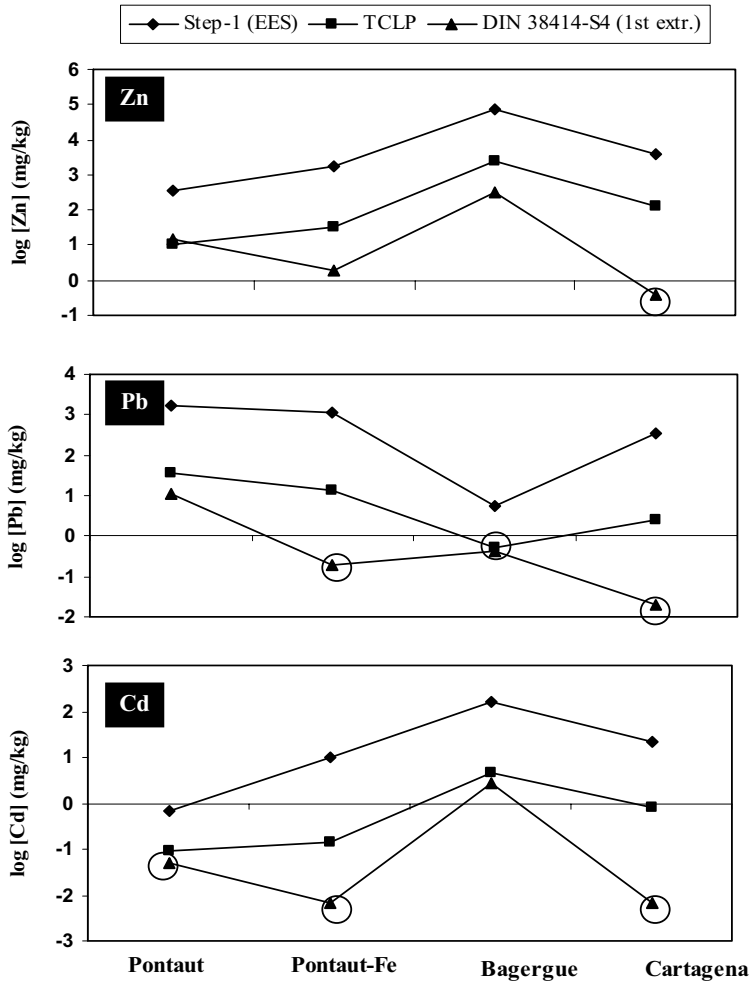

Figure-2.5.- Comparison of more mobile metal content obtained in Step-1 BCR scheme (Step-1 (EES)), TCLP and DIN 38414-S4 (1st extr.) leaching tests. Circled points represent values below the detection limits (see Table 2.7 and Table 2.8).

Note: Step-1 BCR scheme results correspond to <500μm to 63μm sample particle size.

As it can be seen, TCLP results are in agreement with those obtained with Step-1 of BCR scheme for all the metals studied (Zn, Pb and Cd) in the four mining wastes. However, TCLP metal concentrations in all samples are lower than those obtained by Step-1 BCR due to the more reduced solid:liquid ratio and acid acetic solution pH used during the extraction.

The lixiviation with water (DIN 38414-S4) obviously release much lower amounts of metals compared with the other leaching tests applied as a result of sample pH that mainly controls the amount of metal released into solution. In a general way, an increase in waste pH provides a decrease in metal leachability as it is clearly shown in Cartagena mining tailing where due to its basic pH (7.83) all metal concentrations in DIN extracts are below the detection limits ($Zn<0.4$ $mg \cdot Kg^{-1}$, $Pb<0.02$ $mg \cdot Kg^{-1}$ and $Cd<0.007$ $mg \cdot Kg^{-1}$).

From data obtained it could be concluded that some differences exist between the potential leachability under a weak acid extractant and the release as determined by the material itself without external pH influences. Particularly in such cases, significant changes in lixiviation may occur as a result of different sample pH values.

2.4. CONCLUSIONS

In this study the usefulness of single and sequential chemical extractions for predicting metal mobility (Zn, Pb and Cd) from mining wastes has been tested.

Fractionation of the metals was studied by applying the optimised BCR three-step sequential extraction procedure that was previously evaluated by using a certified reference material, BCR-701. From the results of the partitioning analysis it can be concluded that the behaviour of metal distribution between the two size fractions studied (<500μm to 63μm and <63μm) is similar, except for metal content extracted in Step 3 that increase with particle size reduction. A significant proportion of zinc and cadmium (up to 70% for Cd and 25% for Zn, respectively) is present as exchangeable and acid-soluble species in samples collected in Bagergue and Cartagena. Otherwise, a predominance of reducible lead had been found in all the samples with values from 36 to 90% of the pseudototal metal content.

Moreover, two leaching tests have been applied (Toxicity Characteristic Leaching Procedure and DIN 38414-S4) in order to study easily mobilised forms in the mining wastes. Although, the harm caused by material dumped cannot be only determined on the basis of analytical values of the eluates, these tests provide preliminary

information of waste behaviour when it come into contact with a weak acid or water.

Results from lixiviation tests show that wastes sampled in Pontaut present a higher lead mobility whereas in Bagergue and Cartagena samples, cadmium and zinc are the more mobile metals. Furthermore, all the mining wastes studied could be classified as hazardous according to US-EPA regulations regarding TCLP.

Comparison among the more mobile metal fractions obtained form Step-1 of BCR scheme, TCLP and DIN 38414-S4 leaching tests, shows that TCLP results are lower but in agreement with Step-1 of sequential extraction whereas metal concentrations in DIN 38414-S4 extracts decrease a lot and strongly depends on sample pH value.

In summary, according to the experimental results, the extraction procedures used are a useful tool to compare metal mobility in different mining wastes and therefore, their possible environmental implication.

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CHAPTER 3

Assessment of metal bioavailability to vegetation samples

Combination of data from extraction procedures and analysis of plants growing on mining tailings provides a good basis for identifying the input of metals into the environment.

Single extraction procedures based on the use of DTPA seems to be useful for the determination of the metal fraction that a plant can possibly taken up, if pH values of mining tailings are not too low (pH<5). In contrast, extractable metal fractions determined by employing the Step-1 and Step-2 of BCR scheme (Chapter 2) are a poor indicator of metal bioavailability.

On the other hand, information on particular physico-chemical characteristics and mineralogy of mining tailings appears to be necessary to understand metal mobility and bioavailability behaviour in each particular case.

The contents of this chapter have been accepted to be published in Environ. Pollut. (In press).

E.Marguí, I.Queralt, M.L.Carvalho, M.Hidalgo, *Assessment of metal availability to vegetation (Betula pendula) in Pb-Zn ore concentrates residues with different features.*

3.1. INTRODUCTION

Besides knowledge of the total heavy-metal content as well as the main chemical, physical and mineralogical characteristics of the mining tailings, it is essential to have information on the mobility and bioavailability of the heavy metals in order to estimate the impact to the environment arising from these sources [3.1]. As commented in Chapter 2, the easiest method and most often used to assess heavy-metal mobility in soils and sediments are laboratory extraction experiments, both single and sequential extraction procedures [3.2].

In sequential extraction procedures various extractants are applied successively (each extractant is chemically more active than the previous one) for selective fractionation of metals associated with the different constituents of the solid sample. Commonly, the first one or two extracted fractions are taken to be the labile pool of the metal that is potentially mobile and therefore bioavailable.

On the other hand, single extraction procedures using chelating agents (usually EDTA or DTPA) are also widely used in soil science in studies of fertility and quality of crops, for predicting the uptake of essential elements or for diagnosis of deficiency or excess of one element [3.3]. To a lesser extent, they have been applied to elements considered as pollutants, such as heavy metals [3.4]. However, to date, no methods have been considered as universally applicable for the assessment of soil-plant transference.

It is interesting to note that metal bioavailability depends not only on the growing media but also on biological specificity and individual susceptibility of the plant. Consequently, knowing that a plant by itself can influence the uptake of a certain metal, one can only estimate the bioavailable fraction as the fraction of metal form that a plant can possibly take up, but never the total fraction that a plant actually takes up [3.5]. As a result, to provide and effective evaluation of the set of bioavailable trace elements, both studies based on extraction procedures and plant analyses (metal content in plant matrices) should be conducted together.

In the present work, the possibility of using two operationally defined procedures commonly used to assess metal mobility and availability in soils and sediments (the first two extractions of the BCR protocol and the DTPA procedure) was investigated by correlating extracted metals using these procedures and accumulated metals in a plant species (*Betula pendula*) grown in the same mining spoils. In previous studies, *Betula pendula* has been often found on soils contaminated with metals and has

shown tolerance to zinc and lead [3.6]. Accordingly, the usefulness of this birch as Pb and Zn bio-indicator has also been tested through this research.

Furthermore, chemical and physical properties including pH, organic carbon content, particle size distribution, total heavy metal concentrations (Pb, Zn, Cu, As and Cd) and mineralogical composition were determined in the studied mine tailings in order to better understand metal mobility and bioavailability behaviour in each particular case. Statistical analysis of the collected data, carried out with principal component analysis and cluster analysis methods, revealed existing similarities or differences among the different mining tailings of concern.

On the whole, the aim of the present research is to investigate, under field conditions, the effect of the form in which metals are present in the mine spoils on their mobility and biovailability to vegetation specimens.

3.2. EXPERIMENTAL

3.2.1. Samples studied

The mining tailings and vegetation specimens used to fulfil the purpose of this study were collected at the Val d'Aran mining district, specifically in the treatment factory of Pontaut (for more detail see Chapter 1).

Nowadays, at the Pontaut treatment factory, the remains of eight flotation ponds (approximately 15 m^2 each one) are still full of the ore concentrate residues. Some spontaneous and invasive vegetation (such as *Betula pendula* specimens) has started the reforestation of the mining landfills placed in the area.

Sampling of the mining wastes was carried out specifically in each of these eight flotation ponds (from PT-1 to PT-8), see Figure 3.1.

A typical feature of these kinds of spoils is the high heavy metal content, mostly Pb and Zn, and also the presence of chemicals and residues from the ore processing operations which can produce possible changes in the parent ore material. Flotation tailings were collected using a polypropylene shovel, and subsequently transferred to clean polypropylene bags.

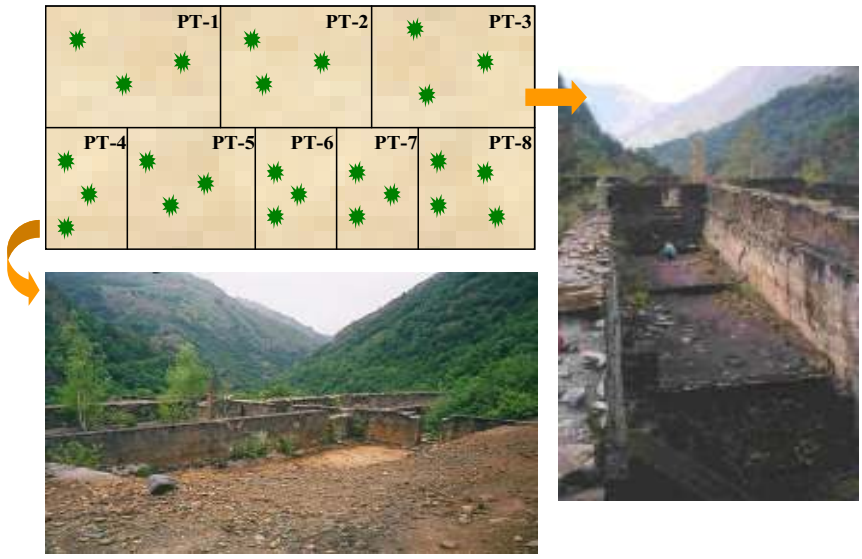


Figure-3.1.- Schematically view of the flotation ponds related with the present study.

Sampling of vegetation specimens was carried out in early autumm (just before shedding) to assure maximum metal accumulation. Vegetation was very scarce on the dump, so only *Betula pendula* species were sampled at each pond (Figure 3.1). Leaves were sampled from the upper third of 1 to 4 plants and a composite sample was prepared for analysis. For comparison purposes, a sample of the same species was also collected in the surroundings, but far from the contaminated mining areas (blank sample). All the vegetation specimens were stored in a clean, labelled, polyethylene bags, closed tightly, and kept in a plastic container to avoid contamination during transportation.

3.2.2. Analytical methods

3.2.2.1. Vegetation specimens

Once at the laboratory, the mixed leaf samples were washed thoroughly with deionised water to remove superficial dust and oven-dried at 55±5ºC for 24 hours. To reduce particle size they were ground in an agate ball mixer mill using a grinding time in the range 2-5 minutes. Once plants were powdered and dried, they were kept in labelled capped polypropylene flasks until analysis.

As pointed in the introduction section, atomic absorption (FAAS, ETAAS) and atomic emission (ICP-OES, ICP-MS) techniques combined with wet or dry ashing procedures are commonly used to determine various metal concentrations in vegetation specimens. However, energy-dispersive X-ray techniques (EDXRF) have

been proven useful for metal determinations in environmental samples such as plants [3.7, 3.8]. In this work, a procedure using EDXRF was performed to determine the elemental composition of the vegetation specimens considered. A more accurate study of the suitability of XRF techniques as an alternative to classical destructive analytical methods for elemental determination in vegetation matrices will be discussed in Chapters 5-7.

Three sub-samples of each powdered plant were pressed into pellets of 2.0 cm in diameter, without any chemical treatment. Multielemental measurements were carried out using an energy-dispersive X-Ray fluorescence spectrometer in a triaxial geometry [3.9]. The X-Ray generator was operated at 50kV and 20mA with an acquisition time of 1000s. The characteristic radiation emitted by the elements present in the sample was detected by a Si(Li) detector with a energy resolution of 130 eV at 5.9 keV. Quantitative calculations were made through the fundamental parameters method. The precision and accuracy of the elemental analysis were checked using a certified reference material (NIST SRM 1571: "Orchard leaves" from National Institute of Standards and Technology). The results obtained from the analysis of three replicates are summarized in Table 3.1. Good agreements were achieved between certified values and data obtained by EDXRF in all cases.

	K [a]	**Ca** [a]	**Mn**	**Fe**	**Sr**	**Zn**	**Pb**
Certified	1.47±0.03	2.09±0.03	91±4	300±20	37±1	25±3	45±3
EDXRF	1.5 (s=0.3)	2.1 (s=0.3)	96 (s=5)	297 (s=10)	35 (s=2)	25 (s=3)	44 (s=3)

[a] Concentration is expressed as percentage (%)

Table-3.1.- Certified and determined values ($mg \cdot Kg^{-1}$) for NIST SRM 1571 (Orchard leaves).

3.2.2.2. Mining tailings

Air dried samples were oven-dried at 50ºC, sieved at <500 μm and stored in polypropylene containers until analysis.

A Crison CLP pH meter was used for pH measurement using a ratio 1:2.5 (waste:MilliQ water) and organic carbon content was obtained by titration (with Fe^{2+}) of the $K_2Cr_2O_7$ that had not been consumed in the oxidation of the organic compounds of the sample in strongly acidic conditions [3.10]. Grain-size distribution

of the tailing samples was assessed with laser–diffraction equipment (MasterSizer-E, Malvern Instruments) and an X-Ray diffractometer (D-5005, BRUKER/AXS) was used to determine mineralogical composition.

A microwave-assisted digestion procedure, using a Milestone ETHOS PLUS microwave with temperature control, was developed for "pseudototal" determination of Pb, Zn, Cu, As and Cd. About 500 mg of sample was weighed and placed in a PTFE reactor. Three millilitres of 65% HNO_3 Suprapur and 9 ml of 37% HCl were added. Then it was heated following a two-stage digestion program: Stage-1 (10 min to reach 200ºC) and Stage-2 (15 min at 200ºC). After cooling, sample digest was filtered with a Whatman 42 filter paper, transferred into a 25 ml flask and brought to volume with MilliQ water.

The first two steps of the BCR method and a single extraction procedure using DTPA were applied to study Pb and Zn bioavailability in the flotation tailings. These elements were chosen as pollution indicators on account of their occurrence in the mining wastes and their high accumulation on the vegetation specimens studied. Details of the experimental protocols are available in Chapter 2 (BCR scheme) and in Figure 3.2 (DTPA protocol).

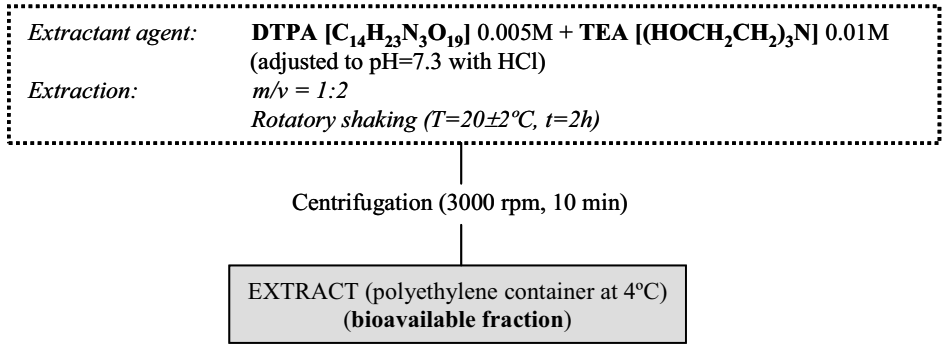


Figure-3.2.- Scheme of the DTPA extraction procedure [3.2].

To keep samples in suspension during extraction, a rotatory shaker (DINKO Systems) was used and a Mixtasel P-Selecta centrifuge was employed for separation of extracts.

In previous work [3.11] (see Chapter 2), the BCR Sequential extraction procedure was evaluated using the certified reference material BCR-701 and good precision (<12%) and accuracy (±15%) were obtained.

Both, digests and extracts, were analysed by a Sequential Inductively Coupled Plasma Emission spectrometer (Liberty RL, Varian) with V-groove nebulizer. The selected wavelengths were: Zn (202.551 nm), Pb (220.353 nm), Cd (214.438 nm), As (188.979 nm) and Cu (324.754 nm). Standard solutions were prepared by serial dilution of 1000 $\mu g \cdot L^{-1}$ Spectroscan solutions of the appropriate element. To avoid matrix-effects of the different extractant reagents, matched standards were used for metal analysis in all cases.

Blank extractions and digests (without sample) were carried out for each set of analyses. Duplicate samples were used in all the anlaysis done.

Throughout the work, analytical grade or suprapur quality reagents were used as well as ultrapure water obtained from a Milli-Q purifier system.

3.2.3. Statistical analysis

Data analysis takes an important place in environmental chemistry. Often, the objects of interest (samples) are described by different types of parameters (explanatory variables). Exploratory data analysis is then applied to obtain information about the similarity between the samples and the relationship between explanatory variables. At this stage of the data analysis, it is important to achieve a good visual representation of the data. In some cases this representation of a situation may be the end product desired. In many other cases, the exploratory data analysis results will serve to achieve an insight into to prepare more focused sampling plans or to formulate hypotheses about the processes taking place, which are then studied further with other data analytical methods. In the exploratory analysis, data are frequently analysed by principal component analysis (PCA) and cluster analysis [3.12].

In the present study, cluster analysis was applied to evaluate similarity of sampling sites with respect to all the variables studied. Furthermore, linear regression and Pearson correlation coefficients were calculated to examine relationships between variables. All calculations were done with the software program Statistica 4.1.

3.3. RESULTS AND DISCUSSION

3.3.1. Vegetation specimens

Elemental composition determined in *Betula pendula* specimens is reported in Table 3.2.

	K[a]	Ca[a]	Mn	Fe	Cu	Zn	Rb	Sr	Pb
PT-1	0.62	2.38	80	960	32	2770	15	84	530
PT-2	0.78	1.70	270	1190	29	1660	6.3	56	370
PT-3	0.40	2.50	96	640	19	2500	5.9	71	170
PT-4	0.73	2.54	149	320	11	2700	23	110	100
PT-5	0.63	2.66	90	370	12	2300	13	47	80
PT-6	0.44	2.51	122	370	13	2940	16	67	116
PT-7	0.48	3.00	77	260	10	3100	23	70	69
PT-8	0.48	2.80	123	290	11	2600	10	86	81
Blank sample	1.22	1.04	670	220	11	225	10	30	8

Data are expressed as mean of 3 replicates in $mg \cdot Kg^{-1}$ (RSD was lower than 15%).
[a] concentrations are presented as percentage values.

Table-3.2.- Elemental composition of Betula pendula specimens at the different sampling sites.

Heavy metal contents are similar to those obtained for other vegetation species from Pb/Zn mining areas [3.13, 3.14]. Comparing the elemental content of *Betula pendula* collected on the mining tailings with the corresponding metal content in the sample collected far from the mining area (blank sample), we can conclude that an accumulation process occurred, particularly for Zn and Pb. Relative Absorption Coefficients (RAC) defined as metal content in polluted samples divided by metal content in the blank specimen were calculated. *Betula pendula* RAC reached values in the range from 7 to 14 for Zn and 8 to 67 for Pb.

On the other hand, from the data obtained, metal accumulation seems to be directly associated with an exclusion of K and other nutritional elements, and to an increase of Ca content. Strongly negative correlations were obtained between Zn and K, Mn concentrations ($r = -0.78$ and $r = -0.60$, respectively) and instead, a positive significant correlation exists between Zn and Ca contents ($r = 0.79$, $p < 0.05$).

3.3.2. Mining tailings

3.3.2.1. General properties

The general characteristics of the mining tailings are shown in Table 3.3.

			Particle size distribution (%)			*Pseudototal metal contents (%)*				
	pH	**%C**	**Clay**	**Silt**	**Sand**	**Zn**	**Pb**	**As**	**Cu**	**Cd**
PT-1	3.83	0.31	7.3	79.1	13.6	0.29	13.00	480	0.26	5.5
PT-2	4.82	0.68	8.8	85.9	5.3	0.73	7.97	370	0.33	6.1
PT-3	5.85	1.72	8.7	88.0	3.3	1.75	3.43	350	0.40	7.8
PT-4	6.37	0.72	7.3	90.9	1.8	2.47	6.64	310	0.40	24
PT-5	5.47	4.79	3.9	54.9	41.2	22.90	1.31	302	0.41	526
PT-6	6.61	0.31	4.3	85.4	10.3	1.77	1.22	210	0.19	11
PT-7	6.05	0.84	3.1	56.7	40.2	3.75	0.83	270	0.15	40.5
PT-8	5.98	0.76	5.4	89.0	5.6	4.04	6.09	254	0.31	38

Data are expressed as mean of 2 replicates.
[a] Concentrations are presented as $mg \cdot Kg^{-1}$.

Table-3.3.- General properties of the mining tailings.

All the samples present an acidic pH (from 3.83 to 6.61) and an organic carbon content usually bellow 0.9%, a value similar to that found in other mine spoils [3.15]. However, the sample collected at PT-5 presents higher carbon content with a value near 5%.

It is apparent that the silt fraction represents a significant portion of the samples granulometric composition (up to 57%), particularly in PT-4 (90.9%). Otherwise, clay contents were lower than 9% in all cases.

As regards to "pseudototal" heavy metal burden, notable differences were observed depending on the location of the sampling stations. Tailings from PT-1 to PT-4 are strongly enriched in Pb, especially in PT-1 (up to 13%). Conversely, high contents of Zn are found (up to 22.9%) in samples from PT-5 to PT-8. Total Pb and Zn concentrations are accompanied with other minor elements also contained in the ores such as Cd, Cu and As. Total Cd concentrations were significantly correlated with total amounts of Zn ($r = 0.99$, $p < 0.05$). There was also a strong correlation between total As and total Pb content ($r = 0.81$, $p < 0.05$).

Mineralogical investigations of the samples indicate, in most cases, the presence of quartz, chlorite, muscovite, anglesite, goslarite and plumbojarosite but no galena (PbS) or sphalerite (ZnS), the main sulfides of the ore vein. In general, chemical structures of these sulphides can be broken up through dissolution or oxidation during weathering processes. So, heavy metals form the ores could be converted from an inert state (PbS, ZnS) into an active one ($PbSO_4$, $ZnSO_4$), and could enter into the environment easier. The sample collected at PT-5 is the only that contains sphalerite in its mineralogical composition (see Figure 3.3).

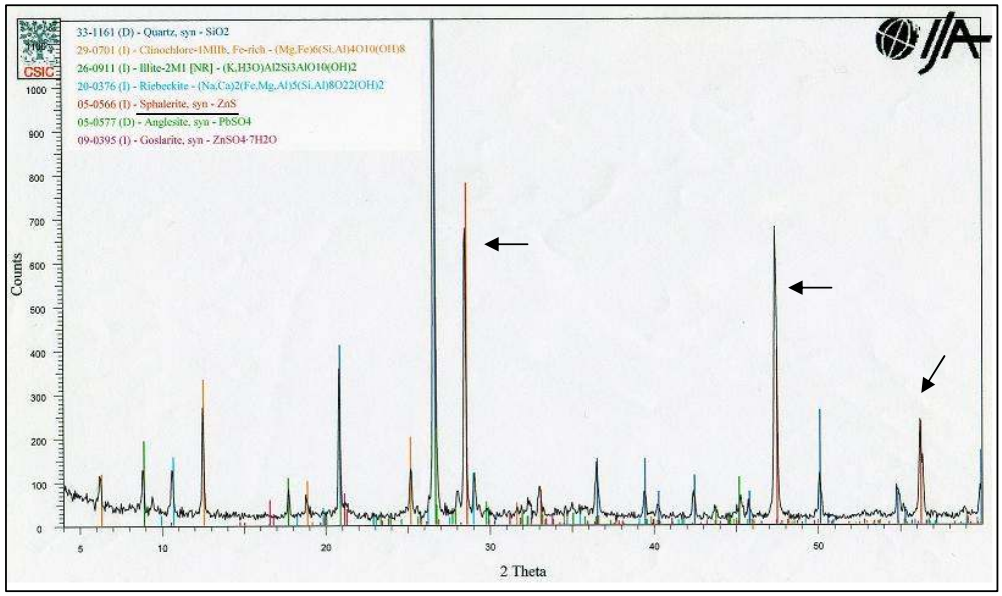


Figure-3.3.- Mineralogical composition of tailing sample PT-5 obtained by XRD (D-5005, BRUKER/AXS)

3.3.2.2. Extraction procedures

As commented in the experimental section, Pb and Zn were chosen as pollution indicators on account of their occurrence in the mining wastes and their high accumulation on the vegetation specimens studied. The results obtained in the extraction procedures are given in Table 3.4.

		PT-1	PT-2	PT-3	PT-4	PT-5	PT-6	PT-7	PT-8
BCR (S1)	***Pb***	1.1	4.5	32.9	40.7	5.0	58.9	41.6	2.6
	Zn	6.6	11.6	31.6	34.8	7.3	59.3	37.3	50.5
BCR (S2)	***Pb***	21.4	40.0	52.5	42.2	86.9	37.5	56.0	45.2
	Zn	7.6	34.2	34.3	18.6	2.1	17.2	12.3	18.1
DTPA	***Pb***	0.1	0.1	0.5	0.1	0.1	0.4	0.3	0.04
	Zn	1.2	0.4	0.6	0.7	0.1	1.1	1.0	0.4

Data are expressed as mean of 2 replicates

Table-3.4.- Extractable contents of Pb and Zn in the mining tailings studied. Results are expressed as percentage of the pseudototal values (see Table 3.3).

It is suggested in the literature that the first extract of the BCR method (CH_3COOH 0.11M as extractant) corresponds to the more mobile metal fraction representing the metal bound in exchangeable positions, water soluble or carbonate-bound. In the mining tailings studied, large amounts of Pb and Zn were released in this fraction (650-27000 $mg \cdot Kg^{-1}$ for Pb and 195-20000 $mg \cdot Kg^{-1}$ for Zn) that translate into high percentages of the pseudototal metal content (up to 58.9% and

59.3% for Pb and Zn, respectively). Likewise, in the second step of the extraction procedure ($NH_2OH{\cdot}HCl$ 0.5M as extractant), that represents the metal bound to iron and manganese oxides, high metal contents were obtained, above all in the case of lead with percentages up to 86.9%. According to these values it can be concluded that the first two extraction fractions account for the greater part of the total Pb and Zn in the mining tailings. A possible explanation for this fact is that metals from the decomposition of sulphides (PbS, ZnS) have been transformed into more mobile fractions as $PbSO_4$ or $ZnSO_4$ or could be adsorbed or complexed by iron oxyhydroxide colloids resulting from pyrite oxidation of the parent ore material [3.16]. Thus explains the low percentages of Zn extracted in the first two fractions of BCR scheme at PT-5 sample (9.4%) which, as has been said before, was the only tailing containing sphalerite in its mineralogical composition.

Regarding the DTPA procedure, the percentages extracted for both metals were lower than 1.5%. However, these percentages accounts for considerable concentrations of "available" metals (up to 157 $mg{\cdot}Kg^{-1}$ for Pb and 390 $mg{\cdot}Kg^{-1}$ for Zn).

3.3.2.3. Statistical analysis

Study of the existing similarities or differences among the mining tailings studied was performed on the basis of the experimental data obtained (general properties plus extraction behaviour of sampling sites) by using cluster analysis. Euclidean distances were used as a similarity measurement and the Ward's method as agglomeration rule (Figure 3.4).

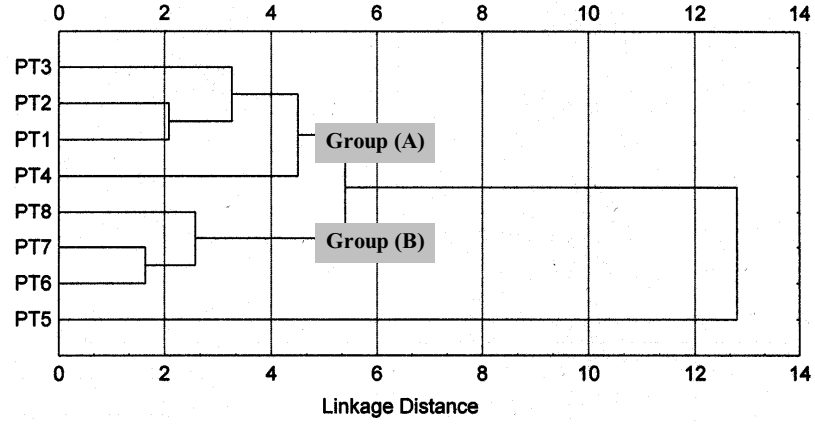


Figure-3.4.- Dendrogram showing clustering of sample sites. Group (A): Zn concentrate remains, Group (B): Pb concentrate remains.

At a cursory glance, two clearly distinct groups (A and B) can be observed. The samples assembled in the group A (from PT-1 to PT-4) are characterised by high Pb/Zn ratios in contrast to the samples joined in group B (PT-6, PT-7 and PT-8). This fact, seem to be indicative of the possible origin of the flotation tailings. Thus, samples from PT-1 to PT-4 may correspond to Zn concentrate residues (high Pb/Zn ratios) whereas the other samples are related to Pb concentrate spoils. On the other side, sample PT-5 keeps it isolated from the other sites. This tailing sample is strongly characterised by its high organic carbon content and the presence of unchanged sphalerite in its mineralogical composition.

3.3.3. Tailing-Plant relationships

In order to study if the metal content in *Betula pendula* specimens varies according to the estimated metal mobility and availability of the mining tailings evaluated from the extraction procedures commented above, single correlation analyses were performed between the extractable contents and the metal concentrations in vegetation specimens.

Best correlations were obtained when DTPA was used as extractant (Figure 3.5).

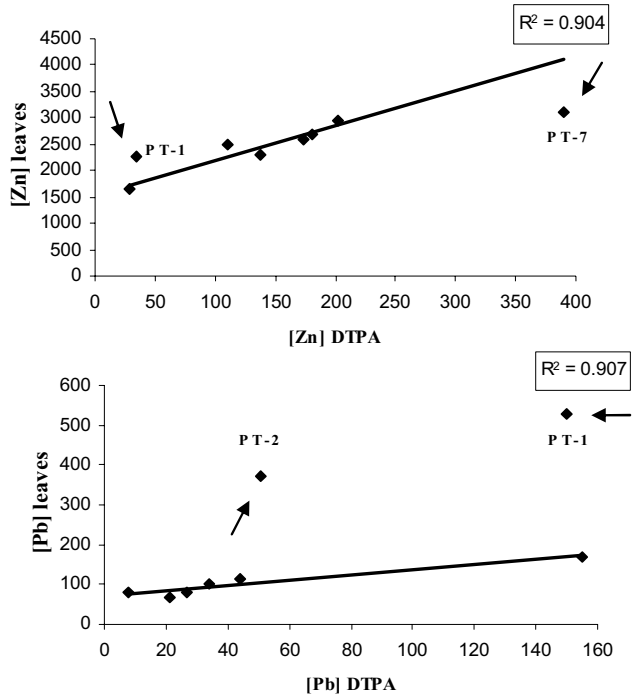


Figure-3.5.- Relationships between *Betula pendula* metal contents and DTPA-extractable metals in mining tailings. (Concentrations are presented as $mg \cdot Kg^{-1}$).

In both cases, it is evident that mining tailings with a pH lower than 5 (PT-1 and to a lesser extent PT-2) lie further from linearity. This fact could be explained since at these sample pH values DTPA is mostly protonated (pKa: 1.9, 2.9, 4.4, 8.7, 10.5) [3.17]. This fact leads to a lesser extraction power and an underestimated availability of the metal extracted following the DTPA extraction procedure (Figure 3.5). As it can be seen, in samples PT-1 and PT-2, the acidic pH plays a major role in the metal accumulated by vegetation specimens as it has been pointed in previous works [3.18].

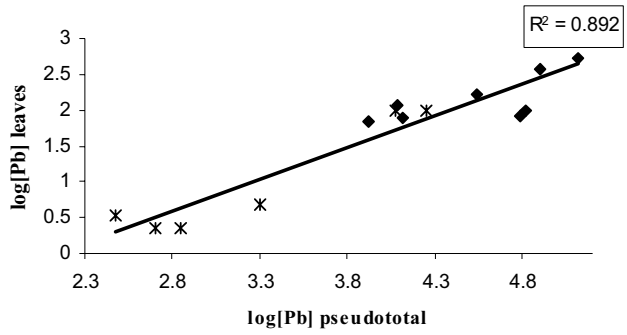
On the other hand, the sample collected at PT-7 showed the highest Zn content in leaves but lower than expected, probably because the Zn accumulation threshold is reached. If these sampling sites are excluded from calculations, R^2 of the trend line are up to 0.904 for Zn and 0.947 for Pb respectively.

Correlation coefficients obtained by using BCR extractions corresponding to the two first steps (S1 and S2) were poor compared with that obtained by DTPA, above all concerning to the extractable metal using $NH_2OH{\cdot}HCl$ 0.5 M as extractant (S2), with values of $R^2<0.3$ in all cases. Therfore, results demonstrated a poor relationship between Zn and Pb extracted using this procedure and the concentrations found in vegetation specimens. Similar results were obtatined in the literature [3.19, 3.20].

Indicator plants are ones whose metal uptake is in response to the amount of metal in the soil environment. They can therefore be used as indicator of the source and the intensity of contamination [3.21]. In previous studies, birch species (*B. papyrifera, B. pendula and B. pubescens*) have been reported to be tolerant to heavy metals [3.22]. In order to study the usefulness of *Betula pendula* as Pb and Zn bioindicator in the mining area, correlations between accumulation and metal burden in the tailings (pseudototal metal content) were carried out. It was not possible to find out a clearly defined relationship concerning to zinc, but in contrast lead metal uptake seems to be in response to the amount of this metal in the tailings. These results are in agreement with those obtained from a former sampling monitoring at different locations of the Aran Valley mining district (Figure 3.6).

As it can be seen, a wide linear tendency ($R^2=0.892$) was found between Pb content in the tailings (from 300 $mg{\cdot}Kg^{-1}$ to 1.3%) and those measured in *Betula pendula* leaves (from 2.3 to 530 $mg{\cdot}Kg^{-1}$). In addition, visual symptoms of lead phyto-toxicity in *Betula pendula* specimens such as chlorosis (brown spots in the

leaves and leaf margins) were not found. Thus, the results from the pilot study of the *Betula pendula* collected around the Aran Valley mining district show that it could be used as lead pollution indicator.



♦ Tailings collected at settling ponds from Pontaut factory (Figure 3.1).
∗ Former sampling monitoring at different locations of the Aran Valley mining district

Figure-3.6.- Variation plot showing the relationship between Pb burden at different sites of the mining area studied and *Betula pendula* lead contents.

3.4. CONCLUSIONS

Information on particular physico-chemical characteristics and mineralogy of mining tailings appears to be necessary to understand metal mobility behaviour. An investigation of eight different ore concentrate remains from an abandoned treatment mining factory revealed that the main sulphides of the ore vein (PbS and ZnS) have been converted into other species (mostly $PbSO_4$ and $ZnSO_4$) with a higher potential mobility and therefore, a larger source of lead and zinc pollution.

Despite the chemical limitations of these ore spoils, some species of vegetation, mainly *Betula pendula*, were found growing on them. Analysis of metal contents in *Betula pendula* leaves demonstrate a high metal accumulation particularly for lead and zinc, whose concentrations were, in some cases, over twenty times background values of this species. The possibility of using *Betula pendula* as indicator of metal pollution has been also tested, and encouraging results were obtained for Pb.

On the other hand, bioavailability studies based on the use of the conventional DTPA extraction procedure recommended for soils and sediments seems to be useful for mining tailings assessment if pH values are not too low (pH>5). In contrast, the extractable metal fractions determined by employing the Step-1 and Step-2 of the three-step sequential extraction procedure proposed by Standard Measurements and

Testing Programme of the European Community (BCR method) are a poor indication of bioavailability and, in general, it was not possible to determine clearly relationships between metal extracted using this operational procedure and metal concentrations determined in vegetation.

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CHAPTER 4

Lead isotope measurements by ICP-QMS to identify metal accumulation in vegetation samples

The use of stable lead isotope ratios proves to be a useful approach to study lead accumulation in vegetation specimens growing on wastes and soils of abandoned mining areas. The results of this investigation highlight that quadrupole based ICP-MS technique provides sufficient precision and accuracy for isotopic analysis of this kind of samples as long as instrumental bias factors are properly studied and corrected.

The contents of this chapter were submitted for publishing in:

Talanta

E.Marguí, M.Iglesias, I.Queralt, M.Hidalgo, *Precise and accurate determination of lead isotope ratios in mining wastes by ICP-QMS as a tool to identify their source.*

Sci. Total Environ (In press)

E.Marguí, M.Iglesias, I.Queralt, M.Hidalgo, *Lead isotope ratio measurements by ICP-QMS to identify metal accumulation in vegetation specimens growing in mining environments.*

4.1. INTRODUCTION

The incorporation of the heavy elements into plants is mainly achieved by uptake from the soil through the roots. However, uptake may also occur from deposition of heavy metals on the leaves from soil or aerosol particles [4.1]. Taking into account this fact, lead accumulated on vegetation specimens growing in contaminated areas can come from different lead sources such as the ground media or atmospheric deposition (including the past leaded-petrol emissions).

Elements are taken up by plants with the same isotopic proportions in which they occur in the soils and in precipitation. Therefore, isotope abundance ratios are excellent provenance indicators or tracers in the biosphere [4.2]. The isotopic composition of lead is variable in nature as only one (^{204}Pb) of the four stable isotopes of this element (^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb) is not the end product of the decay of thorium and uranium isotopes. So, although primordial lead had a fixed isotopic composition, the amounts of ^{206}Pb, ^{207}Pb and ^{208}Pb have increased over time relative to ^{204}Pb [4.3]. Taking this into account, the use of variations in lead stable isotope ratios has become a well-established diagnostic technique to characterise sources of Pb contamination and has been applied to evaluate the global impact of various anthropogenic sources in different environmental matrices including sediments [4.4], soils [4.5], river water [4.6], peat bogs [4.7], tree rings [4.8], plants [4.9] and mussels [4.10].

The fitness for purpose of a given analytical technique chosen for carrying out isotopic analysis is related to the compliance of some desired features such as that the uncertainty of the measured lead isotope ratios has to be better than the expected spread in the isotope ratios for a group of samples due to inherent variations of lead source. As commented in the introduction section of this thesis, isotope ratios have been traditionally determined through thermal ionisation mass spectrometry (TIMS). This technique provides the precision and accuracy required in isotopic analysis. Nevertheless, exhaustive sample preparations, tedious sample handling protocols, long analysis periods (2 to 24 h), and inflexible analytical routines have led to an unfeasible application of TIMS in some environmental studies where results are drawn from a large number of samples. For this reason, inductively coupled plasma mass spectrometry (ICP-MS) has increasingly been used in isotope ratio measurements [4.11,4.12] during last years. Although ICP-MS analysis cannot achieve the precision and trueness in isotope ratio measurements similar to that

attainable in TIMS, several advantageous features make the use of ICP-MS a suitable alternative: a fast sample throughput, low sample analysis cost, instrument robustness, simplified sample preparation and high ionisation efficiency.

This work highlights the suitability of the determination of stable lead isotope ratios by quadrupole based ICP-MS to study the potential sources of the anomalous lead content in vegetation specimens (*Buddleia davidii*) growing on the wastes and soils of two abandoned Pb/Zn mining areas. Circumstantial evidence (see chapter 3) pointed strongly to contamination from mining activities as the prime source of lead but sources such as the past leaded-petrol emissions could not be excluded. According to that, lead isotope ratios of *Buddleia davidii* were compared to those obtained in the isotopic analysis of the different kinds of wastes collected at the areas of study. The determined isotopic composition was also compared to that measured in several natural soils and in a lead-petrol sample, in order to assess the more probable source of the accumulation of lead in vegetation species.

To obtain reliable analytical results, also the main instrumental bias factors were properly evaluated and corrected.

4.2. EXPERIMENTAL

4.2.1. Samples studied

The samples used in the present research were collected in the mining districts of Val d'Aran and Osor. As pointed out in Chapter 1, since the mining closures mines and the areas affected by the mining operations have not been reclaimed, at present, the areas surrounding the factories are still covered by remains of ore concentrates and wastes. In addition, some spontaneous vegetation has started to reforest the soils and mining landfills located in these areas. Among the higher plants we find *Buddleia davidii,* or the butterfly bush, which is an invasive plant of Chinese origin, brought to Europe in the nineteenth century for use as a garden shrub. This plant spreads by producing abundant viable seeds and is now established in old quarries on masonry walls and waste ground in both urban and rural areas [4.13]. At present, this vegetation species has spread all over the mining districts studied.

Mining wastes were sampled at three sites in the Val d'Aran (VA) and four at the Osor location (O). From the first mining district eleven samples were collected including two samples of galena (ore vein, OV (VA)), eight samples of wastes from an old landfill with the remains of the Pb-Zn concentrates (CR (VA)) and one mine

tailing from a mining dump (MD (VA)). In Osor, seven samples were collected comprising two wastes from a mining dump (MD (O)), two wastes from a landfill with the remains of the Pb-Zn concentrates (CR (O)), one sample of galena (OV (O)) and, finally, two soils probably contaminated by mining activities (CS (O)).

For comparison purposes, five natural soils (NS) and a leaded-petrol sample were also collected and analysed to determine the lead isotopic composition. On the other hand, to study which isotope ratios have to be monitored in mining waste samples in order to identify properly mining lead origin a total of eleven samples were also collected in the mining district located in Cartagena and comprise nine tailings from several ponds (fine wastes accumulations) and two marine sediment samples likely contaminated by mining activities.

Surface samples (mining wastes, soils and sediments) were taken using a polypropylene shovel, and subsequently transferred to clean polypropylene bags.

Concerning the vegetal specimens, leaves (L) and flowering tops (F) of *Buddleia davidii* were sampled from the upper third part of the specimens and a composite sample was prepared for analysis from each sampling point. Control samples (CS) were collected in the surroundings of the Val d'Aran but far from the mining activities (opposite side of Garona river, see Chapter 1), with the corresponding soil sample (SS).

All vegetation specimens were stored in clean polyethylene bags and kept in a plastic container to avoid contamination during transportation.

4.2.2. Sample treatment and procedures

In the laboratory, mine tailing and soil samples were oven-dried (50ºC), sieved (<500µm) and stored in polypropylene containers until analysis. Leaves and flowering tops of *Buddleia davidii* specimens were washed thoroughly with deionised water to remove superficial dust and then oven-dried at 55±5ºC for 24 hours. To reduce particle size they were ground in an Agate ball mixer mill for 2-5 minutes. Once plant tissues were powdered and dried, they were kept in capped polypropylene flasks until analysis.

- Sample dissolution

Solid samples (mining wastes, soils and vegetation specimens) had to be brought into solution in order to meet the introduction system requirements of the ICP-QMS

instrument used for later lead isotopic analysis. In all cases, an ETHOS PLUS Millestone microwave with HPR-1000/10S high pressure rotor (Sorisole, Bergamo, Italy) was employed for acid digestion of samples. All the reagents used were of analytical grade Suprapur quality: nitric acid (Suprapur, Merck, Darmstadt, Germany) and hydrogen peroxide and hydrochloric acid (Trace Select, Fluka, Gilligham, Dorset, UK). Moreover, water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA) was used throughout the study.

For sample digestion of mining tailing and soil samples, about 500 mg of sample was weighed and placed in a PTFE reactor with 4mL HNO_3 (65%) and 12 mL HCl (37%). Then the reactor was sealed and heated following a two stage digestion microwave program. The heating procedure consists of a first stage of 5 minutes to reach 180ºC and a second stage of 10 minutes at 180ºC. After cooling, sample digests were filtered through a Whatman 42 filter, transferred into a 25 ml flask and brought to volume with MilliQ water.

As far vegetation specimens, 500 mg of sample was placed in a PTFE reactor with 9mL HNO_3 (65%) and 1 mL H_2O_2 (33%). When the foam caused by the decomposition of the organic matter disappeared, the vessel was sealed and heated following the same two-stage digestion microwave programme described above. After cooling, sample digests were transferred into a 25 ml flask and brought to volume with MilliQ water.

The lead contained in the leaded-petrol sample (10mL) was extracted for a period of 24 hours (rotatory shaking) with HNO_3 (65%) by using a ratio 1:1 (liquid:liquid).

- Solution analyses

A sequential inductively coupled plasma atomic emission spectrometer (ICP-AES, Liberty RL, Varian) was used to determine the lead content in the mining waste, soil and plant extracts. Standard solutions were prepared by serial dilution of a lead stock solution of 1000±0.5 $mg \cdot L^{-1}$(Pure Chemistry, ROMIL, UKAS calibration).

The performance of the experimental procedures was evaluated by analysing lead content in reference materials, including the sediment material BCR-701 from the Community Bureau of Reference (BCR, now the Standards, Measurements and Testing Programme) and the plant material GBW 07602 Bush branches and leaves from the National Research Centre for Certified Reference Materials, Beijing, China. In Table 4.1, certified and determined lead contents are presented. Determined

results are expressed as the mean of four parallel sample determinations with the corresponding standard deviation ($mg \cdot Kg^{-1}$).

	Certified	Determined
BCR-701	454 ± 19	446 (s=4)
GBW 07602	7.1 ± 0.7	6.5 (s=0.6)

Table-4.1.- Certified and determined lead contents in BCR-701 and GBW 07602 reference materials.

In both cases, good agreements were achieved between certified/indicative values and data determined.

4.2.3. Lead isotopic analysis

A quadrupole-based ICP-MS system (Agilent 7500c, Agilent Technologies, Tokyo, Japan) equipped with an octapole collision reaction cell was used for the isotope ratio measurements (see Figure 4.1). In this work, the collision / reaction cell was used only as an ion focussing lens since it was not filled with any pressurised gas.

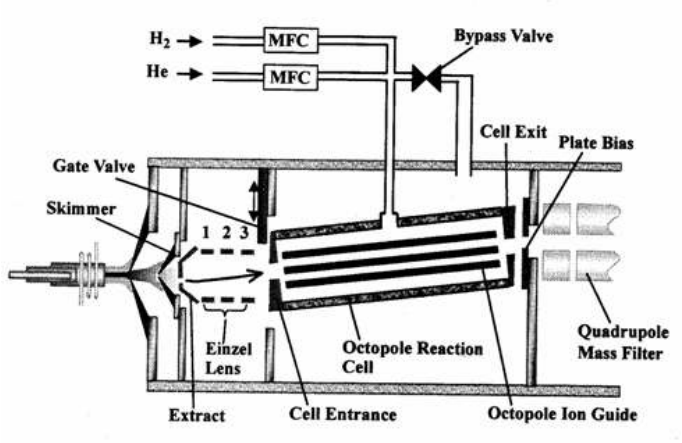


Figure-4.1.- Scheme of the Agilent 7500c ICP-QMS.

In the configuration used, a quartz concentric nebuliser, a scott type double path spray chamber, a shielded torch system and an electron multiplier detector were employed. Typical operating parameters were selected based on the manufacturer's recommended procedures (Table 4.2). Scan conditions were adjusted in order to obtain optimum precision and accuracy, as described in section 4.3.1.

RF Power	1500 W
Gas flow rates	
Plasma	15 L min^{-1}
Makeup	0 L min^{-1}
Nebulizer	1.17 L min^{-1}
Sampling cone	Ni, 1mm aperture diameter
Skimmer cone	Ni, 0.4 mm aperture diameter
Ion Lenses	
Extract lens	3.2 V
Einzel lens (1,2 and 3)	-80V, 10V, -80V
Octapole parameters	
OctP RF	190 V
OctP Bias	-7.8 V
Quadrupole parameters	
AMU Gain	127 V
AMU Offset	124 V
QP Bias	-5 V
Detector parameters	
Analog HV	1670 V
Pluse HV	1060 V

Table-4.2.- Operating parameters settings for the Agilent 7500c ICP-QMS.

The certified reference material NIST SRM 981 ("Common Lead Isotopic Standard", National Institute of Standards and Technology, Gaitherburg, MD, USA) was employed to test the mass bias correction and the whole isotopic measurement procedure developed [4.14].

As will be discussed later, Tl isotopes were used as internal standard. All working solutions were prepared from a Tl stock solution of 1000±0.5 $mg \cdot L^{-1}$ (Spectroscan, Technolab AS, Norway). On the other hand, an Hg stock solution of 1000±0.5 $mg \cdot L^{-1}$ (Spectroscan, Technolab AS, Norway) was employed to study the isobaric interference from ^{204}Hg on ^{204}Pb measurements.

4.2.4. Statistical analysis

The statistical analysis of the obtained results was performed using the program SPSS 12.0 for Windows®. Data interpretation was carried out by using discriminating and cluster analysis [4.15].

In the discriminating analysis performed a set of functions based on linear combinations of the predictor variables (lead isotope ratios) was generated to provide the best discrimination between groups (lead sources). On the other hand, cluster analysis was employed as statistical procedure to form groups of similar objects (samples) according to their isotopic composition.

4.3. RESULTS AND DISCUSSION

4.3.1. Analytical performance

The operating conditions for ICP-QMS measurements were optimised daily by using a solution containing 10 $\mu g \cdot L^{-1}$ of Li, Tl, Y, Ce and Co and monitoring the intensities of the isotopes ^{205}Tl, Y^{89}, Li^{7}, and the intensities at mass 156 (corresponding to $^{140}Ce^{16}O^{+}$) and mass 70 (corresponding to $^{140}Ce^{2+}$) so as to monitor percentage of doubly charged ions and of oxide ions, respectively.

In addition, every working day, a 30 $\mu g \cdot L^{-1}$ lead solution (NIST SRM 981) was analysed in order to check the sensitivity and the resolution of lead isotopes peaks to address peak tailing effects coming from adjacent peaks.

The potential of the different lenses in the ion optics play an important role in isotope ratio measurements as it has been quoted in previous works [4.16, 4.17]. In Figure 4.2 the measured $^{206}Pb/^{208}Pb$ isotope ratio is plotted versus the potential of the Einzel 2 lens in the Agilent 7500c quadrupole ICP-MS. As it is shown, by only increasing the Einzel 2 lens potential from +5 to +16 V the measured $^{206}Pb/^{208}Pb$ ratio decreases by about 2%. The ion lenses settings, selected to obtain maximum analyte signal intensity, are collected in Table 4.2.

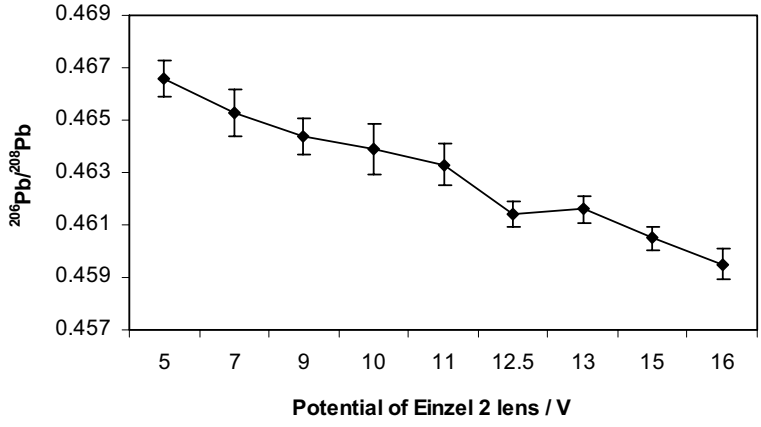


Figure-4.2.- Dependence of the measured $^{206}Pb/^{208}Pb$ ratio on the potential of the Einzel 2 lens (potential of other lenses: Extract:3.2V, Einzel 1: -80V, Einzel 3: -80V). Error bars represent the standard deviation between 5 replicates.

Quality assurance of the isotope ratios measurements involved the evaluation of the main parameters affecting accuracy and precision of analytical data, including

scan conditions (Precision) and dead time, mass bias and interference effects (Accuracy).

4.3.1.1. Precision

The precision of isotope ratio measurements is influenced by noise instability originated in both the ICP and the quadrupole mass filter [4.18]. It should be noted that in the present study, a concentric nebuliser in the self-aspiration mode was used for sample introduction, so discrete noise components caused by rotation of the peristaltic pump rollers were effectively eliminated. RF power and gas nebuliser flow rates were also selected so as to minimise relative standard deviation (RSD), see Table 4.2.

Moreover, integration time for each isotope ratio was optimised in respect to the lowest RSD obtainable as detailed in Figure 4.3.

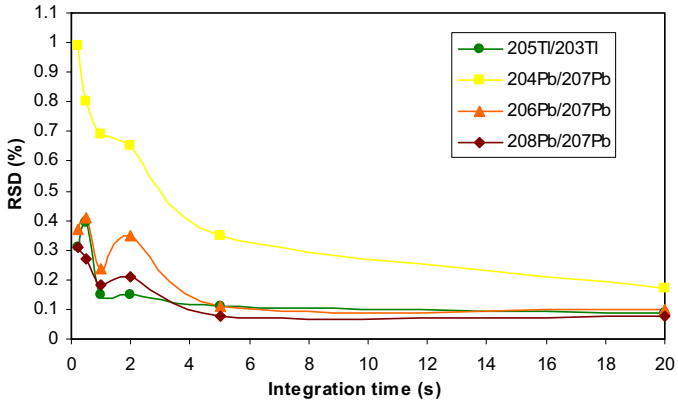


Figure-4.3.- Integration time *versus* relative standard deviation (RSD) using a 20 µg L^{-1} NIST SRM 981 solution spiked with 20 µg L^{-1} of Tl (internal standard).

All measurements were done using three points per peak and five replicates of each sample. As expected, the higher the integration time the lower the standard deviation until a constant value was reached; from these results, an integration time of five seconds was fixed for all the isotopes except for ^{204}Pb which requires a longer time to get a similar precision. Ten seconds were finally chosen as a compromise between precision and analysis time.

As a result, a total time of 9 minutes was needed for each sample analysis obtaining RSD values which are in agreement with those reported in the literature

using other quadrupole based instruments (RSD≈0.1% except in the case of isotope ratios involving ^{204}Pb, RSD≈0.2-0.3%) [4.17,4.18,4.19].

4.3.1.2. Accuracy

To achieve good accuracy of isotope ratio measurements by ICP-QMS three important instrumental bias factors have been studied and properly corrected, including the mass discrimination mainly due to the space charge effects [4.17], the dead time of the detection system as well as spectral interferences.

- Mass discrimination

Mass discrimination is an instrumental bias as a result of a different transmission of ions according to their masses from the point at which they enter the sampling device until they are finally detected by the electron multiplier [4.18]. As pointed in the introduction section of this thesis (I.4), several different processes contribute to this deviation including mass fractionation in the sampling processes or space charge effects in the region of the skimmer cone (due to the preferential transport of heavier isotopes) [4.20].

Mass bias may be corrected by using a certified isotopic standard (external correction) or by the measurement of an isotope ratio of an element with known isotopic composition in the same mass range, as an internal standard. The bias is then applied as a part of a mathematical model (lineal, power or exponential algorithm) to correct the analyte ratio [4.21].

In the present work, mass bias correction has been carried out by internal correction using Tl as standard (^{203}Tl/^{205}Tl). It is known that the use of an inappropriate mathematical model in estimation of mass bias may lead to a certain degree of inaccuracy. Consequently, the different correction algorithms (lineal, power or exponential) were tested, so as to study its suitability in our particular case, by using thallium for the correction of lead isotope ratios measured for NIST SRM 981. Results obtained in the comparison study are summarised in Table 4.3. As it is shown, the relative errors after correction are very similar using the three fitting functions leading to the conclusion that any of the three algorithms are valid for correction of mass bias. Finally, the power law function was selected for measurements since it is one of the most commonly used according to the literature [4.18].

	Lineal	Potential	Exponential
Bias factor (K)	$K = \frac{R_{\exp} - R_{theo}}{R_{theo}\Delta m}$	$K = \left(\frac{R_{\exp}}{R_{theo}}\right)^{\frac{1}{\Delta m}} - 1$	$K = \frac{-1}{\Delta m}\ln\left(\frac{R_{\exp}}{R_{theo}}\right)$
Corrected isotope ratio	$R_c = \frac{R_{\exp}}{1 + K(\Delta m)}$	$R_c = \frac{R_{\exp}}{(1+K)^{\Delta m}}$	$R_c = \frac{R_{\exp}}{e^{K\Delta m}}$
K	-0.0043	-0.0043	-0.0043
204/207	-0.062	-0.077	-0.077
206/207	0.18	0.18	0.18
208/207	-0.0088	-0.0088	-0.0097

Notes: R_{exp}(experimental isotope ratio), R_{theo}(theoretical isotope ratio), R_c(corrected isotope ratio), ΔM (mass difference between the isotopes involved in the ratio), K (mass bias factor)

Table-4.3.- Comparison of relative errors $(R_{corrected}-R_{certified})/R_{certified}$ (%) in estimation of mass bias corrections (lineal, potential and exponential algorithms) using the NIST-981 (30μg·L^{-1} Pb) and Tl (20 μg·L^{-1}) as internal standard (^{203}Tl/^{205}Tl).

In Figure 4.4 measured, corrected and certified values for each isotope ratio studied are displayed. As it is shown, measured value deviations from the certified values, which result from mass bias drift, range from 0.4% to 2%. Whereas, after correction, the errors are reduced to 0.01%-0.2%. Thus, the internal correction used in this study efficiently eliminates the mass bias drift effect.

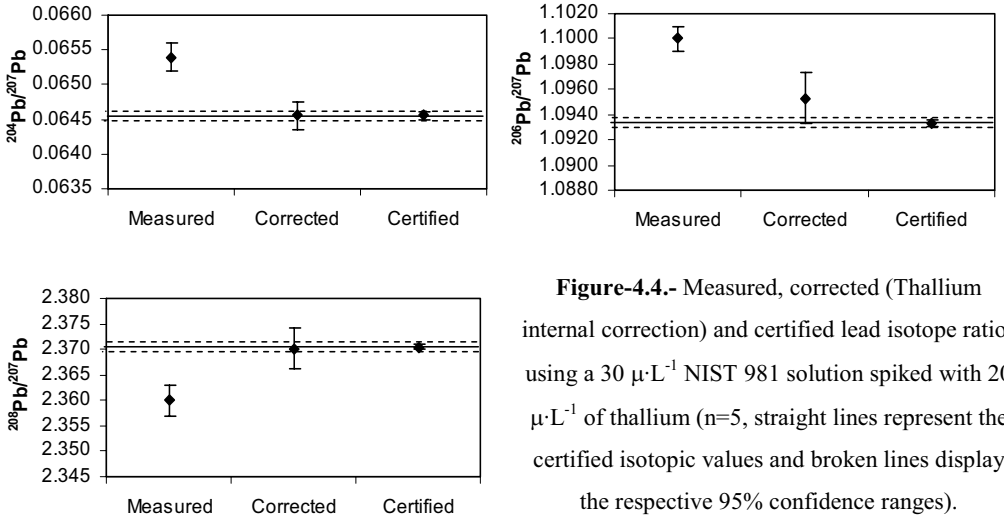


Figure-4.4.- Measured, corrected (Thallium internal correction) and certified lead isotope ratios using a 30 μ·L^{-1} NIST 981 solution spiked with 20 μ·L^{-1} of thallium (n=5, straight lines represent the certified isotopic values and broken lines display the respective 95% confidence ranges).

In order to compute the propagate uncertainty on the lead isotope ratios corrected for mass bias, the uncertainty on the mass discriminating factor (K) has to be taken into account. According to the expressions used in the potential correction (see Table 4.3), the propagated uncertainty results in:

$$S\left(R_{Pb(c)}\right) = R_{Pb(c)}\sqrt{\left(\frac{S(R_{Pb(\exp)})}{R_{Pb(\exp)}}\right)^2 + \left(\frac{S\left(R_{Tl(\exp)}\right)}{R_{Tl(theo)}e^{\frac{\Delta M_{Pb}}{\Delta M_{Tl}}\left(\ln\frac{R_{Tl(\exp)}}{R_{Tl(theo)}}\right)}}\right)^2} \qquad (4.1)$$

Where S: standard deviation, $R_{Pb(c)}$: corrected lead isotope ratio for mass bias, $R_{Pb(exp)}$: measured lead isotope ratio, $R_{Tl(exp)}$: measured Tl isotope ratio ($^{203}Tl/^{205}Tl$), $R_{Tl(theo)}$: theoretical Tl isotope ratio ($^{203}Tl/^{205}Tl$), ΔM: mass difference between the isotopes involved in the ratio.

Therefore, despite that the internal correction increased the accuracy of the isotope ratio values, the precision decreased due to the additional deviations of thallium isotope measurements. In the present study, the overall precision of bias corrected Pb isotope ratios (expression 4.1) ranges from 0.15-0.18%, except for the ratios involving ^{204}Pb that present higher values (≈0.3%) due to the lower abundance of this isotope. Analogous RSD values have been reported by other authors using similar instrumentation [4.22].

- Dead time

The electron multiplier detector employed in this study could use an analog mode (high concentrations) or a pulse counting mode (low concentrations) for ion signal measurement. In our case, the detection was made in all cases using the pulse mode so as to work under more sensitive conditions.

Firstly, it was necessary to study which was the maximum lead concentration that can be measured before the instrument automatically switches to the analog mode. For this purpose, solutions containing increasing concentrations of common lead (NIST SRM 981) spiked with 20 $\mu g \cdot L^{-1}$ of Tl were measured and the "corrected" isotope ratios were plotted with the corresponding confidence range (n=5, 95% confidence level), see Figure 4.5.

The results obtained showed that concentrations lower than 50 $\mu g \cdot L^{-1}$ should be used since at higher levels the more abundant lead isotope (^{208}Pb) is already measured in the analog mode. On the other hand, at 10 $\mu g \cdot L^{-1}$ the measured uncertainty is noticeable, particularly for the less abundant isotope (^{204}Pb). Consequently, a working range from 20 to 50 $\mu g \cdot L^{-1}$ was used throughout this work.

It is important to note that at high count rates different effects can cause pulse counting systems to register fewer events than actually occur [4.20]. As a consequence, signal responses become non-linear above certain concentration level (Dead time of the detector). As it can be seen in Figure 4.5, from 20 to 50 $\mu g \cdot L^{-1}$, the dependence of isotope ratios upon analyte concentration is non-existent, suggesting that at this lead levels dead time correction of the detector was not necessary.

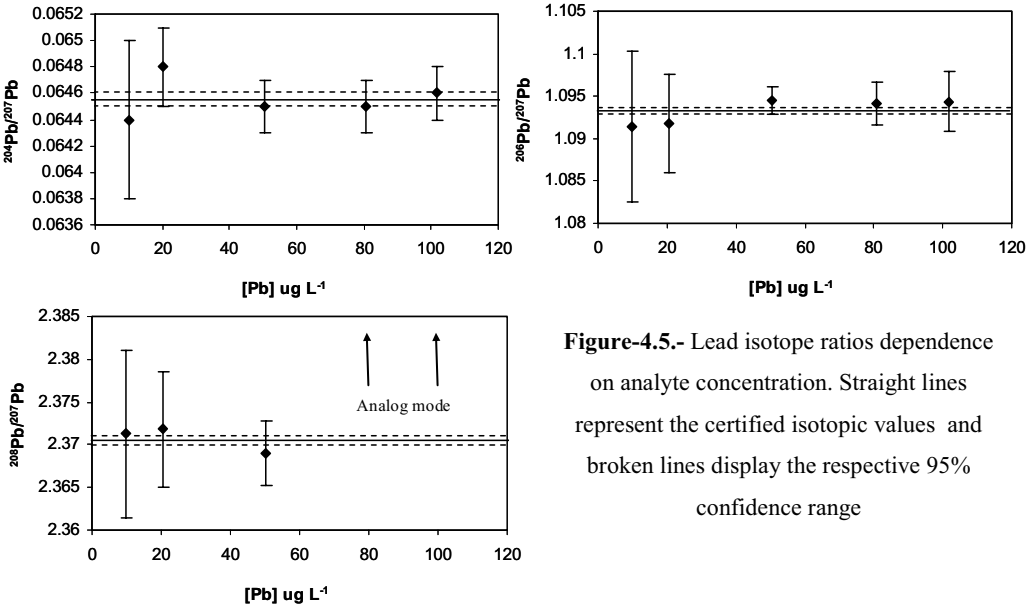


Figure-4.5.- Lead isotope ratios dependence on analyte concentration. Straight lines represent the certified isotopic values and broken lines display the respective 95% confidence range

- Interferences

The measurement of ^{204}Pb by mass spectrometry is subject to an isobaric interference from ^{204}Hg [4.23]. Owing to the possible presence of this metal in the mining waste samples studied, the measured ^{204}Pb ion beam was corrected for the Hg contribution.

Two expressions were evaluated to correct the measured intensity at mass 204 by measuring the ion beam of ^{202}Hg:

$$^{204}Pb = I(204) - \frac{Abundance(^{204}Hg)}{Abundance(^{202}Hg)} \times I(^{202}Hg) \quad (4.2)$$

$$^{204}Pb = I(204) - \left(\frac{\frac{Abundance(^{204}Hg)}{Abundance(^{202}Hg)}}{1 + K\Delta M} \right) \times I(^{202}Hg) \quad (4.3)$$

Where Abundance (^{204}Hg)/ Abundance (^{202}Hg)=0.23.

The mass discrimination factor between 202 and 204 masses (expression 4.3) was neglected since a preliminary study indicates that isotope lead ratios obtained with and without mass bias correction were found not to be significantly different at 95% confidence level. According to that, it was considered appropriate to use the expression 4.2 for 204 ion beam correction, due to its higher simplicity.

Table 4.4 collects the isotope ratios values attained with a 20 µg·L^{-1} lead solution spiked to 20 µg·L^{-1}with an Hg solution, after and before mercury correction. Results clearly demonstrate the necessity of 204 ion beam correction for the Hg contribution. Besides, the isotope ratio values obtained after Hg correction are in agreement to those measured for a solution without Hg, leading to the conclusion of the effectiveness of the equation used for ^{204}Hg correction.

	^{204}Pb/^{207}Pb	**^{204}Pb/^{206}Pb**	**^{204}Pb/^{208}Pb**
With Hg before correction[a]	0.1574 ± 0.0004	0.1203 ± 0.0003	0.0628 ± 0.0002
With Hg after correction[a]	0.0630 ± 0.0002	0.0482 ± 0.0001	0.0252 ± 0.0001
Without Hg before correction	0.0633 ± 0.0003	0.0483 ± 0.0003	0.0253 ± 0.0002
Without Hg after correction	0.0632 ± 0.0003	0.0483 ± 0.0003	0.0252 ± 0.0001

[a] Lead solution was spiked to 20 µg L^{-1} with a high purity Hg solution.

Table-4.4.- Effect of 204 ion beam correction for ^{204}Hg contribution using a NIST SRM 981 solution of 20 µg L^{-1} (n=5, 95% confidence level).

On the other hand, it is necessary to assess whether matrix interferences make a significant contribution to the offset of the measured mass discrimination corrected isotope ratio from its true value. This may be done by scanning the mass region of interest for a procedural blank. In this study, no correction for matrix interferences was considered necessary as lead concentration in procedural blank solutions

represents <0.2% of the Pb content in samples. Besides, Tl content measured in a procedural blank ([Tl]<0.07 $\mu g \cdot L^{-1}$) indicated that its contribution to the isotopic measurement was also negligible.

4.3.1.3. Reproducibility

As mentioned previously, the whole procedure developed was evaluated using the isotopic standard NIST SRM 981, and all the lead isotope ratios of this standard were determined every working day. The results of the determinations during a four month period (n=10) are presented in Figure 4.6 with the corresponding confidence range (n=5, 95% confidence level).

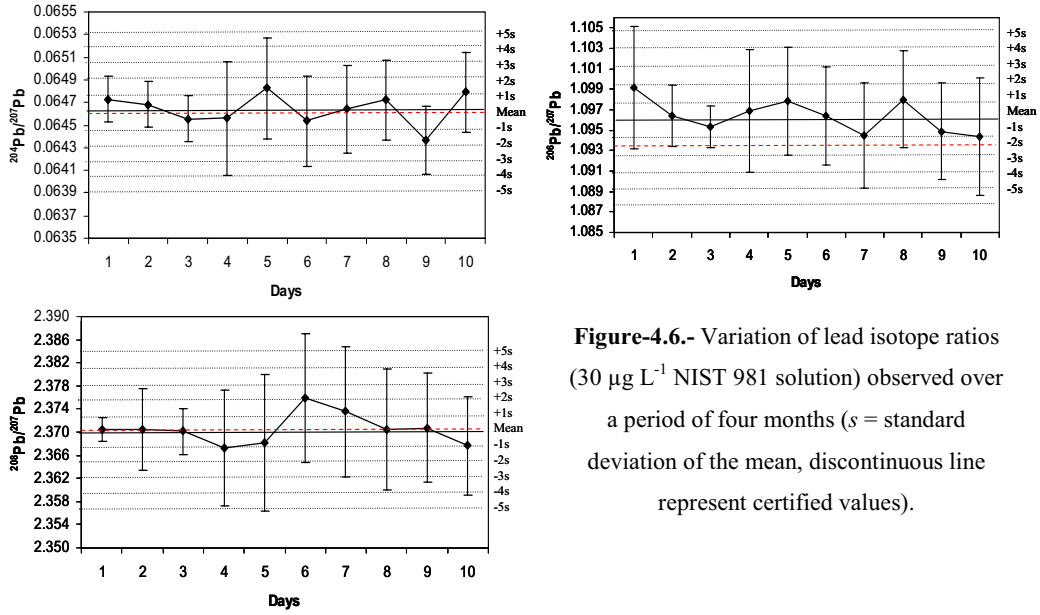


Figure-4.6.- Variation of lead isotope ratios (30 µg L^{-1} NIST 981 solution) observed over a period of four months (*s* = standard deviation of the mean, discontinuous line represent certified values).

The standard deviation for long-term measurements was slightly lower to that obtained for short-term (a single working day) measurements, with a RSD values ranging from 0.11 to 0.14%, except for the ratios involving ^{204}Pb which presents a RSD values from 0.21 to 0.27%.

A similar study was performed measuring the lead isotope ratios of a mining waste sample on three different days over a period of three month (see Table 4.5). As it is shown, results obtained for measurements (n=5) on three different days agree well and no statistically significant differences at the 95% confidence level were found.

	$^{204}Pb/^{207}Pb$	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
Initial	0.0640±0.0005	1.155±0.004	2.439±0.007
1 month	0.0640±0.0006	1.155±0.007	2.439±0.012
3 month	0.0643±0.0008	1.147±0.013	2.431±0.016

Table-4.5.- Variation of lead isotope ratios (mining waste sample) over a period of three months. Data are expressed as the mean value of five replicates with the respective 95% confidence range.

4.3.2. Lead content in samples

Remarkable differences were observed for lead burden in the different wastes collected due to the different nature of the materials (see Table 4.6).

The highest concentrations were found in the remains of the Pb-Zn concentrates with values of Pb up to 13 %. In general the mine tailings from the Val d'Aran are substantially more enriched in lead than those collected at the mining district of Osor. In both cases, the lead concentrations determined are of the same order of magnitude than those reported from similar mining areas [4.24, 4.25]. On the other hand, the results of the survey revealed that the soils collected at Osor exhibited lead contents of the same order of magnitude as wastes from the mining dump. From these concentrations and the location of the soils, it was therefore reasonable to assume that the high lead amounts were derived principally from the mining operations.

As commented in the Experimental Section, five natural soils were also collected for comparison purposes and the average of lead concentration recorded was 13 $mg \cdot Kg^{-1}$. The lead concentration found in the control soil was significantly higher, with a mean value of 158 $mg \cdot Kg^{-1}$. However, both concentrations are within the range of lead content for surface soils in different countries reported in the literature [4.1].

	Val d'Aran		Osor			Natural Soils (NS)	Control Soil (SS)
	Mining dump (MD)	Concentrate remains (CR)	Mining dump (MD)	Concentrate remains (CR)	Contaminated soils (CS)		
Min.		8300 ± 200	320 ± 10	28000 ± 600	770 ± 10	10 ± 0.5	
Max.		130000 ± 4000	2050 ± 40	38970 ± 20	1200 ± 100	15 ± 1	
Mean	20000 ± 1000	50612	1185	33485	985	13	158±4
SD[a]		42161	1224	7757	304	4	
Nº samples	1	8	2	2	2	5	1

Standard deviations between duplicate samples are represented as (± SD).
[a] Standard deviation between the means of all the samples analysed.

Table-4.6.- Descriptive statistics for lead concentrations ($mg \cdot kg^{-1}$) in mining tailings and soil samples.

Regarding vegetation specimens, considerable lead contents were found in *Buddleia davidii* specimens growing on the mine tailings of the Val d'Aran area (Table 4.7). Comparing the metal content of vegetation samples from the mining districts with the corresponding metal content in the control sample using the Relative Absorption Coefficient (RAC: content in plant growing on mining affected area / content in control sample), it can be concluded that an accumulation process

has occurred. Relative absorption coefficients reach values up to 106 and 80 for leaves and flowering tops, respectively.

	Val d'Aran (VA)		Osor (O)		Control sample	
	Leaves	Flowering	Leaves	Flowering	Leaves	Flowering
Min.	47,5 ± 0,1	86 ± 2	7,3 ± 0,4	12,4 ± 0,4		
Max.	607 ± 2	650 ± 20	40 ± 2	45,45 ± 0,01		
Mean	197,3	333,5	20	28	5,7 ± 0,5	8,1 ± 0,1
SD[a]	193	199	18,0	16,6		
Nº samples	8	6	3	3		

Standard deviations between duplicate samples are represented as (± SD).
[a]Standard deviation between the means of all the samples analysed.

Table-4.7.- Descriptive statistics for lead concentrations ($mg \cdot Kg^{-1}$) in *Buddleia davidii* specimens (leaves and flowering).

In order to identify if the anomalous lead content in the vegetation specimens is mainly derived from mining activities rather than from other possible lead sources including past leaded gasoline emissions, the Pb isotopic composition of plants and wastes was carried out and a comparison study was performed.

4.3.3. Lead isotope ratio analysis

As it is shown from the results obtained in the last section, a characteristic feature of all the samples collected in the mining districts studied is the large amount of lead, ranging from a few $mg \cdot Kg^{-1}$ up to $g \cdot Kg^{-1}$.Therefore, after measuring of the Pb concentration in sample digests, the solutions were adjusted to have a lead concentration of about 30 $\mu g \cdot L^{-1}$, according to the suitable working range (from 20 $\mu g \cdot L^{-1}$ to 50 $\mu g \cdot L^{-1}$) established previously.

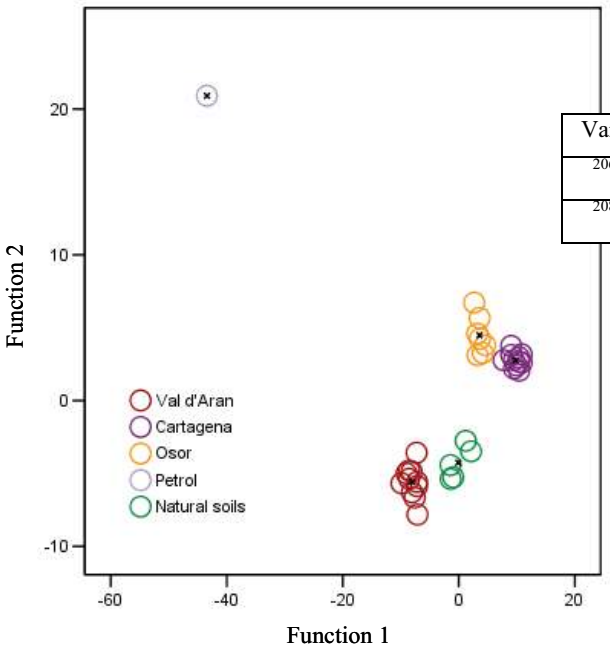
Once dilutions were performed, they were spiked with a high purity Tl solution to 20 $\mu g \cdot L^{-1}$ so as to correct for mass bias drift. It is relevant to note that diluted digests spiking was carried out just before the analysis to avoid possible redox reactions under laboratory conditions that can greatly affect the precision and accuracy of Pb and Tl isotope ratio values [4.23].

For each sample, two independent replicates were pre-treated and analysed five times separately. The mean value and respective confidence level (95% confidence level) were then calculated.

To avoid sample-to-sample memory effects a rinse step of 20s using Milli-Q water was performed.

Quality assurance (precision and accuracy) of the measuring procedure was addressed by analysing a 30 μg· L^{-1} NIST SRM 981 solution at the beginning and at the end of the analysis of each batch of samples.

In this study, the lead contamination in vegetation specimens collected at the two mining areas probably comes from one of three main sources: past leaded-petrol emissions, natural background lead and, obviously, the lead contained in the ores from the mining operations. Firstly, it was considered appropriate to study which isotope ratios have to be monitored in collected samples in order to identify properly lead source. For this purpose a discrimination analysis using Wilk's lambda method [4.15] was performed on lead isotope ratios measured in the collected mining waste samples (including those taken from the mining district of Cartagena), soils and the leaded-petrol sample. Two functions based on linear combinations of the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ variables were generated indicating that using only these two isotope ratios is possible to discriminate properly between lead sources (see Figure 4.7). This point is the main interest for isotopic analysis by techniques less sensitive and precise than thermal ionisation mass spectrometry as ICP-MS, since 206, 207 and 208 isotopes are easier determined than ^{204}Pb due to the lower abundance of this isotope.



	Function 1	Function 2
Variance (%)	75.9	24.1
$^{206}Pb/^{207}Pb$	1.701	-0.640
$^{208}Pb/^{207}Pb$	-1.069	1.470

Figure-4.7.- Discriminating analysis (Wilks' Lambda method) of isotope ratios in samples with different sources of lead.

In Figure 4.8 the biplot $^{206}Pb/^{207}Pb$ versus $^{208}Pb/^{207}Pb$ obtained in the isotopic analysis of mine tailings, soils and the leaded-petrol sample is displayed. Results are expressed as the mean of two independent replicates analysed five times with the respective confidence range (n=5, 95% confidence level).

As can be seen, a correlation between the pair of ratios exists. As suggested in the literature, the presence of a straight line appears to represent the simple mixing of two dominant components, known as end members, which would indicate different sources of lead [4.26, 4.27]. This fact could be explained by taking into account the possible variations of the relative abundance of lead isotopes over time, as commented in the introduction section.

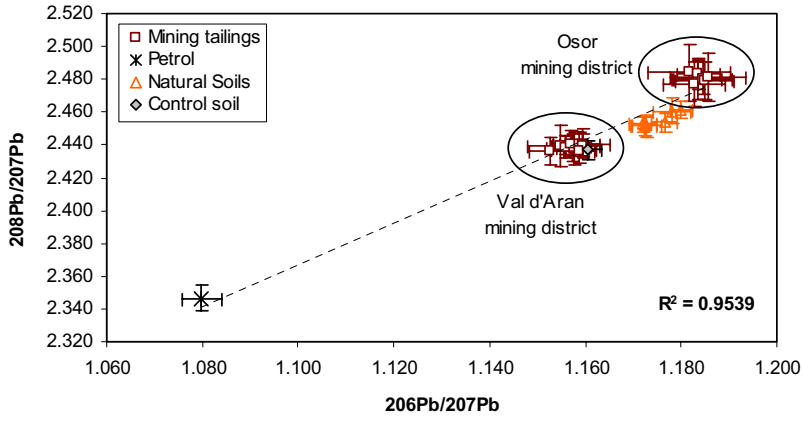


Figure-4.8.- Plot of $^{206}Pb/^{207}Pb$ ratios against $^{208}Pb/^{207}Pb$ for isotopic analysis of mine tailings, soils and the leaded-petrol sample.

It is interesting to note that the measured changes in isotope ratios are much greater than the uncertainties in the measurements and that they are significant in all cases. This trend emphasises that the implemented ICP-QMS method is sufficiently precise to discriminate among different lead origins. Besides, it is also possible to differentiate the two mining districts studied. All the mine tailings from the Val d'Aran can be assembled in a single group with Pb ratio ranges from 1.153±0.004 to 1.159±0.006 ($^{206}Pb/^{207}Pb$) and from 2.436±0.009 to 2.441±0.009 ($^{208}Pb/^{207}Pb$). In a similar way, the samples collected at Osor are grouped in values ranging from 1.182±0.009 to 1.186±0.008 ($^{206}Pb/^{207}Pb$) and from 2.47±0.01 to 2.48±0.02 ($^{208}Pb/^{207}Pb$). However, the Pb isotopic composition of the different kinds of samples within a mining district (including galena ore samples, Pb-Zn concentrate remains and tailings from the mining dumps) was indistinguishable. On the other hand,

resemblance between the composition of likely contaminated soil samples collected at Osor and those from mine tailings corroborate the pollution of these samples by mining activities.

Old lead ores were formed at an early stage in the earth's evolution so the decay of Th and U contribute little to the lead before these elements become isolate from the lead ore. Thus, as a general rule, old lead ores are generally characterised by low $^{206}Pb/^{207}Pb$ ratios, whereas more radiogenic samples of lead, those that have been mixed with thorium and uranium for a long period of time, have ratios of 1.18 and above [4.27]. According to that, it could be deducted that galena (lead ore) from Val d'Aran is geologically older than that from Osor, which present higher radiogenic isotope ratios. This statement is consistent with the geological data for the age of the ore deposits in the Eastern part of Spain. Whilst Val d'Aran deposits were formed during Cambro-Ordovician time (about 450-550 mya old) [4.28], the mineral deposits of Osor where generated during the Tertiary and the calculated ages are quite younger (aprox. 45-50 mya) than for Val d'Aran deposits [4.29].

In Figure 4.9 (A) the isotopic composition of vegetation specimens has also been included in the biplot.

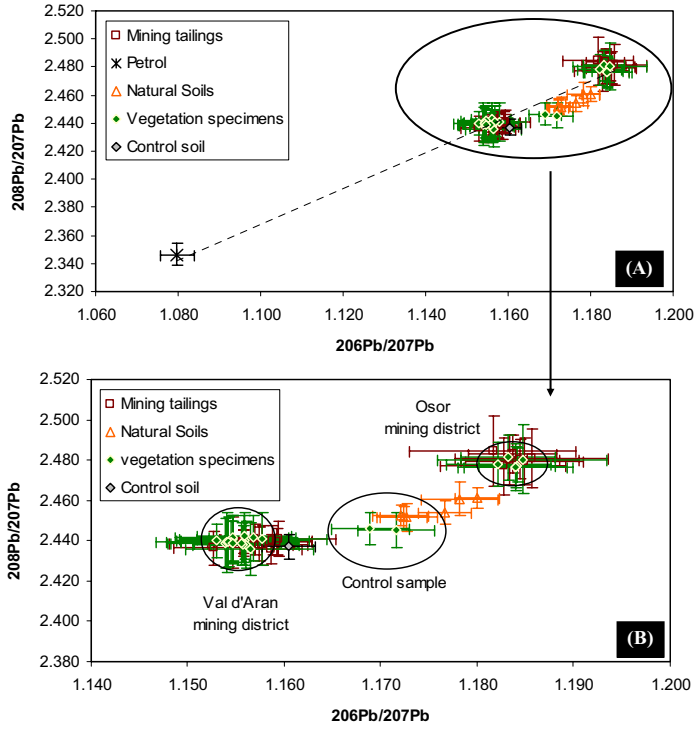


Figure-4.9.- Plot of $^{206}Pb/^{207}Pb$ ratios against $^{208}Pb/^{207}Pb$ for isotopic analysis of all samples studied.

The obtained data set shows that there was not a noticeable contamination of *Buddleia davidii* specimens from petrol emissions: lead isotope ratios determined in the samples are substantially different from those obtained in the analysed leaded-petrol sample analysed. The results for the latter are of a low $^{206}Pb/^{207}Pb$ ratio of about 1.080, as reported in other works [4.30, 4.8]. The significant reduction in the use of alkyl-Pb additives in gasoline during the past years has led to a gradual increase of the $^{206}Pb/^{207}Pb$ ratio in environmental samples affected by atmospheric deposition. For example, the $^{206}Pb/^{207}Pb$ ratio in moss samples collected at different localities in Norway have risen from 1.120 (1977) to 1.143 (2000) [4.31]. However, the ratios determined in the vegetation specimens studied in the present work are substantially higher (from 1.154 to 1.183) than current atmospheric deposition values.

Moreover, as shown in Figure 4.9 (B), the lead isotopic composition of *Buddleia davidii* samples collected at the mining areas is close to that determined for the mine tailings from the same areas, suggesting that the anomalous amounts of Pb in the plants are derived from mining activities rather than from other lead sources.

It is interesting to note that the results for the control sample do not match with the groups formed for the vegetation specimens collected at the Osor and Val d'Aran mining districts. On the contrary, the isotopic composition of this sample is similar to those measured for natural soils studied which are representative of the estimated natural background composition. Therefore, it seems feasible to discriminate between the control sample and lead contaminated specimens taking into account only the values of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ isotope ratios. This trend is more clearly shown in the dendrogram obtained after performing a Cluster analysis of the isotope ratios of vegetation specimens and mine tailings, using the average linkage between groups as a distance measurement [4.15] (Figure 4.10). Three distinct groups, corresponding to the samples collected at the Val d'Aran mining district, those sampled at Osor and the control sample can be observed.

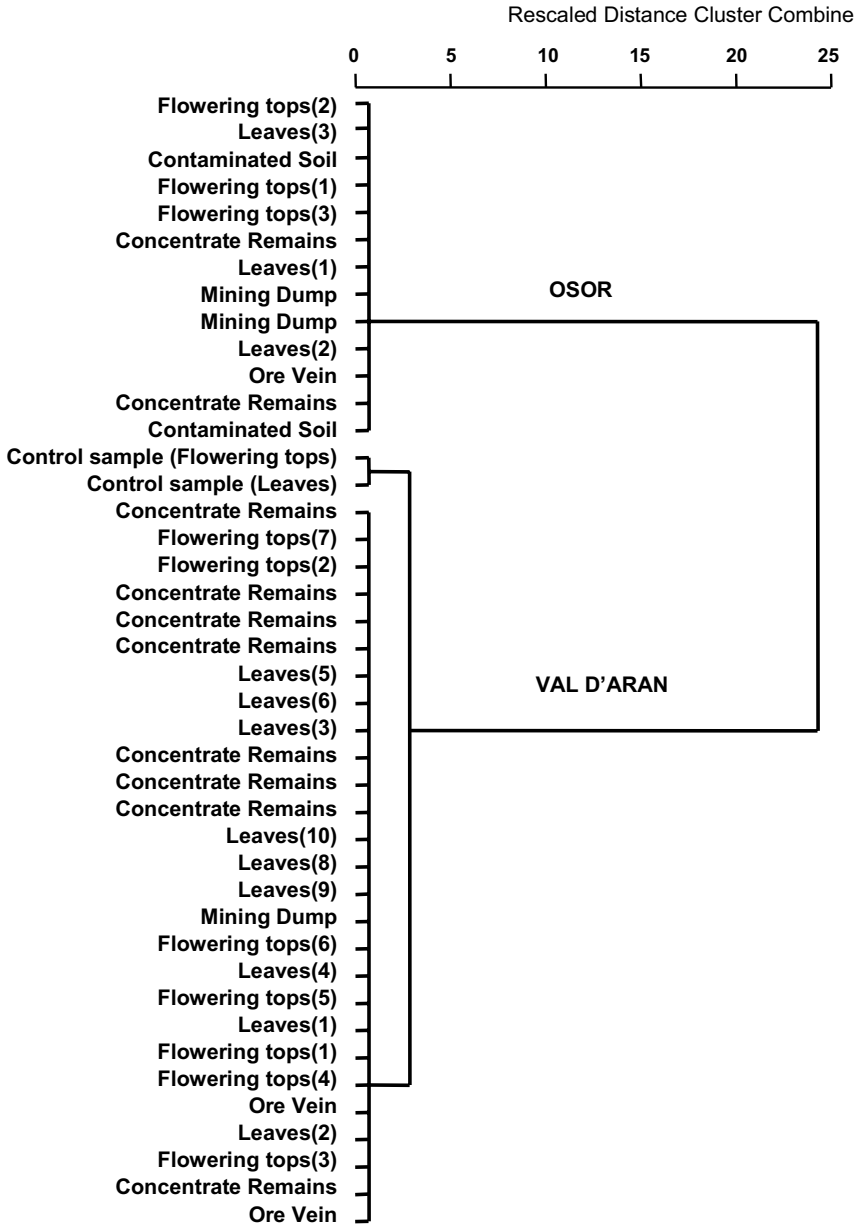
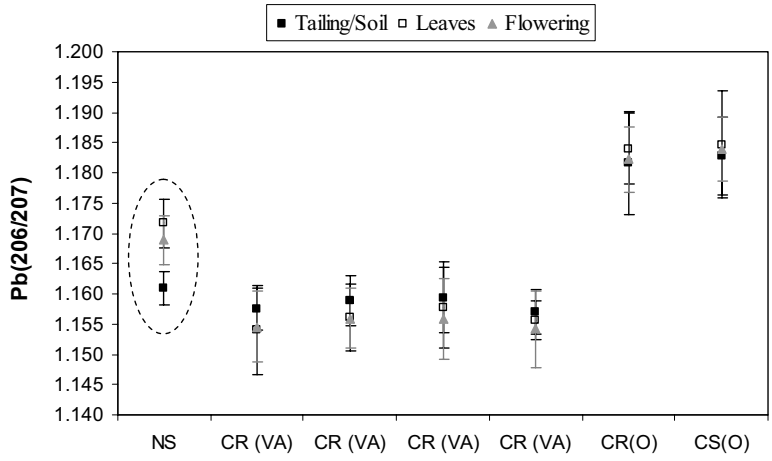


Figure-4.10.- Cluster analysis applied on the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios of vegetation specimens and mining tailings, using average linkage between groups (n=42).

In order to gain a more complete information of the similitude between lead isotope ratios from *Buddleia daviddi* tissues (leaves and flowering tops) and those from the mine tailings or soil samples where they are growing, a graphical representation compared the $^{206}Pb/^{207}Pb$ values obtained in both environmental matrices (Figure 4.11).

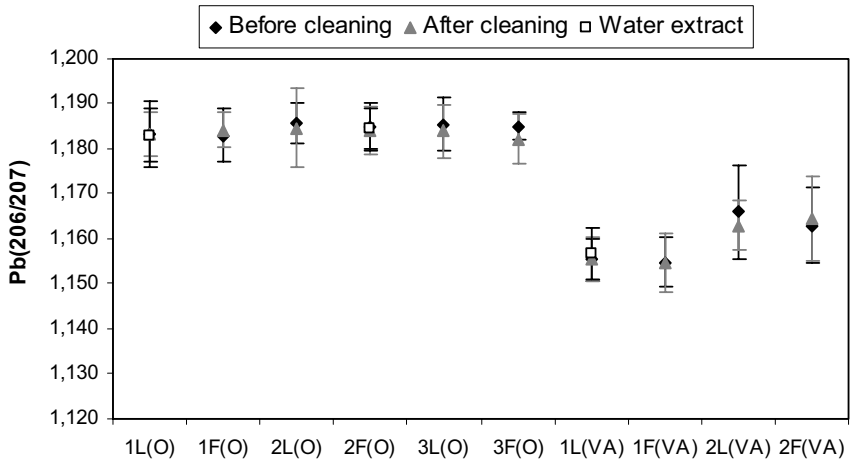


Notes: NS: Control soil, CR: Remains of Pb-Zn concentrates, CS: contaminated soil, (VA): Val d'Aran, (O): Osor.

Figure-4.11.- Comparison of $^{206}Pb/^{207}Pb$ ratios in mining tailings, soil samples and corresponding vegetation specimens. Results are expressed as mean of two independent replicates analysed five times with the respective confidence range (n=5, 95% confidence level).

The results showed that the $^{206}Pb/^{207}Pb$ ratios in mine tailings and the corresponding *Buddleia davidii* samples growing on these areas were identical. A different trend was observed in the case of the control sample, which presents $^{206}Pb/^{207}Pb$ ratio different from that of the corresponding soil sample, indicating that in this case the lead uptake from the soil did not play a major role. This fact could be explained if one considers the high mobility of lead contained in the mining wastes. As pointed out in Chapter 3, the oxidation of the ore vein (PbS) into more mobile species ($PbSO_4$) frequently occurs and, in such a case, the lead could be more easily taken up by vegetation specimens. Moreover, it has to be kept in mind that the acidic pH of mining waste samples (from 3.8 to 6.6) also facilitates the metal availability to vegetation specimens as it has been addressed in previous works [4.32].

Furthermore, in order to study the possible presence of lead in the surface of leaves and flowering tops of *Buddleia davidii*, an analysis of extracted water soluble metals was performed. For this purpose, 1g of intact vegetation specimen was immersed in 10 mL of deionised water for 10 min. The lead concentrations analysed in the water extracts was, in most cases, below 20μg·L^{-1}. The $^{206}Pb/^{207}Pb$ values obtained in the analysis of the leaves and flowering tops of *Buddleia davidii* before and after water cleaning were indistinguishable and were also identical to those from the isotopic analysis of water extracts (Figure 4.12).



Notes: (VA): Val d'Aran, (O): Osor, F: Flowering tops of *Buddleia davidii*, L: Leaves of *Buddleia davidii*.

Figure-4.12.- Graphical view of $^{206}Pb/^{207}Pb$ ratios obtained before cleaning, after cleaning and in water extracts of Buddleia davidii specimens (isotopic composition of water extracts was carried out whenever [Pb]>20 μg·L^{-1}, according to the suitable working range: 20-50 μg·L^{-1})

4.4. CONCLUSIONS

Elevated concentrations of Pb were found in the different mine tailings collected from Val d'Aran and Osor mining districts, indicating a potential environmental hazard. Similarly, *Buddleia davidii* specimens growing in these areas presented a higher lead content compared to the natural burden which is characteristic of this vegetation species (control sample). Circumstantial evidence pointed strongly to contamination from mining activities as the prime source of lead, but sources such as the past leaded-petrol emissions could not be excluded.

The use of isotopic composition to identify the origin of the anomalous lead content in vegetation specimens proved to be a powerful technique. The precision

and accuracy attained with the methodology proposed based on ICP-QMS made it possible to satisfactorily discriminate among the likeliest lead sources in the studied areas.

The results show that the lead in the leaves and flowering tops of *Buddleia davidii* specimens from the mining areas did not have the same isotopic composition as a specimen from an uncontaminated site (control sample) but was isotopically identical to the mine tailings collected. This trend suggests that the lead accumulation in plants is primarily derived from the mining operations rather than from other lead sources such as the past leaded-petrol emissions.

From the data obtained, it was concluded that the abandoned mining areas studied (above all the Val d'Aran mining district) can become important sources of lead contamination for the occurring vegetation growing in the soils and on the mining landfills located in these areas.

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CHAPTER 5

Comparison of EDXRF and ICP-OES after microwave digestion for element determination in vegetation samples

Sample decomposition procedures used in classical plant analysis influence the results of element mass fraction determination, depending on the analyte of interest and the matrix composition of the sample. In contrast, the energy dispersive X-ray fluorescence technique has proven to be an effective tool for screening and determining simultaneously the mass fractions of several elements in vegetation matrices. Since matrix digestion stage is not required in direct XRF analysis, the risk of having poor recoveries for some elements or contamination are avoided. Moreover, the proposed method is less time consuming and requires fewer amounts of reagents. The latter is of great significance in environmental studies where conclusions are based on the analysis of a large number of samples.

The contents of this chapter are published in:

E.Marguí, I.Queralt, M.L.Carvalho, M.Hidalgo, *Comparison of EDXRF and ICP-OES after microwave digestion for element determination in plant specimens from an abandoned mining area.* Anal. Chim. Acta 549 (2005) 197-204.

5.1. INTRODUCTION

The use of vegetation species in monitoring studies as bioindicators of the degree of pollution with metallic elements is already a widespread approach in environmental researches [5.1,5.2], as it has already been quoted in the previous chapters. In such studies, conclusions are drawn on the basis of a large number of samples. Hence, it is of significance that the analytical procedures used should be rapid and simple, with no detriment to adequate accuracy and precision in analyte determination [5.3].

Atomic spectrometry is the likeliest technique used for metal determination in this kind of samples [5.4]. The market of this type of instruments offers basically instrumentation designed for the analysis of liquid samples. Therefore, solid samples have to be brought to solution in order to satisfy the needs of introduction systems of most spectroscopic techniques used.

For some kind of samples the dissolution is not a problem and it may be readily achieved using mixtures of strong acids (generally nitric and sulphuric) and oxidants (commonly hydrogen peroxide). However, plant samples often require a more complete decomposition procedure to ensure efficient mobilization of metals into solution due to the presence of high contents of silicon [5.5]. Most of the usual wet methodologies proposed rarely include the particular silicon problem leading to poor recoveries of some elements because of the analyte binding with the insoluble residue. In such a case, an additional hydrofluoric acid attack and evaporation to dryness is necessary to assure a complete dissolution of the analytes [5.6].

In recent years, the use of microwave ovens for acid digestion of many types of solid samples is well documented [5.7,5.8] and is commonly regarded as an alternative for the classical open air hot plate digestion procedures. This sort of devices offer less risk of contamination, a reduced amount of acids used as well as a substantial decrease of digestion time compared with traditional methods. However, despite of the advantages of microwave methods, the hydrofluoric acid step commonly needed to assess an efficient mineralization of vegetation tissues is more difficult to realize in wet digestion procedures employing a microwave heating system. In pressurized bombs, the evaporation to dryness with the objective to volatilize silicon and mobilize associated elements cannot practically be achieved, leading to an ineffective total destruction of the sample [5.6].

In view of these problems, the implementation of other methods for analyzing vegetal samples has increased over the last few years, including X-ray fluorescence spectrometry (XRF). A great advantage of XRF techniques compared to wet chemical procedures is that the multi-elemental analysis can be directly carried out on solid samples. This avoids the tedious and laborious wet digestion steps and the possible analyte losses and / or sample contamination as well as a considerable decrease in analysis time. On the other hand, despite of the higher detection limits compared to spectroscopic techniques, the precision and accuracy obtained using XRF methods are good enough for the purposes of environmental studies.

The aim of this work was to study the applicability of an EDXRF method to the multi-elemental analysis of different vegetation species as an alternative to conventional destructive methods. The analytical results of the proposed X-ray method were compared with those obtained from the application of two routine microwave assisted digestion procedures followed by ICP-OES determination, that are often applied to analyse vegetation matrices. The statistical evaluation of the results was carried out using robust statistics.

5.2. EXPERIMENTAL

5.2.1. Samples studied

In order to fulfil the purpose of this study, a sampling of four higher plants species (*Betula pendula, Buddleia daviddii, Quercus robur* and *Pinus sylvestris*) growing on the waste landfills of the treatment factory of Pontaut (Val d'Aran mining district) was carried out. Leaves and flowering (in the case of *Buddleia davidii*) were sampled from the upper third of each plant. For comparison, samples of the same species were also collected in the surrounding areas, but far from the mining activities (control sample).

All the vegetation specimens were stored in clean, polyethylene bags, closed tightly, and kept in a plastic container to avoid contamination during transportation. Once at the laboratory, leaves and flowering samples were washed thoroughly with deionised water to remove surface dust and oven-dried at 55±5 ºC for 24 hours. To reduce particle size the samples were ground in an agate ball mixer mill for 2-5 minutes. Once plant tissues were powdered and dried, they were kept in capped polypropylene flasks until analysis.

5.2.2. Energy dispersive X-ray fluorescence analysis (EDXRF)

As commented before, one of the advantageous features that make XRF techniques an attractive alternative to destructive analytical methods is the possibility of performing analysis directly on solid samples. In this case, three sub-samples of each powdered plant were pressed into pellets of 2.0cm in diameter without any chemical treatment. Each pellet was glued on a Mylar film, on a sample holder and placed directly on the X-ray beam of the energy dispersive X-ray spectrometer used for elemental determination.

EDXRF spectrometers have the great advantage of displaying information on several elements of the sample at the same time (simultaneous analysis). Therefore, they are particularly effective for the screening and identification of most of the elements in the studied samples.

The spectrometer used in this work for the multi-elemental measurements of vegetation specimens consists of an X-ray tube equipped with a secondary target of molybdenum (secondary mode of operation) [5.9]. The X-ray tube, the secondary target and the sample are oriented in a triaxial geometry (Figure 5.1). This arrangement allows obtaining a decrease in the measured continuum, taking advantage of the effect of polarization of the X-ray beam scattered from the secondary target. The spectrometer is a self-constructed system, using a Philips X-Ray generator (PW 1140/00/60 3 kV).

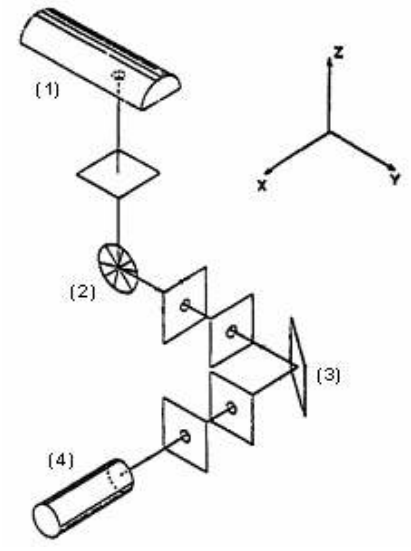

Figure-5.1.- Experimental EDXRF set-up (triaxial geometry): (1)X-ray tube, (2) secondary target in Mo, (3)sample, (4) Si (Li) detector.

To reduce the background and improve the detection limits, some special details were taken into account. A silver foil was placed between the primary X-ray beam and the secondary target to attenuate the low energy radiation of the bremsstrahlung, which does not contribute to ionize the secondary target but rather increases the continuum in the low energy range of the measured x-ray spectrum. The other important features of the system are two collimators made of silver, to collimate the secondary beam impinging on the sample. For further reduction of the background in the measured spectra that

might arise from the excitation of constructive elements of the spectrometer, special attention was paid to the collimation of the detection beam by using a pair of 1mm silver collimators at a distance of 15mm from each other. The diameters were such that only the irradiated area of the sample is "viewed" by the active area of the detector. A Si(Li) detector with a 30 mm^2 active area and 8 μm beryllium window and 135 eV resolution at 5.9 keV was used.

Quantitative calculations were performed using the fundamental parameters method [5.10]. The calibration of the whole system was carried out by measuring two biological certified reference materials: NIST SRM 1575 (Pine needles) and CRM 279 (Ulva lactuca). The X-Ray generator was operated at 50kV and 20mA and a typical acquisition time of 1000s was used.

5.2.3. Microwave wet digestion and further ICP-OES analysis

In this work, the study on powdered plant samples was carried out using two digestion mixtures commonly applied to vegetation specimens' decomposition [5.11,5.12].

All reagents used in the digestions were analytical grade Suprapur quality: hydrofluoric acid and hydrogen peroxide (Trace Select, Fluka, Gilligham, Dorset, UK); nitric acid (Suprapur, Merck, Darmstadt, Germany) and boric acid (Extrapure, Riedel-de-Haën, Germany). Ultrapure water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA) was used throughout the work.

In Dig A, 500 mg of sample was placed in a PTFE reactor with 9mL HNO_3 (65%) and 1 mL H_2O_2 (33%). When the foam caused by organic matter decomposition disappeared, the vessel was capped and heated following a two-stage digestion programme using an ETHOS PLUS Millestone microwave with HPR-1000/10S high pressure rotor (Sorisole, Bergamo, Italy). The heating procedure consists of a first step of 5 minutes to reach 180ºC and a second step of 10 minutes at 180ºC. After cooling, sample digests were transferred into a 25 mL flask and brought to volume with MilliQ water.

In the case of Dig B, 500 mg of sample were treated with 9 mL HNO_3 (65%), 1 mL H_2O_2 (33%) and 1.5 mL HF (40%); the digestion programme was the same as in Dig A. Following the decomposition with HF, extracts were additionally treated with 5 mL of 5% H_3BO_3 solution and heated during 5 min at 100 ºC to allow the

complexation of fluoride. Finally, digests were transferred into a 25 mL flask and brought to volume with MilliQ water.

The internal quality control of the digestion procedures was achieved by analysing duplicate samples and method blanks for each batch of samples run. For all the elements, the results for blanks were within 0-1% of the values measured.

A sequential inductively coupled plasma atomic emission spectrometer (Liberty RL, Varian) with a V-groove nebulizer was used for the analysis of elements studied in the plant digests. The wavelengths selected were: Zn (202.551 nm), Pb (220.353 nm), Mn (257.610 nm), Fe (259.940 nm), Ca (317.933 nm), Cu (324.754 nm), Sr (421.552 nm) and K (766.490 nm). Reagent-matched standards prepared by serial dilution of 1000 $mg \cdot L^{-1}$ Spectroscan solutions of the appropriate element were used for metal analysis in each digestion method to avoid matrix effects of the different reagents employed.

5.2.4. Statistical analysis

For all vegetation specimens studied, a comparison between the performance of the EDXRF method proposed and microwave digestion procedures was carried out by using a LR method (Linear regression method) [5.13]. On the other hand, principal component analysis (PCA) was applied in order to reveal some structure in the obtained data set. For this purpose the software program STATISTICA 4.1 was used.

5.3. RESULTS AND DISCUSSION

5.3.1. Evaluation of EDXRF method

In Figure 5.2, a representation of two measured spectra obtained with EDXRF analysis on leaves of *Betula pendula* is displayed. As it can be seen at a cursory glance, the spectra allow discriminating between a sample growing on the mining waste landfill studied from one sample growing far from the mining activities (control sample), which is significantly useful for environmental purposes. The main elements that can be identified in the vegetation samples are K, Ca, Mn, Fe, Cu, Mn, Fe, Cu, Zn, Pb and Sr.

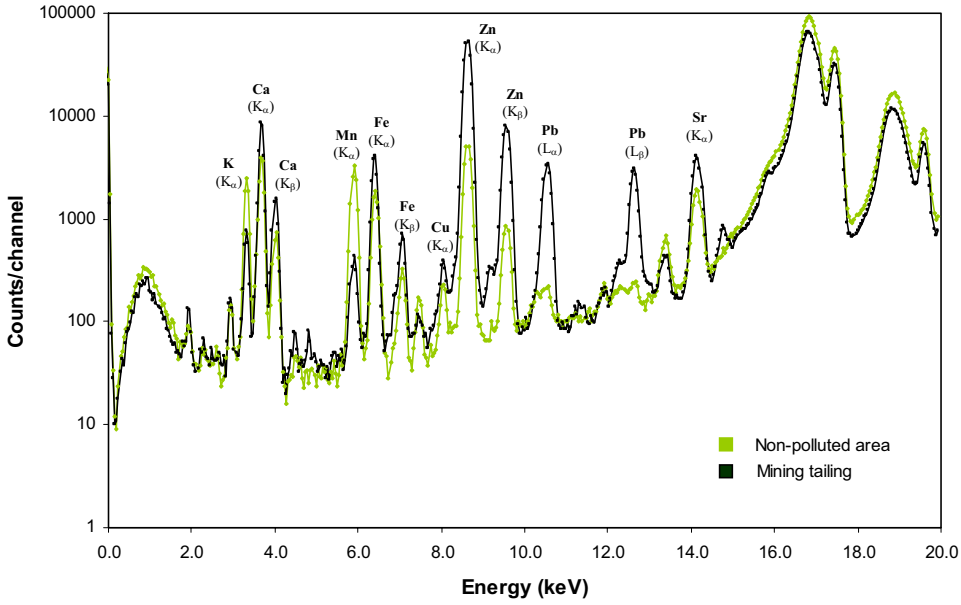

Figure-5.2.- Spectra obtained with EDXRF analysis on leaves (*Betula pendula*) collected on the mining landfill with the corresponding content in a specimen sampled far from the mining activities (Control sample).

In order to state the really capability of EDXRF for determination of these elements in vegetation specimens, analytical figures of merit of the alternative method (including precision, accuracy and limits of detection) were evaluated.

The precision and accuracy of the EDXRF proposed method was checked by analyzing a certified reference material with a matrix composition similar to real samples studied (NIST SRM 1571: "Orchard leaves" from National Institute of Standards and Technology). The results obtained from the measurement of three replicates are displayed in Table 5.1.

	K[a]	Ca[a]	Mn	Fe	Cu	Zn	Sr	Pb
Certified	1.47±0.03	2.09±0.03	91±4	300±20	12±1	25±3	37±1	45±3
EDXRF	1.5±0.3	2.1±0.3	96±5	297±10	14±3	25±0.3	35±2	44±0.3

[a] Concentrations are expressed as percentage (%).

Table-5.1.- Results of analysis of certified material NIST SRM 1571 (Orchard leaves) by EDXRF Mehod (n=3). Results are expressed as $mg \cdot Kg^{-1}$.

Good agreement was achieved between certified values and the results obtained by EDXRF in all cases, with recoveries ranging from 99 to 106%.

The limits of detection (DL) in this study, determined according to Van Grieken et al. [5.14], are given in Table 5.2.

	K	Ca	Mn	Fe	Cu	Zn	Sr	Pb
DL	10	20	4	3.1	1.2	1.2	1.1	1.1

Table-5.2.- Limits of detection ($mg \cdot Kg^{-1}$) obtained in the present work calculated according to Van Grieken et al. [5.14].

It is apparent that energy dispersive X-ray fluorescence is a sensitive technique for detection of mineral elements in vegetation samples at $mg \cdot Kg^{-1}$ concentration levels. Values of DL for K and Ca (light elements) are higher than the other elements (10 and 20 $mg \cdot Kg^{-1}$, respectively) but they are satisfactory for the intended purpose, since both elements are present at very high concentrations in plant tissues.

In view of the above points, detection limits, precision, accuracy and graphical information with EDXRF method were satisfactory for assess this kind of environmental analysis.

5.3.2. Comparison of EDXRF method with microwave wet digestion procedures

In order to compare the data obtained with the EDXRF method proposed with the results from the two microwave digestion procedures previously described, a correlation analysis with a linear regression (LR) method was carried out.

In Figure 5.3 an example of the lineal relationship found between the lead content in 32 vegetation specimens obtained with Dig A (HNO_3+H_2O_2) and with the EDXRF method is presented.

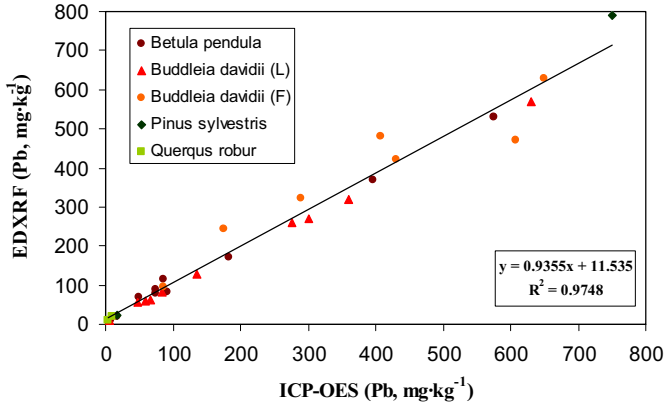

Figure-5.3.- Relationship between the results obtained with the reference method (HNO_3+H_2O_2) and with the EDXRF method for Pb (n=32).

For the comparison R^2, intercept (A) and slope (B) obtained for each element were evaluated. If the results from the different methods are equal these parameters should have the values R^2=1, B=1 and A=0.

As it is shown in Table 5.3, for Ca, Sr, Pb and Zn "A" was found not to be significantly different from 0 and "B" was found not to be significantly different from 1 for the model EDXRF=A+B* DigA. This leads to the conclusion that results obtained using the two methodologies are similar.

			Parameters for LR analysis				
Analyte	Range of concentration	Samples	R^2	A	=0	B	=1
K	4000-13870	36	0.9210	1533.8	No	0.925	Yes[a]
Ca	5300-32200	36	0.9735	-242.2	Yes[a]	1.079	Yes[c]
Mn	21-730	36	0.9952	10.899	No	1.399	No
Fe	90-2700	36	0.9754	44.307	Yes[a]	1.255	No
Cu	6-76	34	0.9698	3.990	No	1.036	Yes[a]
Zn	33-3100	36	0.9821	-21.922	Yes[a]	1.131	Yes[c]
Sr	12.8-110	35	0.9095	5.978	Yes[b]	0.953	Yes[a]
Pb	9-790	32	0.9748	11.535	Yes[a]	0.935	Yes[b]

Regression analysis: LR model, EDXRF=A+B*Dig A (A: Intercept, B: Slope).
[a] 95% confidence level, [b] 98% confidence level, [c] 99% confidence level.

Table-5.3.- Range of concentration ($mg \cdot Kg^{-1}$) of elements determined and values of parameters for LR analysis between EDXRF method and Dig A (HNO_3+H_2O_2).

On the contrary, at the same confidence levels, statistically significant differences were found for K, Mn, Fe and Cu. For these elements lower concentrations were obtained using Dig A. This fact could be explained taking into account the appreciable content of silicon in plant specimens, especially in the case of leaves. This fact is of prime importance when an efficient mineralization procedure has to be considered for these media, where Si is generally involved as silica which may be in colloidal form. In these conditions, according to the literature [5.6], some elements could not be fully recovered (especially Al, Fe, Cu and Mn) due to the binding of analytes with this silica residue [5.5]. This trend is consistent with the qualitative analysis of the solid residue from Dig A, made by scanning electron microscopy equipped with EDS analyzer, which showed a detectable signal of the elements of interest and confirmed silica as the major component.

In order to dissolve the elements which may be retained by the insoluble residue, Dig B (which includes a hydrofluoric acid treatment) was performed. LR parameters between data obtained following this procedure and EDXRF method are summarized in Table 5.4.

			Parameters for LR analysis				
Analyte	Range of concentration	Samples	R^2	A	=0	B	=1
K	4000-13870	35	0.8979	1739.9	No	0.865	Yes[c]
Ca	5300-32200	35	0.3967[d]	-	-	-	-
Mn	21-730	35	0.9895	9.5847	Yes[c]	1.244	No
Fe	90-2700	35	0.9587	105.521	No	1.164	No
Cu	6-76	30	0.9678	-0.1988	Yes[a]	1.312	No
Zn	33-3100	35	0.9648	-60.194	Yes[a]	1.319	No
Sr	14-110	32	0.7262[d]	-	-	-	-
Pb	5-790	34	0.9881	11.514	Yes[b]	1.035	Yes[a]

Regression analysis: LR model, EDXRF=A+B*Dig B (A: Intercept, B: Slope).
[a] 95% confidence level, [b] 98% confidence level, [c] 99% confidence level.
[d] R^2 is significantly different from 1.

Table-5.4.- Range of concentration ($mg \cdot Kg^{-1}$) of elements determined and values of parameters for LR analysis between EDXRF method and Dig B (HNO_3+H_2O_2+HF).

As can be seen, significant differences between the two methodologies exist in all the elements (except for Pb). It is interesting to note that after applying Dig B a new precipitate, mainly composed by calcium and fluoride, appeared (Figure 5.4). According to that, the poor recoveries of elements of interest could be explained by co-precipitation of the analytes with this solid residue.

Figure 5.5 corroborates the precipitation of Ca using Dig B as decomposition procedure. It seems that when concentration of calcium exceeds a certain value (approximately 8500 $mg \cdot Kg^{-1}$) the precipitation of this element takes place, and then the differences between results from EDXRF measurement and employing Dig B increase dramatically.

These results highlight that the method of matrix destruction used strongly depends on the chemical composition of the sample and on the element to be determined. According to that, to obtain reliable analytical results using classical destructive analytical methods, the study and use of a suitable digestion procedure for each specific matrix and analyte under study should be performed. The latter

becomes quite unfeasible in environmental studies, where several plant species are used as pollution indicators of different elements.

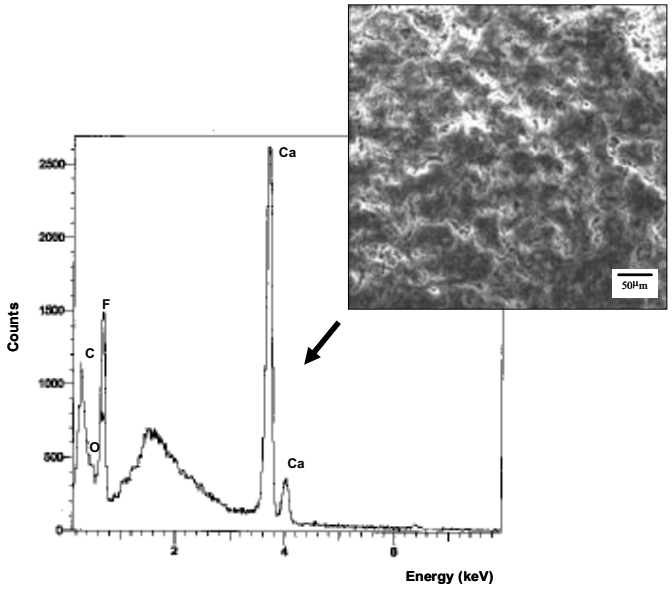

Figure-5.4.- Secondary electron image and spectrum of solid residue obtained after Dig B (HNO_3+H_2O_2+HF) by SEM-EDS.

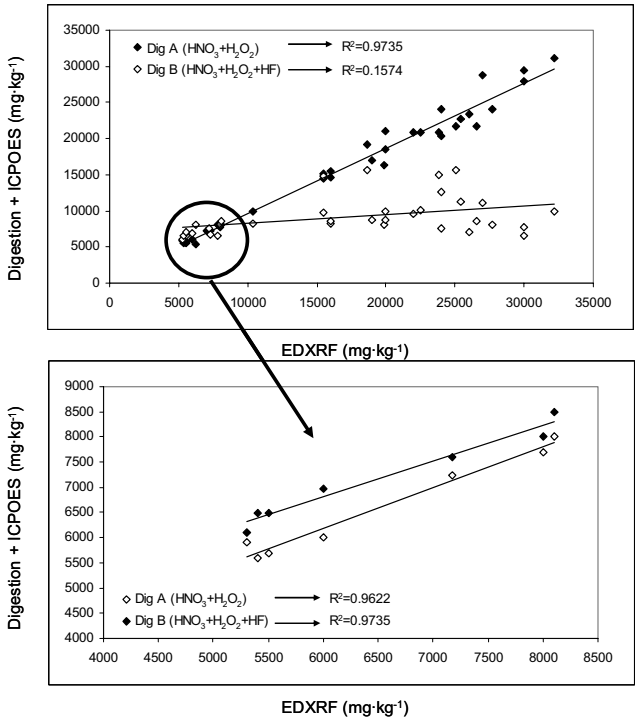

Figure-5.5.- Correlation plot of the Ca concentration (mg·Kg^{-1}) in the digests (Dig A and Dig B) analysed by ICP-OES (y) versus EDXRF (x).

A comparison of the precision achieved with the non-destructive technique and the two digestion protocols was also performed (Figure 5.6). As shown, relative standard deviations (RSD) obtained with EDXRF method were satisfactory with values for all elements <8%, and in most cases <5%. Although these values are higher than those from wet digestion procedures (mainly due to sample inhomogeneities) they are good enough for monitoring purposes. It is also relevant to notice that the RSD values obtained in this work are similar to other reported values involving X-ray fluorescence techniques [5.15,5.16].

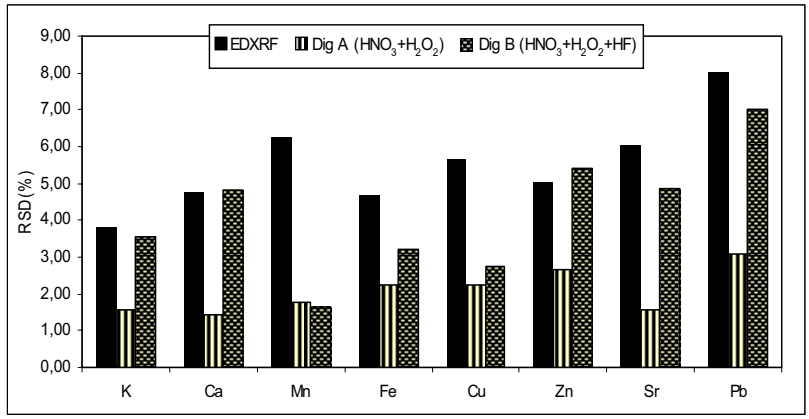

Figure-5.6.- Average of relative standard deviation (RSD) values obtained for each element by using EDXRF method and microwave-assisted decomposition (Dig A and Dig B).

5.3.3. Interpretation of the results in the environmental context

The elemental content for the different species of vegetation determined by EDXRF is shown in Table 5.5. Concentration values obtained are similar to those obtained for other vegetation species from analogous Pb /Zn mining areas [5.17,5.18].

(A)		K	Ca	Mn	Fe	Cu	Zn	Sr	Pb
Betula pendula	**Leaves**	6000	23800	170	470	16	2500	67	160
Buddleia davidii	**Leaves**	8000	23900	60	600	26	560	59	180
Buddleia davidii	**Flowering**	9400	6100	30	1200	54	560	24	300
Quercus robur	**Leaves**	7500	11800	200	130	9	200	23	14
Pinus sylvestris	**Needles**	7900	7200	400	820	25	500	16	400

Concentrations are expressed as mean of all vegetation specimens collected at the mining landfill: *Betula pendula* (n=10), *Buddleia davidii* (n=10), *Quercus robur* (n=2), *Pinus sylvestris* (n=2).

(B)		K	Ca	Mn	Fe	Cu	Zn	Sr	Pb
Betula pendula	**Leaves**	12200 (s=200)	10400 (s=400)	670 (s=10)	220 (s=3)	11.3 (s=0.5)	225 (s=3)	30 (s=2)	8 (s=1)
Buddleia davidii	**Leaves**	13000 (s=900)	18600 (s=300)	50 (s=6)	270 (s=30)	15 (s=2)	33 (s=2)	50 (s=4)	6 (s=1)
Buddleia davidii	**Flowering**	13900 (s=20)	7800 (s=40)	30 (s=1)	670 (s=30)	38 (s=3)	43 (s=4)	25 (s=2)	6 (s=1)
Quercus robur	**Leaves**	8500 (s=30)	16000 (s=300)	470 (s=20)	90 (s=1)	8.5 (s=0.1)	36 (s=3)	26 (s=2)	5 (s=1)
Pinus sylvestris	**Needles**	6200 (s=20)	18000 (s=300)	400 (s=20)	210 (s=10)	2 (s=0.5)	260 (s=5)	47 (s=3)	<DL

Table-5.5.- Elemental content ($mg \cdot Kg^{-1}$) for the different vegetation species collected at the mining area (A) and far from the mining activities (control sample) (B), determined by EDXRF.

For the comparison of the elemental content of species from the mining area (A) with the corresponding metal content in plants growing at the clean region (B), the relative absorption coefficients (RAC: ratio of the content in plant growing on a supposed contaminated area / content in non-affected plant) were calculated. From the results, we can conclude that an accumulation process occurred for all the species collected at the mining waste landfill, mainly for Pb and Zn. Relative absorption coefficients of these elements reach values up to 400 to 17 for Pb and Zn respectively. This accumulation tendency can also be observed from the PCA analysis of the results of all the samples studied collected at the mining landfill (n=34) using relative absorption coefficients for each element as variables. Figure 5.7 shows a combined diagram obtained overlapping scores and loadings plots for the main two factors extracted (PC1, PC2), representing 94.6 % of the total variance. It is apparent that all the vegetation specimens collected at the mining area tend to cluster together near the Pb and Zn variables and far from the other elements. This trend suggests the potential Pb and Zn accumulation in the studied species.

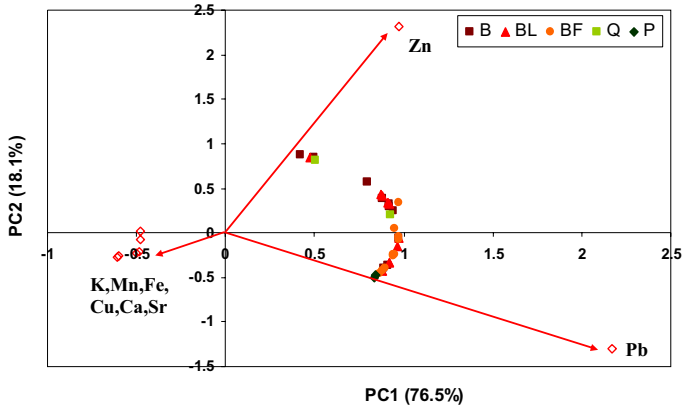

Figure-5.7.- Biplot (loadings + scores) of the two first PCs. Notes: (B)*Betula pendula*, (BL)*Buddleia davidii* leaves, (BF)*Buddleia davidii* flowers, (Q)*Quercus robur*, (P)*Pinus sylvestris*.

On the other hand, as already quoted in the study of *Betula pendula* specimens (Chapter 3), metal accumulation seems to be directly associated with a decrease in nutritional element contents (e.g. K and Ca) as it can be deduced by comparing the concentrations obtained for the specimens collected at the mining area with those from the control sample (Table 5.5, Figure 5.2). Analogous experience was reported by other authors who state that interactions of chemical elements may also refer to the ability of one element to inhibit or simulate the absorption of other elements in plants [5.18].

Finally, in order to visualize data structure, a PCA analysis on element content of all samples studied was carried out. Prior to statistical analysis, the variables (element contents) were standardized so as to compensate for the different scales. Three significant components were extracted for the data set (73% of the total variance), see Figure 5.8.

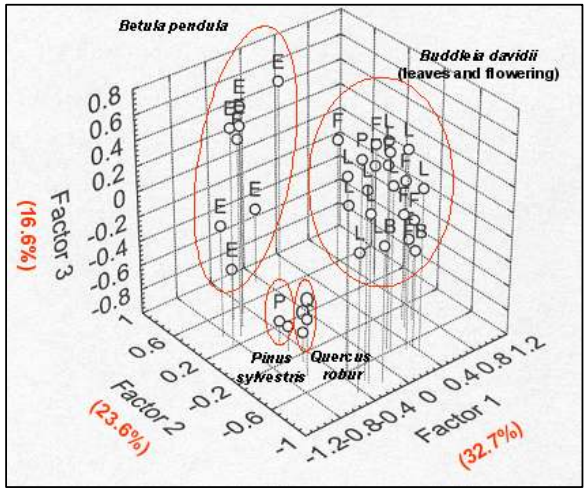

Figure-5.8.- PCA analysis on element content of vegetation samples collected (n=34).

The ordination of the samples by factor scores shows that by monitoring the eight elements determined (K, Ca, Fe, Mn, Cu, Sr, Pb, Zn) it is possible to distinguish between the different vegetation species studied.

5.4. CONCLUSIONS

In this study the usefulness of a non-destructive methodology based on EDXRF for the simultaneous determination and monitoring of some major and minor elements (K, Ca, Fe, Mn, Cu, Sr, Pb, Zn) in different vegetation species growing on a mining landfill has been tested.

The precision and accuracy of the proposed method was checked by analyzing a reference plant material (NIST SRM 1571: Orchard leaves). Good agreements were achieved between certified values and data obtained, with recoveries ranging from 99 to 106%.

A comparison of elemental content determined by EDXRF method with those obtained by employing two routine microwave assisted digestions commonly applied to vegetation specimens decomposition (Dig A: HNO_3+H_2O_2 and Dig B: HNO_3+H_2O_2+HF) plus ICP-OES has been performed. Statistical evaluation shows that similar results were obtained for Pb, Zn, Sr and Ca using Dig A procedure. The opposite trend was observed for K, Mn, Fe, Cu which present a lower content in the digests. Additional investigations indicate that these discrepancies were caused by the presence of a silica residue. The addition of HF (Dig B) neither brings these elements into solution. A solid residue is formed mainly composed by Ca and F according to the results obtained by Scanning Electronic Microscopy (SEM).

Taking into account these facts, it could be concluded that sample decomposition procedure used influences the results of element determination and this depends on the analyte of interest and the main matrix element composition of the sample. In contrast, the EDXRF method proposed proved to be an effective tool for metal determination in vegetation specimens since it allows obviating the matrix destruction stage and the poor recoveries of some elements. Moreover, this method is less time consuming, requires less amounts of reagents and it is easy to control because almost does not require supervision. This is of great importance in environmental studies where conclusions are based on the analysis of a large number of samples.

From data obtained about metal content in vegetation specimens collected on the mining waste landfill, it could be concluded that a metal accumulation process (mainly of Pb and Zn) occurred in all the species studied, and seems to be directly associated with an exclusion of K and other nutritional elements. The PCA analysis

suggests that K, Ca, Mn, Fe, Cu, Sr, Pb and Zn contents allow distinguishing between the different vegetation species studied.

Keeping in mind all the results obtained, it can be concluded that XRF technique is a convenient analytical technique for the simultaneous determination of chemical composition in different vegetation species as an alternative to the classical destructive analytical methods.

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CHAPTER 6

Suitability of WDXRF for multi-elemental analysis of vegetation samples

A methodology based on WDXRF was evaluated as an useful analytical tool for the sequential determination of some major elements (Na, Mg, Al, P, S, K, Ca), trace elements (Mn, Fe, Co, Zn, As) and non-essential elements (Sr, Pb) in vegetation matrices.

The combination of plant certified reference materials and several synthetic calibrators made of cellulose to simulate the vegetation matrix appears to be an effective means to obtain reliable calibration curves with a good spread of data points over the range of each element to be determined. The achieved results demonstrate that the proposed methodology provides, in most cases, sufficient accuracy and precision for analysing vegetation samples in environmental studies.

The contents of this chapter are published in:

E.Marguí, M.Hidalgo, I.Queralt, *Multielemental fast analysis of vegetation samples by wavelength dispersive X-Ray fluorescence spectrometry: Possibilities and drawbacks.* Spectroc. Acta Pt.B-Atom. Spectr. 60 (2005) 1363-1372.

6.1. INTRODUCTION

As pointed out in the last chapter, XRF technique could be a tentative approach for the determination and quantitation of chemical composition in vegetation specimens as an alternative to the classical destructive analytical methods [6.1].

In general, WDXRF quantitative analysis is carried out by the calibration curve method, obtained with many calibrators. However, for some applications (such as plant specimens) it is difficult to get sufficient certified standards, with matrices similar to those of the studied samples, in order to achieve a good spread of data points over the range of mass fraction for each element to be determined [6.2]. In such cases, the use of standard-less quantitative procedures based on the Fundamental Parameter algorithm are commonly applied [6.3]. On the other hand, the utilisation of standards prepared in the laboratory with commercially available pure elements or compounds has proven to be an efficient alternative for calibration purposes since they are inexpensive and can be easily prepared [6.4]. Plant material consists mainly of C, N, H and O (cellulose matrix). According to that, the use of several synthetic standards made of cellulose could provide a good mean to simulate the vegetation matrix and to obtain reliable calibration curves.

In the case of vegetation samples, the absorption of the measured X-rays by the matrix is relatively small compared to other heavier matrices (i.e., rock or soil samples). Nevertheless, due to the widely variable range of concentrations of K, Ca, Cl and other elements in plant samples, in most cases, correction for the inter-elemental effects have to be done in order to obtain quantitative results. Several methods have been described for matrix effect corrections [6.5]. Among them, the use of the influence coefficients as correction method has been successfully applied in different plant materials [6.6]

The aim of this work was to study the applicability of a quantitative WDXRF (Wavelength-Dispersive X-Ray Fluorescence) method, based on the use of several synthetic cellulose calibrators and the matrix correction method using influence coefficients, for the sequential determination of some major elements (Na, Mg, Al, P, S, K, Ca), trace elements (Mn, Fe, Co, Zn, As) and non-essential elements (Sr, Pb) in vegetation specimens.

Furthermore, the analytical performance and analytical figures of merit, including the limit of determination of the proposed method (LDM) as well as trueness,

repeatability and reproducibility over the time were evaluated to state the capability of the WDXRF method for environmental analytical purposes.

6.2. EXPERIMENTAL

6.2.1. Synthetic calibrators and certified reference materials

The cellulose used to simulate the vegetation matrix was a high-purity product (Cellulose powder, Sigma-Aldrich, Buchs SG, Schweiz) in order to avoid the addition of contaminants. The latter results are of special importance in the case of trace element determinations.

For each synthetic calibrator 10 g of microcrystalline cellulose were employed. Then an appropriate amount of analyte solution was added and the mixture was carefully shaken. Stock solutions of 1000 ± 0.5 $mg \cdot L^{-1}$ (Spectroscan, TECKNOLAB A/S, Norway) were used to prepare spiking solutions concerning to Al, Mn, Fe, Co, Zn, As, Sr and Pb. Whereas, in the case of major elements (Na, Mg, P, S, K, Ca) solutions prepared from analytical grade salts and verified by ICP-OES quantitation were employed ($MgCl_2 \cdot 6H_2O$, KH_2PO_4, K_2SO_4, $Ca(CO_3)_2$; PA-ISO Panreac, S.A.Spain). After drying in an oven at 65-70ºC until constant weigh (about 72 h), the spiked cellulose was homogenized in an agate mortar. Then the calibrators were stored in polypropylene containers and placed into a dessicator until analysis.

The quality of the calibrator's preparation was pursued by cleaning the walls of the container where the calibrators were prepared and checked by metal determination of the extracts, so as to study possible metal loses during the spiking procedure.

Besides the synthetic cellulose standards prepared, four certified reference materials were used for calibration purposes: NIST 1575 "Pine needles" from National Institute of Standards and Technology, USA; GBW07603 "Bush branches and leaves", GBW07604 "Poplar leaves", GBW07605 "Tea" from the National Research Centre for Certified Reference Materials, Beijing, China. These reference materials differ considerably both physically and chemically in order to assure the applicability of the WDXRF method developed to different vegetation tissues, including branches, needles and leaves.

The reference material GBW07602 "Bush branches and leaves" from the National Research Centre for Certified Reference Materials (Beijing, China) was employed to

check the trueness and to estimate the uncertainty of the results. In all cases, the reference standards were supplied in the form of finely ground powder.

6.2.2. Samples studied

Analysis of elemental content in three higher plants species (*Buddleia davidii, Betula pendula, Pinus sylvestris*) was carried out using the WDXRF method developed. These samples were collected from the surroundings of the treatment factory of Pontaut (Val d'Aran mining district) and also from an uncontaminated soil in the same area.

Once at the laboratory, leaves and flowerings samples were washed thoroughly with deionized water and oven-dried at 55 ± 5ºC for 24 h. To reduce particle size they were ground in an agate ball mixer mill for 2-5 minutes. Once the plant tissues were powdered and dried, they were kept in labeled capped polypropylene containers until analysis.

6.2.3. Wavelength dispersive X-ray fluorescence analysis (WDXRF)

Considering the morphology of plant powder and the capacity to be compacted together, the preparation of pellets was performed without addition of a binder. As reported in the literature [6.7], constant pressure and weight have to be applied in order to take into account only analyte concentrations and matrix effects as factors of variability of XRF intensities. Methodology used in the present study consist on weighing 2 g of powdered sample and pressing it at 20t for 60s to obtain a cylindrical pellet of 40 mm in diameter. This procedure was employed both for synthetic cellulose calibrators and plant samples.

Samples were analyzed using a commercial WDXRF instrument (Bruker S4 Explorer) equipped with an Rh anode X-ray tube (50kV, 20mA), four analyzer crystals (OVO-B, OVO-55, LiF 200 and PET) and with two different detectors: a flow proportional counter for light elements and a scintillation counter for heavy elements.

This equipment allows the determination of a wide elemental range (from Be to U) with a typical measuring time of 20-60 s per element. Energy resolution and efficiency for each analytical line were determined both by collimator aperture and analyzer crystal used (i.e., resolution for Cu K_α line was 40eV). In this work,

analyses were made in helium atmosphere to avoid signal attenuation due to air absorption.

In a first stage, all the samples were analyzed to obtain the whole spectra at standard conditions, which were studied to determine elemental composition, sensitivities and detection limits. Secondly, a quantitative model was designed in order to improve the elemental peaks signal for each element to be determined. Both tasks were carried out using the software provided with the instrument (Spectra Plus, Bruker/AXS).

6.3. RESULTS AND DISCUSSION

6.3.1. Automatic qualitative analysis and spectral identification

Automatic qualitative analysis and spectral identification was carried out by using the qualitative scanning mode linked to the equipment, which includes automatic peak and element identification. Listed in Table 6.1 are the instrumental measuring conditions used.

Z	Element	Line	Crystal[a]	Detector[b]	Collimator (º)	kV	mA
11	**Na**	Kα	OVO-55	FPC	0.46	20	50
12	**Mg**	Kα	OVO-55	FPC	0.46	20	50
13	**Al**	Kα	PET	FPC	0.46	20	50
15	**P**	Kα	PET	FPC	0.46	20	50
16	**S**	Kα	PET	FPC	0.46	20	50
19	**K**	Kα	LiF200	FPC	0.46	50	20
20	**Ca**	Kα	LiF200	FPC	0.46	50	20
25	**Mn**	Kα	LiF200	SC	0.23	50	20
26	**Fe**	Kα	LiF200	SC	0.23	50	20
27	**Co**	Kα	LiF200	SC	0.23	50	20
30	**Zn**	Kα	LiF200	SC	0.23	50	20
33	**As**	Kα	LiF200	SC	0.23	50	20
38	**Sr**	Kα	LiF200	SC	0.23	50	20
82	**Pb**	Lβ	LiF200	SC	0.23	50	20

Counting time (min/step)=0.1
[a] OVO-55:W/Si multilayer, PET: Pentaerythrite, LiF200: Lithium fluoride
[b] FPC: Flow proportional counter, SC: Scintillator counter.

Table-6.1.- WDXRF conditions used in the scanning mode (standard measuring conditions).

This kind of analysis enables the analyst to obtain multi-elemental information and appears to be an effective means for primary screening of the studied sample.

Figure 6.1 shows the spectrum obtained from analysis of the reference material NIST-1575 "Pine needles" and a cellulose blank (without element spiking). As it can be seen, the profile of the obtained spectrums is not significantly different leading to the conclusion that the instrument response fro both matrices is similar.

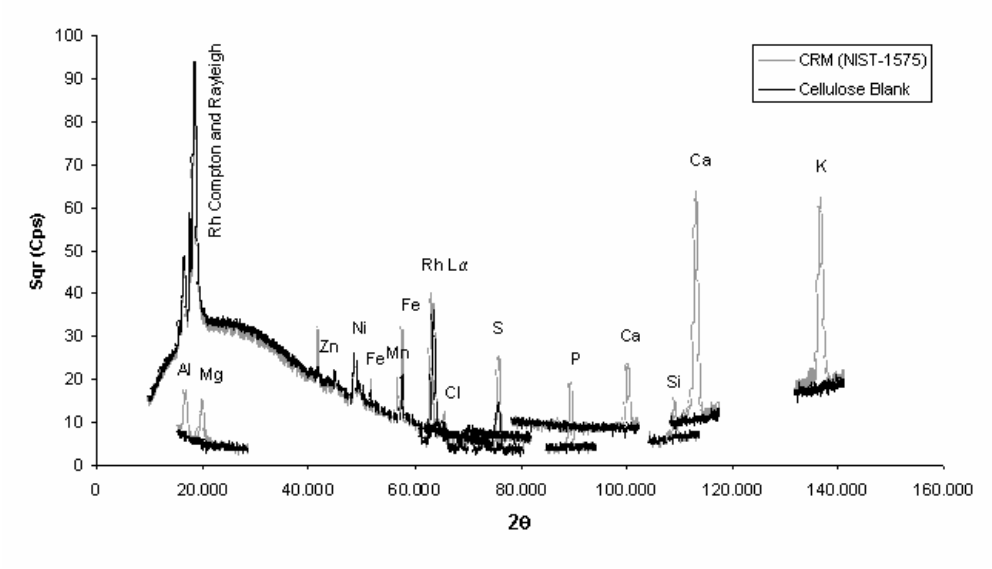

Figure-6.1.- Spectrum obtained from analysis of the reference material NIST-1575 (Pine needles) and a cellulose blank (without element spiking) by using the qualitative scanning mode.

The spectrum of cellulose (considered as the analytical "blank") exhibit identifiable small peaks corresponding to Fe and Zn due to background signal arising from the excitation of these elements in some parts of the spectrometer (instrumental blanks).

On the other hand, a detectable peak was also found for S, surely as a trace in the cellulose employed (sample blank). Carefully characterization of this blanks was performed (n=9) and the value of these blanks contributions were subtracted from the measured intensity of each element.

6.3.1.1. Sensitivity

An important parameter to assess the capability of one technique to analyze a given analyte in a given specimen is the sensitivity of the instrument. In WDXRF practice it is defined as the net intensity obtained per unit of concentration [6.8]. According to that, after measuring the net intensities for the plant reference material

(GBW07603 "Bush branches and leaves") and a cellulose calibrator spiked with 0.1% for 11<Z<20 and 0.05% for 25<Z<82, the experimental sensitivities for each element were estimated (see Figure 6.2). It is important to note that sensitivity does not differ significantly between a plant specimen and a synthetic cellulose calibrator and in both cases it is found that the sensitivity falls off towards the long wavelength limit of the spectrometer (light elements) mainly due to low fluorescence yields and the increased attenuation of low energy characteristic radiation [6.9] . The discontinuities observed result from the use of different analyzing crystals; excitation conditions, etc. (see Table 6.1).

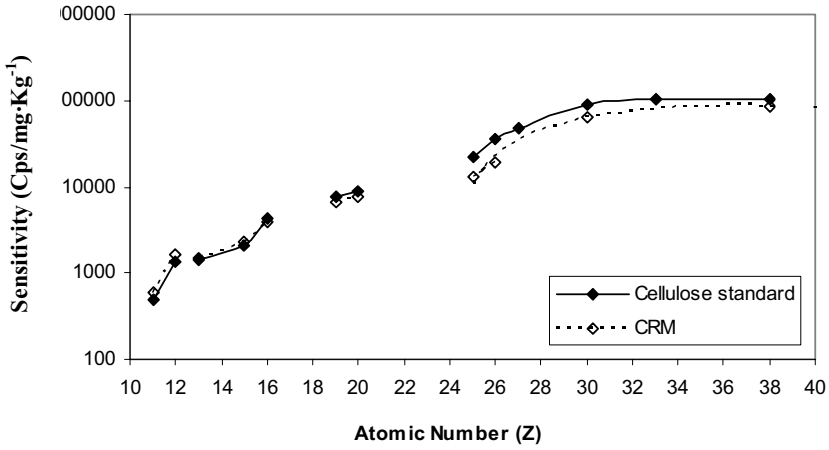

Figure-6.2.- Elemental sensitivity calculated using the qualitative scanning mode for a synthetic cellulose calibrator and the certified reference material GBW 07603 (Bush branches and leaves).

6.3.1.2. Instrumental limit of detection (ILD)

The smallest concentration of the analyte that can be detected in a specimen has to be determined to check if the instrument is sensitive enough to detect all the elements of interest. The instrumental limit of detection (ILD) is defined as the minimum mass fraction of an analyte that can be detected by an instrument in a given analytical context with a 99.95% confidence level [6.10]. Commonly, it is expressed as:

$$ILD = \frac{4.65}{S_i}\sqrt{I_b / T_b} \qquad (6.1)$$

Where S_i: Sensitivity (Cps/concentration), I_b: background intensity (counts) and T_b: background measuring time (s).

It is worth mentioning that this expression takes into account, as an approximation, the square root of the background intensity (I_b) divided by the

background measuring time (T_b), instead of the real fluctuations of the background noise (σ_b):

$$ILD = \frac{4.65}{S_i}\sigma_b \tag{6.2}$$

In the present work, a comparison of the results obtained by using both expressions (6.1 and 6.2) was carried out. Figure 6.3 shows the measurements of peak (I_p) and background (I_b, σ_b) used in ILD calculations.

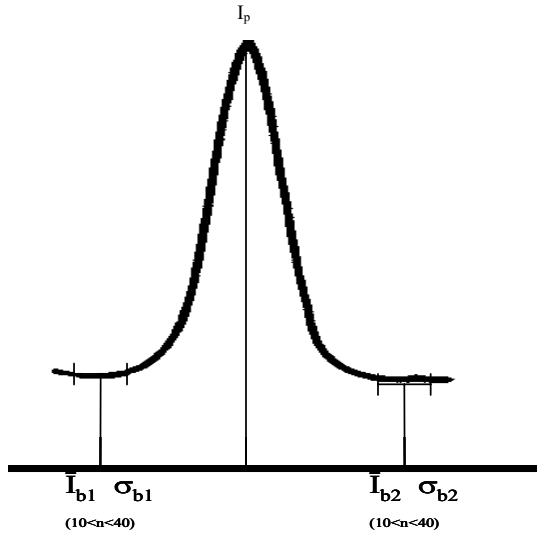

Figure-6.3.- Peak and background measurements used in ILD calculation

(Qualitative scanning mode).

In the expression (6.2), the σ_b is related to the standard deviation calculated from several background intensity measurements (10<n<40) performed in both sides of analyte peak. The value obtained using this approach provides a more realistic estimation of the capability of the instrument to distinguish a peak intensity from the fluctuations of the background noise.

As it can be deduced from the above expressions, the ILD varies with the atomic number (Z) and instrument configuration employed since these parameters are related to the sensitivity. In Figure 6.4 are displayed the results obtained in ILD

calculations using expressions (6.1) and (6.2) for the plant reference material (GBW07603 "Bush branches and leaves") and a cellulose calibrator spiked with 0.1% for 10<Z<21 and 0.05% for 24<Z<83.

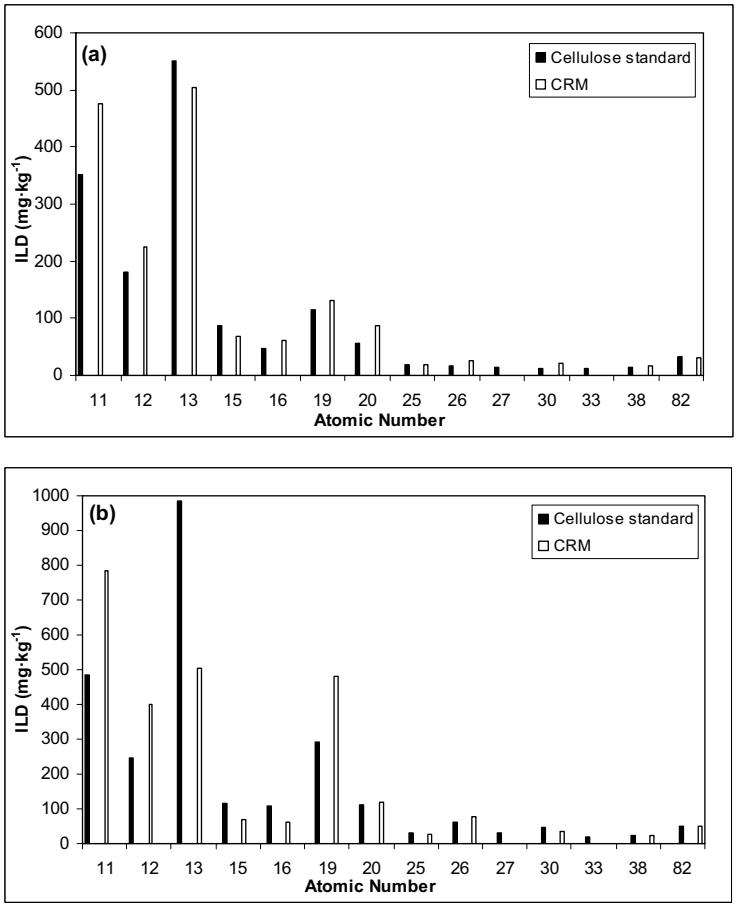

Figure-6.4.- Comparison of elemental instrumental detection limits (Qualitative scanning mode) for a synthetic cellulose calibrator and the certified reference material GBW07603 "Bush branches and leaves": (a) $ILD = \frac{4.65}{S_i}\sqrt{I_b / T_b}$ and (b) $ILD = \frac{4.65}{S_i} \times \sigma_b$

By comparing Figure 6.4 (a) and Figure 6.4 (b), it can be concluded that ILD calculated by using the common applied expression (6.1) yields lower ILD values than those observed taking into account the real background noise (6.2).

As already quoted in the previous section, the sensitivity of the X-ray spectrometer decreased substantially towards the long wavelength extreme and as a result, poorer detection limits are found at this region which corresponds to the lower atomic numbers. The ILD for light elements are at levels of hundredths of one percent rather than parts per million for elements with Z>25.

Slightly lower ILD values are achieved for a synthetic cellulose standard than for a real vegetation specimen (CRM), particularly concerning to Na and Mg. This trend could be explained taking into account a possible small variation in background intensity in both matrices.

In any case, it must be kept in mind that the ILD values obtained with the qualitative scanning mode (standard measuring conditions) are only an approximation of the instrument capability to detect an element in a particular sample type, but they can be improved using high-sensitivity measuring conditions (increasing the counting time, changing the analyzer crystal or collimator and by introducing appropriate filters).

6.3.2. Quantitative analysis

Once qualitative analysis was carried out and taking into account the typical element contents in the plant reference materials employed and the experimental data from Chapter 5 (see Table 6.2), a quantitative XRF method was implemented based on the empirical calibration mode.

Calibration curves were established using four plant reference materials and several synthetic cellulose standards spiked with appropriate amounts of analytes. The concentration range for each element in standards is given in Table 6.3. Combination of certified and synthetic standards enables a suitable concentration range but also a good spread of calibration data points over the range of each element determined.

	Certified reference materials[a]	Mining environment[b]
Na	44-19 600	-
Mg	1700-4800	-
Al	1000-3000	-
P	800-2900	-
S	2400-7300	-
K	8500-16 600	4000-14 000
Ca	4300-22 200	4000-34 000
Mn	45-1240	10-800
Fe	260-1100	50-3000
Co	0.18-0.42	-
Zn	20-55	20-3500
As	0.28-1.25	-
Sr	15-350	10-120
Pb	1.5-50	5-1000

[a] GBW07602, GBW07603, GBW07604, GBW07605, NIST1575.
[b] Experimental data (Chapter 5).

Table-6.2.- Element contents ranges ($mg \cdot Kg^{-1}$) in different vegetation specimens.

	Na	Mg	Al	P	S	K	Ca	Mn	Fe	Co	Zn	As	Sr	Pb
Blank	-	-	-	-	-	-	-	-	-	-	-	-	-	-
STD-1	-	0.1000	-	0.0500	0.1570	-	0.4000	-	0.0050	0.0005	0.0010	0.0005	0.0010	0.0005
STD-2	0.1001	-	-	-	-	0.1266	-	-	-	-	-	-	-	-
STD-3	-	0.1000	-	-	0.1000	-	-	-	0.1000	-	0.1000	-	-	-
STD-4	-	-	0.1000	-	-	-	0.1000	-	-	-	-	-	0.1000	-
STD-5	-	-	-	-	-	-	-	0.1000	-	-	-	-	-	0.1000
STD-6	2.0084	-	0.3000	0.1570	-	2.0163	-	-	-	0.0050	-	0.0050	-	-
STD-7	-	0.5780	-	-	1.4919	-	-	-	-	-	0.3500	-	-	-
STD-8	-	-	-	-	-	-	-	-	0.3000	-	-	-	0.0350	0.1000
STD-9	-	-	-	-	-	-	3.6050	0.1500	-	-	-	-	-	-
STD-10	0.0500	-	0.0500	-	-	0.0633	0.0500	-	-	-	-	-	0.0500	-
STD-11	-	0.0500	-	-	-	-	-	0.0500	0.0500	-	0.0500	-	-	0.0500
STD-12	1.0042	0.2890	0.1500	0.0785	0.7460	-	-	-	-	0.0025	0.1750	0.0025	-	-
STD-13	-	-	-	-	-	-	1.8025	0.0750	0.1500	-	-	-	0.0175	0.0500
STD-14	0.5021	0.1445	0.0750	-	0.3730	-	0.9013	0.0375	0.0750	0.0013	0.0875	0.0013	0.0088	0.0250
STD-15	1.5063	-	0.2250	0.1178	-	1.5122	-	-	-	0.0038	-	0.0038	-	-
STD-16	-	0.4335	-	-	1.1189	-	-	-	-	-	0.2625	-	-	-
STD-17	-	-	-	-	-	-	-	-	0.2250	-	-	-	0.0263	0.0750
STD-18	-	-	-	-	-	-	2.7038	0.1125	-	-	-	-	-	-
STD-19	-	-	-	-	-	-	-	0.0100	0.0100	-	0.0100	-	-	0.0100
GBW07603	1.9600	0.4800	0.2000	0.1000	0.7300	0.9200	1.6800	0.0061	0.1070	-	-	-	0.0246	0.0047
GBW07604	-	0.6500	0.1040	0.1680	0.3500	1.3800	1.8100	0.0045	0.0274	-	0.0037	-	0.0154	-
GBW07605	-	0.1700	0.3000	0.2840	0.2450	1.6600	0.4300	0.1700	0.0264	-	0.0026	-	0.0015	-
NIST-1575	-	0.1180	0.0545	0.1200	0.1250	0.3700	0.4100	0.0675	0.0200	-	0.0065	-	0.0005	0.0011

Table-6.3.- Synthetic cellulose standards and certified reference materials used for calibration of the WDXRF method. Concentrations are expressed as %.

The instrumental conditions used in the quantitative analysis were selected so as to achieve the best signal/background ratio (SBR). According to that, some modifications of conditions used in the scanning mode (Table 6.1) were performed, including the use of a W/Si multilayer crystal (OVO-55) for the determination of Al, a wider collimator aperture (2º) for P determination and finally, a different potential/intensity ratio in the case of K (40kV and 25mA).

Concerning the counting time used for each element, it was chosen as a compromise between SBR and global analysis time. Thirty seconds were finally selected for all element determinations except for Co, Zn and Pb which were analyzed for 100s.

In Table 6.4 the calibration data obtained for vegetation matrices is presented.

Z	Element	Calibration Range (%)	Nº Std.[a]	R^{2}[b]	Sensitivity (kCps/%)[c]	SEC[d]	Influence coefficients
11	**Na**	0.05-2.01	7	0.9984	2.4	0.03	S Kα / Zn Kα
12	**Mg**	0.05-0.65	11	0.9934	13.4	0.02	Na Kα / S Kα
13	**Al**	0.05-0.3	10	0.9894	25.6	0.001	Mg Kα / P Kα / S Kα
15	**P**	0.05-0.2840	8	0.9168	1877.0	0.02	S Kα / Ca Kα / K Kα
16	**S**	0.01-1.49	10	0.9962	28.5	0.02	P Kα / Ca Kα / K Kα
19	**K**	0.063-2.02	8	0.9919	70.5	0.07	P Kα / S Kα / Ca Kα
20	**Ca**	0.05-3.61	11	0.9946	59.7	0.09	K Kα / S Kα
25	**Mn**	0.005-0.17	11	0.9324	310.8	0.01[e]	Fe Kα / Ca Kα
26	**Fe**	0.005-0.3	12	0.9960	324.7	0.006	Mn Kα / Ca Kα
27	**Co**	0.0005-0.005	5	0.9741	283.8	0.0003	No correction
30	**Zn**	0.001-0.35	11	0.9913	454.5	0.01[e]	Fe Kα
33	**As**	0.0005-0.005	5	0.9857	668.1	0.0003	Pb Lβ
38	**Sr**	0.0005-0.1	11	0.9968	851.5	0.001	No correction
82	**Pb**	0.0005-0.1	10	0.9990	291.2	0.001[e]	As Kβ

[a] number of calibrators employed for calibration purposes
[b] correlation coefficient
[c] calibration curve slope
[d] standard error of calibration (%)
[e] using a more limited calibration range (recommended in trace analysis) the SEC values decrease dramatically: Mn (0.005-0.0375%) SEC=0.004; Zn (0.001-0.05%) SEC=0.0006; Pb (0.0005-0.025%)=0.0005.

Table-6.4.- Calibration data for vegetation matrices.

The standard error of calibration (SEC) was derived from linear regression by least-squares fit and in all cases was acceptable according to the concentration range studied.

A matrix correction method using influence coefficients was performed by means of the computerized routine program linked to the equipment ($SPECTRA^{plus}$). The model was based on the Sherman equation and the corrected concentration was calculated according to the following expression:

$$C_i = C_{oi} + m_i I_i \left(1 + \sum_j \alpha_{ij} C_j \right) \tag{6.3}$$

Where C_i: analyte concentration, C_{0i}: intercept, m_i: slope, I_i: analyte intensity, α_{ij}: inter-elemental influence coefficient, C_j: interfering element concentration.

Both the concentration directly converted from measured intensities (C_{direct}) and those obtained after matrix correction ($C_{corrected}$) are compared in Figure 6.5 with the chemical concentration of the calibrators for a major element (S), a minor element (Fe) and a trace element (Pb). Straight lines (slope=1) represent perfect correlation between chemical and XRF concentrations. Results demonstrate the linearity over the whole concentration range studied and also the suitability of the matrix correction method employed.

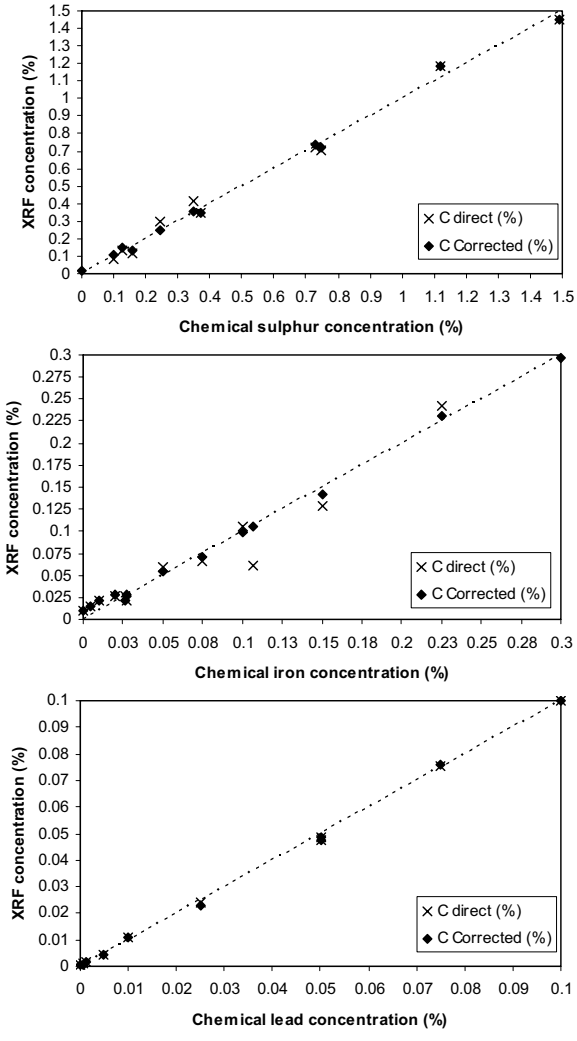

Figure-6.5.- Comparison between C_{direct}, $C_{corrected}$ (determined by XRF) and chemical concentration: (a) sulfur (major element); (b) iron (minor element); (c) lead (trace element).

The limit of determination of the method (LDM) was evaluated for each element. It is defined as the smallest concentration of an analyte in a given sample that can be reliably quantified in practice with a 95.4% confidence level [6.8]. It takes into account the errors introduced by sample preparation, instrument and counting statistics and it is quantitatively expressed as the concentration of an element equivalent to two standard deviations of a set of determinations of the same representative concentration:

$$LDM = 2\sqrt{\frac{\sum_{m=1}^{n}\left(C_n - \bar{C}\right)^2}{n-1}} \qquad (6.4)$$

As it can be seen, LDM value varies according to the concentration present in the specimen on which measurements are made. For this reason, a good practice is to select a representative sample of the set of samples to be analyzed. In our case, the LDM was calculated from a series of ten replicate specimens (n=10) prepared from the same representative sample (flowering of a *Buddleia davidii* specimen) in the same experimental conditions. The LDM values provided in Table 6.5 are suitable both for minor and major elements determination in vegetable samples.

	Concentration	LDM
Na	0.021	0.008
Mg	0.111	0.005
Al	0.059	0.003
P	0.112	0.003
S	0.097	0.004
K	1.32	0.04
Ca	0.59	0.02
Mn[a]	209	6
Fe[a]	860	20
Co[a]	11.4	0.5
Zn[a]	270	5
As[a]	5	1
Sr[a]	42.0	0.7
Pb[a]	396	6

[a] Concentrations are expressed as $mg \cdot Kg^{-1}$.

Table-6.5.- Limits of determination (%) of the WDXRF method evaluated in a vegetation sepcimen (flowering *Buddleia davidii*), n=10.

As many other analytical methods, quantitative X-ray fluorescence analysis is subject to a number of random and systematic errors that contribute to the uncertainty of the analytical result. In this work, the combined analytical uncertainty (including trueness, repeatability and reproducibility over the time) for the developed method was calculated.

6.3.2.1. Analytical performance

- Trueness

The trueness of the WDXRF method was checked by determining, under the same conditions as the samples, the elemental composition in the standard reference material GBW07602 "Bush branches and leaves". The results obtained from the measurement of three replicates are displayed in Table 6.6. Good agreements were achieved between certified values and data obtained by WDXRF with a recoveries ranging from 94-107%, except in the case of P that presents a higher content in comparison with the certified value.

	GBW 07602	
	Certified	**WDXRF method**
Na[a]	1.10 ± 0.06	1.10 ± 0.09
Mg[a]	0.287 ± 0.011	0.272 ± 0.008
Al[a]	0.214 ± 0.018	0.20 ± 0.01
P[a]	0.083 ± 0.003	0.118 ± 0.004
S[a]	0.32 ± 0.02	0.31 ± 0.01
K[a]	0.85 ± 0.03	0.94 ± 0.03
Ca[a]	2.22 ± 0.07	2.27 ± 0.05
Mn[b]	58 ± 3	54 ± 3
Fe[b]	1020 ± 40	1030 ± 60
Co[b]	0.39 ± 0.03	<0.5
Zn[b]	20.6 ± 1.0	22 ± 1
As[b]	0.95 ± 0.08	<1
Sr[b]	345 ± 7	310 ± 10
Pb[b]	7.1 ± 0.7	7.9 ± 0.7

[a] Concentrations are expressed as percentage (%)
[b] Concentrations are expressed as $mg \cdot Kg^{-1}$

Table-6.6.- Concentrations obtained for the certified reference material CBW07602 (Bush branches and leaves) by using WDXRF quantitative method. Experimental values are presented as mean of 3 replicates with the corresponding standard deviation.

- Repeatability

In order to study the global repeatability of the proposed method, 10 pellets of a representative vegetation sample (flowering of a *Buddleia davidii* specimen) were prepared and measured under the same experimental conditions. Calculated relative standard deviations (RSD) of the results are given in Table 6.7 (a). In most instances, RSD values are generally better than 5% and discrepancies greater than 9% were not found.

As far as the estimated repeatability includes the uncertainty due to sample preparation, instrument and counting statistics it could be stated the good precision of the experimental procedure employed.

Additionally, one of the pellets was measured ten times and the relative standard deviation associated was also calculated (see Table 6.7 (b)). This uncertainty

(repeatability) is related to the instrument and counting statistics and it was found that, in most cases, RSD ≤ 2%. These results are comparable to those obtained by spectroscopic techniques (≈ 0.5% FAAS, ≈ 1.5% ICP-OES, ≈ 3-5% ETAAS, ≈ 2-3% ICP-MS) [6.11].

	(a) 10 pellets / 1 measurement		**(b)** 1 pellets / 10 measurements	
	Intensity (KCps)	**RSD (%)**	Intensity (KCps)	**RSD (%)**
Na	0.063	8.8	0.079	4.2
Mg	1.081	2.6	1.119	2.4
Al	1.110	7.1	1.173	1.6
P	9.339	3.2	9.272	3.4
S	5.024	3.7	5.189	1.7
K	99.297	2.3	101.216	2.0
Ca	43.631	1.5	45.056	1.4
Mn	0.585	4.0	0.610	2.0
Fe	24.450	6.0	26.908	1.4
Co	0.419	3.0	0.425	3.5
Zn	12.782	2.6	13.565	0.9
As	12.508	7.9	13.901	1.2
Sr	3.466	2.7	3.525	1.9
Pb	11.490	7.7	12.640	1.1

Table-6.7.- Comparison of the results of 1 measurement on 10 different pellet vegetation specimens and of 10 measurements on the same pellet.

Moreover, from data obtained, it could be deduced that the uncertainty introduced by sample preparation (comparing (a) and (b) values) is acceptable. The elements which are most affected by sample preparation are Na, Al, Fe, As and Pb.

- Reproducibility

Reproducibility of the WDXRF method over the time was evaluated by measuring the same specimen (previously used in the repeatability study) three times over a period of one month (see Table 6.8).

In general, relative standard deviations obtained were lower than 6%, except for Na, Al and P which presented a RSD values ranging from 9 to 20%. A reason that could be considered to explain that fact is the possible deformation of the flat surface of the pellet as pointed out in previous works [6.12]. According to that, the time between pelletizing and analysis should be as short as possible in order to avoid changes in surface of the pellet that could alter the measured intensity of some elements and therefore the calculated analyte concentration.

	Intensity (KCps)			
	1 day	1 week	1 month	**RSD(%)**
Na	0.075	0.063	0.062	10.9
Mg	1.129	1.129	1.060	3.6
Al	1.196	1.212	1.039	8.3
P	9.090	9.113	12.5	19.2
S	5.287	5.303	4.763	6.0
K	100.583	100.530	98.197	1.4
Ca	44.807	44.707	40.053	5.1
Mn	0.607	0.603	0.587	1.8
Fe	26.477	26.383	25.11	2.9
Co	0.431	0.432	0.405	3.6
Zn	13.291	13.169	12.858	1.7
As	13.583	13.603	13.357	1.0
Sr	3.583	3.547	3.813	4.0
Pb	12.312	12.338	12.228	0.5

Table-6.8.- Study of the reproducibility of the WDXRF method during a period of 1 month.

6.3.2.2. Application to real vegetation samples

Application of the developed method to three higher plants species collected in the surroundings of the treatment factory of Pontaut and also in one uncontaminated soil was carried out. Values obtained are expressed as mean of two replicates with the corresponding standard deviation ($mg \cdot Kg^{-1}$) and are displayed in Table 6.9.

In general, good precision was achieved from duplicate samples indicating the good quality of the results obtained. As it can be seen from the table, the developed methodology provides sufficient sensitivity to quantify fourteen elements in different kind of vegetations species. Only in the case of sodium and arsenic the obtained detection limits were higher than the real content in samples.

On the other hand, from the multi-elemental information obtained it is possible to study the variations in element content depending on the growing conditions of the vegetation specimens. For instance, it is feasible the comparison of lead and zinc content in vegetation samples collected in the related mining area with those from a non-polluted site (background), which is significantly useful for environmental purposes.

	Na	Mg	Al	P	S	K	Ca	Mn	Fe	Co	Zn	As	Sr	Pb
BD leaves (mining area)	<500	2980 (s=60)	526 (s=4)	905 (s=3)	5500 (s=100)	5010 (s=80)	23040 (s=40)	60.7 (s=0.4)	580 (s=10)	89 (s=3)	627 (s=7)	<1	62.37 (s=0.09)	279.1 (s=0.5)
BD leaves (background)	<500	2080 (s=60)	632 (s=1)	1190 (s=20)	3200 (s=100)	9700 (s=100)	18900 (s=500)	44 (s=2)	296 (s=6)	8.46 (s=0.06)	33.7 (s=0.4)	<1	60.1 (s=3)	4.1 (s=0.1)
BD flower (mining area)	<500	1230 (s=30)	610 (s=30)	896 (s=5)	1910 (s=20)	9420 (s=30)	8000 (s=100)	18.0 (s=0.6)	997 (s=3)	11.42 (s=0.06)	601 (s=5)	5.2 (s=0.4)	26.1 (s=0.6)	455 (s=2)
BD flower (background)	<500	973 (s=4)	1060 (s=30)	1270 (s=20)	1300 (s=80)	14800 (s=400)	7900 (s=200)	12 (s=1)	400 (s=30)	9.73 (s=0.01)	37.9 (s=0.9)	<1	27.08 (s=0.06)	16.2 (s=0.1)
BE (mining area)	3640 (s=100)	3480 (s=30)	740 (s=10)	1200 (s=9)	1750 (s=50)	6820 (s=50)	18590 (s=40)	296 (s=2)	930 (s=20)	10.2 (s=0.2)	1920 (s=40)	<1	62.0 (s=0.5)	350 (s=10)
BE (background)	<500	1960 (s=20)	662 (s=2)	1500 (s=400)	2120 (s=30)	11690 (s=30)	11790 (s=10)	582 (s=5)	213 (s=3)	10.3 (s=0.3)	247 (s=1)	<1	26.3 (s=0.7)	8.9 (s=0.1)
P (mining area)	520 (s=40)	1930 (s=20)	625 (s=7)	590.2 (s=0.3)	1336 (s=5)	4246.5 (s=0.7)	8140 (s=10)	31.8 (s=0.0007)	970 (s=30)	11.6 (s=0.5)	500 (s=2)	17 (s=4)	24.6 (s=0.5)	690 (s=10)
P (background)	1175 (s=1)	1120 (s=20)	430 (s=10)	1761 (s=4)	520 (s=30)	11380 (s=50)	9700 (s=200)	701 (s=2)	214 (s=1)	10.3 (s=0.1)	507 (s=7)	<1	19.6 (s=0.5)	25 (s=2)

Table-6.9.- Elemental composition of different vegetation species collected in a Pb/Zn mining area and in a uncontaminated soil (background).

6.3.2.3. Comparison of WDXRF and EDXRF capabilities

In view of the results from the study of the quality parameters (trueness, repeatability, reproducibility) it could be pointed that WDXRF is slightly more precise and accurate than other methodologies appeared in the literature using conventional EDXRF instrumentation. With respect to the detection limits, the WDXRF is also somewhat better, especially for low elements [6.11,6.13].

As commented in the introduction section of the thesis, the use of EDXRF-secondary mode of operation (using a secondary target) has been also applied in the analysis of different vegetation matrices. Commonly, in this configuration the primary, the secondary and the characteristic radiation form mutually orthogonal angles (tri-axial geometry). This geometry reduces the background radiation relative to the characteristic radiation from different element in the sample, allowing an improvement of the sensibility and limits of detection compared to conventional EDXRF. In Chapter 5, the results of the implementation of a method based on the use of this type of instrumentation for vegetation sample analysis are reported. Comparing the results assessed by means of EDXRF (secondary mode of operation) with those obtained with the WDXRF developed in this chapter it could be concluded that despite of achieving similar precisions in the obtained data, lower detection limits were assessed using EDXRF instrumentation for heavier elements. However, it has to be kept in mind, that using WDXRF methodology, a total number of fourteen elements (including light elements) can be quantified with sensitivity, in most cases, sufficient for plant analysis. Therefore, more complete information about element composition of vegetation matrices could be assessed compared to EDXRF instrumentation.

On the other hand, it is interesting to remark that neither of these equipments allows the determination of cadmium, an element that is of special importance in environmental studies. In this kind of instrumentation heavy elements have to be analysed using L-series spectral lines (Cd, L_α=3.133keV, L_β=3.528keV). The main problem arising when using L-lines for quantitation is that they lie in the low energy range of the spectrum, commonly overlapped by intense K-lines of major elements. The instrumental sensitivity for L-lines analysis is lower than for K-lines, resulting in higher detection limits that do not fulfil the needs of environmental studies and the severe regulations for Cd and neighbouring elements.

6.4. CONCLUSIONS

In the present study the usefulness of a WDXRF method for the sequential determination of some major elements (Na, Mg, Al, P, S, K, Ca), trace elements (Mn, Fe, Co, Zn, As) and non-essential elements (Sr, Pb) in different vegetation tissues has been tested.

Combination of plant certified reference materials and several synthetic calibrators made of cellulose to simulate the vegetation matrix appears to be an effective means to obtain reliable calibration curves with a good spread of data points over the range of each element to be determined. Results achieved from the quantitative study demonstrate the linearity over the whole concentration range of each element and also the suitability of the matrix correction method employed based on the use of influence coefficients.

Analytical performance of the methodology proposed shows that although the limit of determination of the method (LDM) calculated for the light elements are at levels of tens of $mg \cdot Kg^{-1}$ they are low enough if we consider that these elements are present at very high concentrations in plant tissues.

On the other hand, the results obtained from the evaluation of the combined uncertainty (including trueness, repeatability and reproducibility over the time) for the proposed methodology were satisfactory and comparable to those from other spectroscopic techniques.

To summarize, it could be concluded that the method of direct multi element WDXRF determination in dry powdered plant materials described in this paper is fast, simple and provides sufficient accuracy and precision for this kind of analysis. Therefore, it is expected that the method developed will find wide application in environmental investigations where conclusions are based on the analysis of a considerable number of samples.

6.5. REFERENCES

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CHAPTER 7

High-energy polarised-beam EDXRF for trace metal analysis of vegetation samples

The combination of selective excitation (using different secondary targets) and the reduction of spectrum continuum in polarised-beam EDXRF leads to a significant improvement in the achieved sensitivity and limits of detection of trace elements in vegetation samples. Moreover, the use of a gadolinium anode X-ray tube and a high-energy Ge semiconductor detector overcomes the problems of reduced sensitivity and spectral interferences inherent to the choice of L_α lines for the determination of heavy metals, commonly used in conventional XRF instrumentation. Therefore, the determination of some important pollutant elements such as cadmium is feasible in the low $mg \cdot Kg^{-1}$ range.

The contents of this chapter have been accepted to be published in X-Ray Spectrom (In Press).

E.Marguí, R.Padilla, M.Hidalgo, I.Queralt, R.Van Grieken, *High-energy polarised- beam EDXRF for trace metal analysis of vegetation samples in environmental studies.*

7.1. INTRODUCTION

As concluded in Chapter 6, one of the main drawbacks of XRF instrumentation (EDXRF and WDXRF), restricting a more frequent use for environmental purposes, has been the insufficient sensitivity for some important pollutant elements such as cadmium or adjacent elements such as Ag.

On the one hand, the high background intensities attributable to the high degree of scattering of the X-ray source by organic matrices (including vegetation specimens) hampers the determination of elements at trace levels [7.1]. This background can be reduced very significantly by the use of polarised X-ray sources such as a synchrotron or a primary beam scattered by a secondary target. Further background reduction can be achieved by minimising scattering by configuring a Cartesian geometry between source, sample and detector [7.2]. These principles are now combined in the novel EDXRF instrumentation usually termed as energy-dispersive polarised-beam X-ray fluorescence (EDPXRF). The use of suitable secondary targets (ST) for excitation not only allows reducing the intensity of the measured continuum radiation, thus significantly increasing the signal-to-noise ratio, but also to increase X-ray production due to the choice of quasi-monochromatic radiation with energy close to the photo-electric absorption edge of a given element of interest. Therefore, compared to other EDXRF techniques, it has the advantages of increased sensitivity (net intensity per unit of analyte concentration) and specificity for trace element analysis [7.3,7.4].

On the other hand, the recent development of digital signal processing based spectrometers in combination with enlarged X-ray production using better designs for excitation-detection [7.5] has added the advantage of increasing instrumental sensitivity, thus allowing improving both precision and productivity. The combined use of a gadolinium anode X-ray tube and a high-energy Ge semiconductor detector [7.6] allow performing EDXRF analysis using K-lines of high atomic number elements. In this way, the problems of reduced sensitivity and spectral interferences inherent to the choice of L_α lines for the determination of heavy metals (commonly used in conventional XRF instrumentation) are overcome.

This chapter presents the results of a study aimed to establish the best experimental conditions for the determination of several trace metal pollutants (Cd, As, Pb, Cu, Fe and Zn) in different vegetation species collected in two abandoned mining areas using EDPXRF. Furthermore, an evaluation of using a standard-less

fundamental parameter quantitative analytical procedure in the EDPXRF system is performed.

7.2. EXPERIMENTAL

7.2.1. Samples studied and preparation

Vegetation samples were collected at the surroundings of the abandoned mining areas located in the Aran Valley and Cartagena (see Chapter 1). In the present study, several vegetation species commonly found in each of these regions were selected to assess the impact of mining activities at the different sites (mines, landfills), taking into account the results obtained in previous surveys [7.7, 7.8, 7.9], in which the ability of this species to accumulating heavy metals was demonstrated.

The vegetation species studied were: *Betula pendula*, *Buddleia daviddii*, *Leucobryum sp.*, *Bromus sp.*, *Pleurocarpus sp*. (Aran Valley) and *Salicornia sp*., *Citrus limonium* (Cartagena).

The vegetation specimens were washed thoroughly with deionised water, to remove surface dust, and oven-dried at 55±5ºC for 24 h. The samples were ground in an agate ball mixer mill for 2-5 minutes and transferred to caped polypropylene flasks for conservation.

Compact pellets of 32 mm of diameter were pressed under 20 tons/cm^2 during 1 minute using an ANALIS press (up to 25 tons) and stainless steel dices from SPEAC (England). The spectrometer sample holders are stainless steel cups with a circular hole in the bottom facing the detector and the secondary target. To avoid contamination of the spectrometer measuring chamber, the samples were positioned on a polycarbonate film (0.422 mg/cm^2, SpexCertiPrep) mounted in a double Teflon ring.

Two types of blanks were measured to evaluate the presence of “instrumental” interferences: an empty sample holder and a real sample with concentration of Cd less than 0.2 mg·kg^{-1}.

7.2.2. Experimental conditions

The spectrometer used in this study was the commercially available Epsilon 5 of PANalytical (Almelo, The Netherlands) equipped with a Gd anode X-ray tube, operated at maximal values of 100 kV and 600W, and a high-energy Ge semiconductor detector.

In a similar way of the self-constructed system used in Chapter 5, the optical heart of the Epsilon 5 polarising EDXRF spectrometer is a 3-dimensional, or Cartesian geometry, defined by three orthogonal axes (see Figure 7.1).

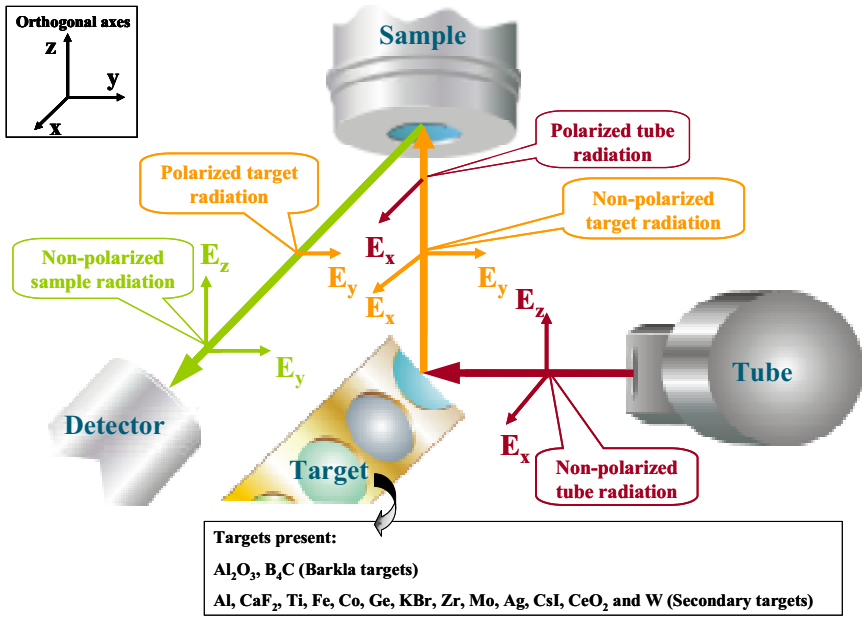

Figure-7.1.- Polarised beam excitation in Epsilon 5 [7.6]

The primary beam (red) from the X-ray tube first irradiates a polarising target placed along the first axis. After scattering at 90º, the X-rays travel along the second axis (orange) to the sample. The spectrum of the sample is recorded by the detector, which is placed along the third axis (green). The effect of this geometry is to eliminate the X-ray tube spectrum by polarisation, reducing thereby the spectral background enormously. Backgrounds can be an order of magnitude lower than conventional EDXRF instrumentation, resulting in much lower detection limits.

In contrast to the equipment used in Chapter 5, the excitation in the Epsilon 5 can be performed by choosing 13 secondary targets, constituted by pure metals or compounds, and two Barkla scatter devices (see Figure 7.1). Therefore, by using targets of different materials it is possible to optimise the excitation source specifically for analyte element of interest leading to a higher specificity and sensitivity for trace element analysis.

The instrument allows selecting two working environments: vacuum or helium flush. Information about the distances in the Cartesian geometric arrangement

between X-ray tube, secondary target, sample and detector is not provided by the manufacturer, but the attenuation of the radiation in vacuum path can be neglected. A summary of the experimental conditions that were studied for the determination of the elements of interest is provided in Table 7.1. Detailed information about Epsilon 5 features can be found elsewhere [7.6].

X-ray tube				
Anode	Gd			
Rating	25-100 kV, 0.5 mA-24 mA, max power 600 W			
Cooling	Internal water cooling			
Detector				
X-tal	30 mm^2 and 5 mm thick Ge			
Window	8 μm Be			
Energy range	0.7-100 keV			
Resolution	<140 eV (1000 cps Mn Kα)			
Cooling	Liquid N_2 cooled			
Target	***Type***	***Filter (μm)***	***Counting time (s)***	***Elements***
Al_2O_3	Barkla	Cu 250, Zr 125, Mo 250	1000	Cd
CsI	Secondary	None	1000	Cd
Ce_2O_3	Secondary	None	1000	Cd
KBr	Secondary	None	600	Fe, Cu, Zn, As
Zr	Secondary	None	600	Pb

See text for explanation on the types of targets and filters evaluated.

Table-7.1.- Specifications and operational conditions studied in this work.

Although the software provided with the instrument includes a set of recommended experimental conditions for the determination of different groups of elements, the analytical performance for the specific case of Cd was assessed for different experimental conditions, in view of its low concentration.

Among the available targets, CsI, Ce_2O_3 and Barkla Al_2O_3 provide suitable excitation energies to increase the X-ray production for Cd-K lines. The use of a Barkla target allows obtaining a non-monochromatic excitation which intensity increases from energies about 15 keV, and with maximum values around 40 keV [7.10]. Additional metal foils can be used as filters between the tube and the secondary target to reduce the background continuum for lower energies, thus improving the signal-to-noise ratio in Cd determination.

The determination of As and Pb is hampered by the well-known mutual spectral interference of these elements. The highest intensity As X-ray characteristic peak (As-K_α, 10.53 keV) can not be accurately fitted in the presence of Pb (Pb-L_α, 10.55 keV). A shortcoming of several quantitation programs (including QXAS software) is

the impossibility to use an interference-free peak for the determination of Pb (Pb-L_β, 12.62 keV). A more accurate determination of Pb can be achieved if the peak area for Pb-L_α is corrected by using the Pb-L_β peak area and a theoretical measured Pb-L_β:Pb-L_α ratio employing Zr as a secondary target for excitation. In the present study, the theoretical intensity ratio between these two groups of lines was determined by measuring a synthetic cellulose standard (to simulate plant matrix) spiked at 8 $g \cdot Kg^{-1}$ of lead. Then, the calculated Pb concentration was used to subtract its contribution to the more selective determination of As using KBr excitation. As reported in a previous work [7.5], the ratio of As-K_α : Pb-L_α X-ray fluorescent production is 1:0.06, thus diminishing the interference of Pb in the As determination (see Expression 7.1).

$$[As]_{corrected} = [As]_{KBr} - 0.06\ [Pb]_{corrected} \quad (7.1)$$

It is interesting to remark that, As-K_α peak fitting is not significantly interfered by the incoherently scattered Br-K_α peak, which main energy is of 11.63 keV.

Spectrum evaluation was performed using the WinAxil code (CANBERRA), and Axil Saved Results files (ASR) were created using a routine kindly provided by Prof. P. Van Espen (University of Antwerp) for the further quantitation with QXAS [7.11]. The scatter peaks were fitted using the QXAS-AXIL code.

7.2.3. Quantitation methods

7.2.3.1. Determination of Cd

Considering that the total sample attenuation coefficient (μ_S) in the case of vegetable samples shall not include significant photoelectric absorption jumps for elements with atomic number higher than Ca, the correction for attenuation can be simple in the case of using Cd-K_α lines for the analysis. If there are no significant differences in matrix composition, a linear calibration of Cd-K_α peak areas, normalized to the X-ray tube operational current, versus the concentration, can be used if samples and standards are prepared and measured under the same conditions (pellet aerial density, measuring time, and sample moisture).

In the case that variations in matrix composition lead to significant differences in sample attenuation, a simple correction can be performed by normalizing the Cd-K_α

peak area to the Compton scatter intensity measured for a metal target (i.e., Zr). The latter is only valid if there are not significant absorption edges in the total attenuation coefficient of the sample between the Compton energy and the energy of the analytical peak, which is the case.

A linear calibration was performed using the procedure provided in ISO 8466:1 [7.12]. A subset of seven samples with known compositions of Cd determined by ICP-MS was selected to establish the calibration. Ten replicates were measured for the initial and final concentrations and four replicates for the rest of the points. The accuracy and precision of the results were evaluated by measuring three replicates of four different pellets of the certified reference material (CRM) NIST-1570a ("Spinach leaves", NIST-1570a, from the National Institute for Standards and Technology, Gaithersburg, MD, USA; Cd concentration of 2.89 ± 0.07 mg·Kg^{-1}) .

7.2.3.2. Determination of As, Pb, Fe, Cu and Zn

For the quantitation of Pb, As, Zn, Cu and Fe, the absence of suitable reference materials and the need of an accurate sample absorption correction for low energies imposed the use of a standard-less procedure. The fundamental parameter approach provided in the IAEA QXAS quantitation package was selected for this purpose.

The QXAS fundamental parameter approach uses the more general expression of Sherman to compensate for sample self-absorption and enhancement effects. The corrections for both effects are calculated using a combination of the estimated concentrations of all detected elements with an hypothetical "dark" (composed by the elements not detected by XRF) sample matrix constituted of two low-Z elements, which can be a-priori defined or estimated from the ratio of measured scatter peak intensities [7.13]. The purpose of the instrumental calibration reduces to the determination of the geometric proportionality factor G for fluorescence (and in the more general case for scatter) by measuring pure metal foils or pellets of known compounds.

The estimation of sample 'dark' matrix was performed using the procedure reported in [7.14]. An experimental calibration of the ratio of incoherent to coherent Zr-K_α scatter peaks for measured compounds having an effective atomic number Z_{eff} between 6.3 and 9.1 served to establish an equation to determine the sample effective number for unknown samples. Later, Z_{eff} was estimated for each sample, and the dark matrix was defined as composed by a combination of two elements producing

such effective atomic number. This hypothetical composition was later supplied to the quantitation program as 'known-dark' matrix. The accuracy and precision of the methodology was evaluated by measuring four replicates of NIST-1570a and of GBW 07603 (Bush branches and leaves), GBW 07604 (Leaves of poplar), GBW 07605 (Tea) from the China National Center for Iron and Steel (Beijing, China).

7.2.4. Detection limits

In XRF practice, detection limit for an element *i* is customarily calculated by using three times the value of the 'noise' signal and the instrumental sensitivity S_i [7.15]. The main noise signal in XRF spectra is the continuum under the peak (N_{cont}). In some cases, some peaks are also noticed in a measurement performed in a blank sample, which are usually called 'instrumental' background (N_{bkgd}). In general, the probability distribution of the results of a series of measurements for both of these signals can be considered as close to a Poisson distribution, and in such case $\sigma_{blank} = \sqrt{N_{blank}}$. Expression (7.2) is then written for the general case of the presence of an instrumental background peak as:

$$DL_i\left(mg \cdot Kg^{-1}\right) = \frac{3\sqrt{N_{cont} + N_{bkgd}}}{S_i} \tag{7.2}$$

7.3. RESULTS AND DISCUSSION

7.3.1. Sample preparation

In EDXRF practice, the measured characteristic radiation for an element *i* must be corrected for sample self-absorption. The absorption correction for the case of using the characteristic radiation E_i and monochromatic excitation with energy E_0 can be expressed as:

$$A(E_0, E_i) = \frac{1 - \exp\left[-\rho d \chi(E_i, E_0)\right]}{\chi(E_i, E_0)} \tag{7.3}$$

$$\chi(E_i, E_0) = \mu_s\left(E_0\right) \Big/ \sin(\psi_1) + \mu_s\left(E_i\right) \Big/ \sin(\psi_2) \tag{7.4}$$

$$\mu_s\left(E\right) = \sum_k w_k \mu_k\left(E\right) \tag{7.5}$$

where ρd is the sample aerial density (g cm^{-2}), $\mu(E)$ is the total attenuation coefficient for the energy *E*, w_k is the mass fraction of any element *k* present in the sample, Ψ_1

an Ψ_2 are the average angles of excitation and detection, respectively. The index *s* denotes sample, and from expression (7.5) is clear that an accurate absorption correction implies the knowledge of sample matrix composition.

Samples are often classified depending on the value of the product $\rho d\chi(E_i, E_0)$ as "thin" ($\rho d\chi(E_i, E_0) < 0.1$), "thick" ($\rho d\chi(E_i, E_0) > 4.6$) or otherwise of intermediate thickness. It is easy to prove that for "thin" samples, the absorption correction becomes only proportional to sample aerial density, while for "thick" samples the absorption correction is proportional to $1/\chi(E_i, E_0)$.

Pellets of different aerial densities were prepared from the CRM NIST 1570a to assess the type of attenuation correction for Cd-K_α (Figure 7.2).

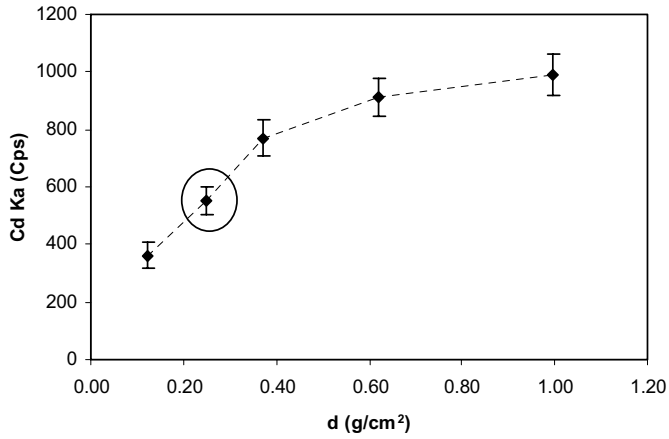

Figure-7.2.- Assessment of the sample thickness type for Cd (Barkla-Al_2O_3 excitation).

The obtained results evidence that the condition of "thin" sample can be reached for aerial densities lower than 0.2 g cm^{-2}, which is unpractical, due to the fragility of the obtained pellets. Working at a density corresponding to the condition of "thick" sample is also impossible, since the required weight is about 8 grams, and the available material is scarce. Therefore, the availability of material imposes to work under the conditions of intermediate thickness.

7.3.2. Determination of Cd

Among the available targets, CsI, Ce_2O_3 and Barkla Al_2O_3 provide suitable excitation energies to increase the X-ray production for the Cd-K lines. However, the use of the secondary targets of Ce_2O_3 and CsI was discarded. In both cases, spectral

interferences arising from escape peaks (either Ge-K_α or Ge-K_β) of the scatter peaks of the target material could hamper the fitting of the Cd-K_α peak (see Table 7.2).

Target scatter peaks		Escape peak energies	
Scatter peak	E (keV)	E – 8.89 (Ge-K_α)	E – 10.98 (Ge-K_β)
Ce- K_α, Coherent	34.71	24.83	23.73
Ce- K_α, Incoherent	32.95	**23.07**	21.97
Ce- K_β, Coherent	39.25	29.37	28.27
Ce- K_β, Incoherent	37.19	27.31	26.21
I- K_α, Coherent	28.61	18.72	17.63
I- K_α, Incoherent	27.24	17.36	16.26
I- K_β, Coherent	32.29	22.40	21.31
I- K_β, Incoherent	30.68	20.80	19.70
Cs- K_α, Coherent	30.97	21.085	19.99
Cs- K_α, Incoherent	29.45	19.57	18.48
Cs- K_β, Coherent	34.98	25.09	**24.00**
Cs- K_β, Incoherent	33.19	**23.32**	22.22

Table-7.2.- Spectral interferences hampering the fitting of Cd-K_α (23.17 keV) peak.

The Barkla Al_2O_3 scatter was selected for Cd excitation and the additional use of three different filters (see Table 7.1) was evaluated to improve the signal-to-noise ratio by reducing the measured continuum. The spectra corresponding to the measurement of CRM NIST 1570a under these conditions are shown in Figure 7.3.

The instrumental sensitivity is higher when using unfiltered excitation and therefore a smaller uncertainty is expected due to better counting statistics in Cd-K_α peak area. On the other hand, the use of Mo filter seems to be the best choice to attain larger signal to noise ratio.

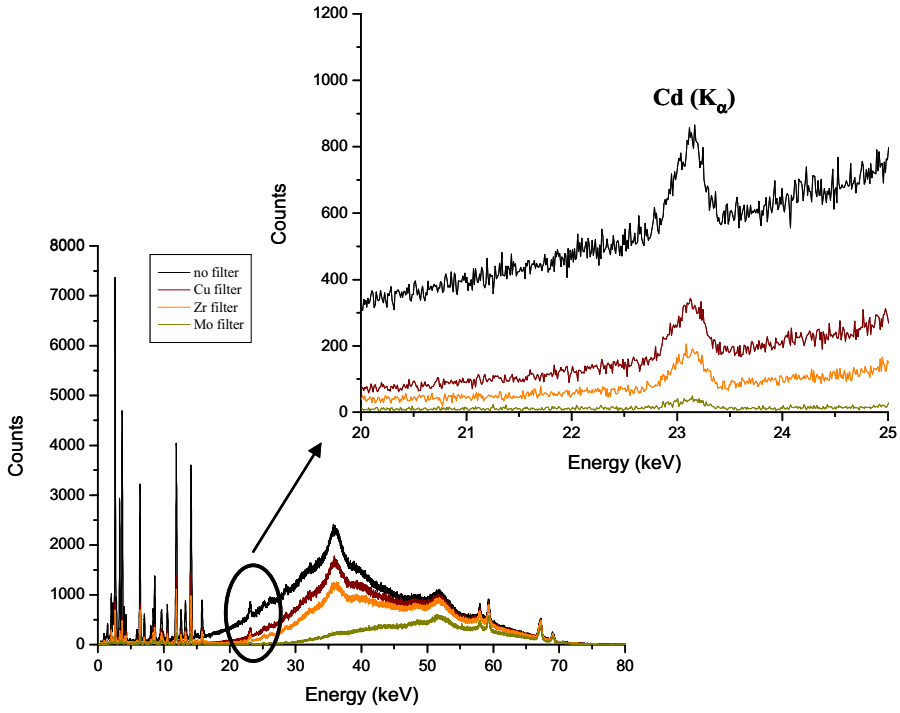

Figure-7.3.- Spectra of NIST 1570a using Al_2O_3 Barkla scatter for Cd excitation (HV = 100kV, I = 5mA, t_{meas} = 1000s).

To evaluate the detection limits, ten measurements of two independent pellets of a vegetation sample with a concentration of less than 0.2 mg·Kg^{-1} of Cd (determined by ICP-MS) were measured under the evaluated excitation conditions. A low-intensity background peak was observed, and the obtained results are summarized in Table 7.3.

Filter	*Average* N_{Cd}	$\sigma_{N\,Cd}$	*Average* N_{Cont}	$\sigma_{N\,Cont}$ (n=20)
None	2229	349	60819	2942
Cu	1079	183	16717	705
Zr	621	82	6922	335
Mo	200	55	810	44

Table-7.3.- Values of the instrumental background and continuum values for the evaluated excitation conditions (Al_2O_3-Barkla scatter, HV = 100 kV, I = 5 mA).

The values of $\sigma_{N\,Cd}$ and $\sigma_{N\,Cont}$ are calculated as the standard deviation of the 20 replicate values. It is evident that the results of the continuum in the first three cases can not be considered as a Poisson distribution, since the standard deviation is significantly greater than the square root of their average value. The determination of continuum counts is less accurate in the case of having a slope-shaped continuum (see Figure 7.3), and there might be two reasons conditioning such large variations. Firstly, the fitting of a small area peak leads to a less accurate definition of the peak width, and therefore of the limits of the region over which the continuum counts are summed. Secondly, if the continuum profile slope varies or shifts, the variation of the continuum counts at the boundary channels of the region defined for calculating the continuum is larger. An experiment was performed to find out which of these causes might have the largest influence in the observed effect.

Two different samples were measured in two measuring routines. The first sample corresponded to a large intensity Cd-K_α peak ([Cd]=7.9 ± 0.4 mg·Kg^{-1}), while the second was the same blank sample measured before ([Cd]< 0.2 mg·Kg^{-1}). In the first measuring routine, each sample was measured ten times without changing the target or the sample (consecutive replicates), and in the second case, the measurements were performed by changing the sample before the measurement (alternate replicates). There were no significant differences in continuum standard deviation while measuring the sample with large Cd-K_α peak area, thus excluding the influence caused for differences in the performance of the fitting routine. The fact that the values of Cd-K_α instrumental background peak from the previous experiment (Table 7.3) do fulfil the expected Poisson distribution reinforces the conclusion that the fitting algorithm is robust. Apparently, neither the changes of the sample lead to slope variations or shifts of the continuum profile. It is logical to suppose that such variations are inherent to the use of the Barkla secondary target, and that there are other unrevealed contributions to the uncertainty of the estimation of the continuum in this region.

A contradiction arises to choose the optimal conditions for Cd determination among the four evaluated excitation arrangements. Apparently, the best reproducibility can be achieved in the case of the higher instrumental sensitivity. Regarding the uncertainty in the determination of Cd-K_α net peak area, the assessment of the contribution due to instrumental background and due to continuum

shall be discussed in details. As far as the values of the background net peak area can be assumed as Poisson-distributed, its contribution to the uncertainty of the Cd-K_α net peak area can be assumed as $\sqrt{N_{Bkgd}}$. The contribution due to continuum uncertainty, which, for a single measurement, is usually considered as $\sqrt{N_{Cont}}$ is not strictly valid, since it was proven that the continuum counts cannot be considered as Poisson-distributed. A more 'safe' estimate can only be based on the use of $\sigma_{N\,Cont}$.

A comparison of the relative error in measured Cd-K_α for a sample with an ICP-MS determined concentration of 7.9 ± 0.4 mg·Kg^{-1} is shown in Figure 7.4.

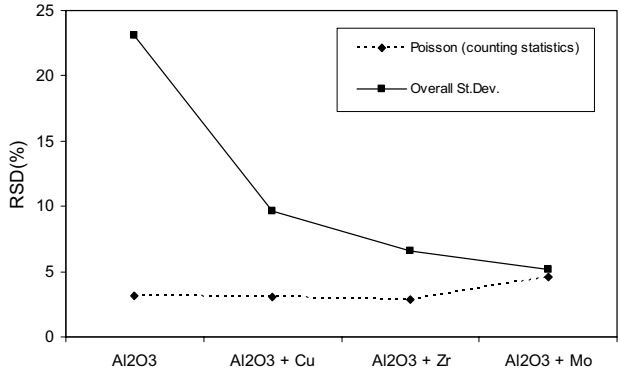

Figure-7.4.- Assessment of relative uncertainty in Cd-K_α estimation:
Poisson (counting statistics): $\sigma_{Cd-K\alpha} = \sqrt{N_{Cd} + N_{Cont} + N_{Bkgd}}$,
Overall St. Dev.: $\sigma_{Cd-K\alpha} = \sqrt{N_{Cd} + \sigma^2_{Cont} + N_{Bkgd}}$

The detection limits were calculated according to expression (7.2), but using $\sigma_{N\,Cont}$ instead of $\sqrt{N_{cont}}$. The large fluctuation in the continuum observed for the first three excitation conditions causes larger values of the detection limit (see Figure 7.5).

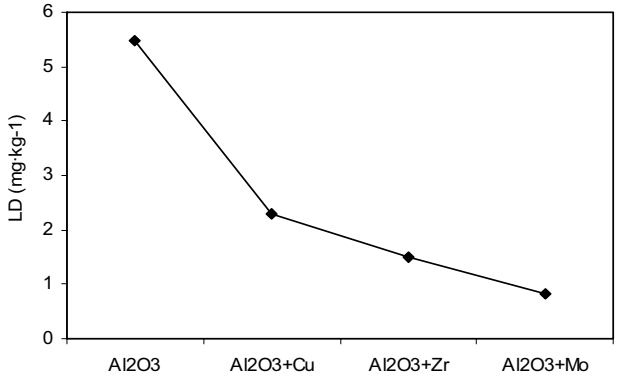

Figure-7.5.- Detection limits for the determination of Cd (HV=100kV, I=5mA, t_{meas}=1000s)

As a conclusion, the combined use of Barkla-Al_2O_3 with the Mo filter was selected as the best excitation condition.

Different values of X-ray tube current (mA) and voltage (kV) were also tested to achieve the maximum Cd-K_α intensity. Operational values of 100 kV and 5 mA were chosen for the Cd determination in vegetation specimens. Concerning the measuring time (1000 s), it was selected as a trade-off between an acceptable repeatability of measurements and total analysis time.

The linearity of the calibration curve for Cd (see Figure 7.6) was tested according to the ISO 8466:1. Satisfactory results were obtained for the cadmium concentration range studied (0.89-11.6 mg·Kg^{-1}).

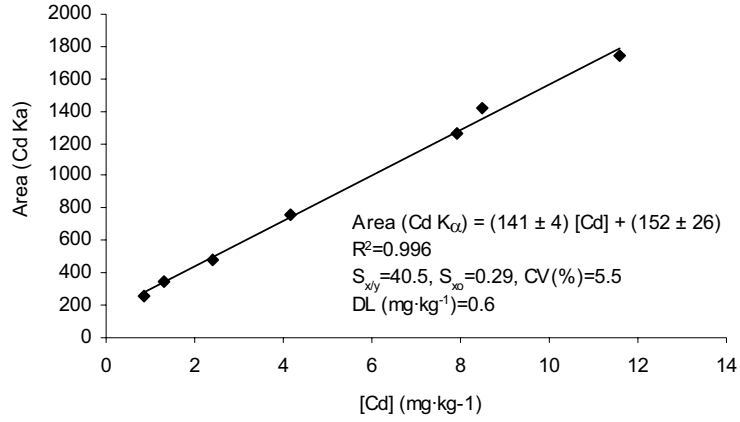

Figure-7.6.- Calibration curve for cadmium.

Besides, in this figure, the limits of detection of the calibaration curve (calculated from ISO8466:1 specification [7.12]) and some figures of merit (standard deviation of the predicted y-value for each x in the calibration curve ($S_{x/y}$, Counts), the standard deviation of the method (S_{xo}, mg·Kg^{-1}) and the variation coefficient of the method (CV, %) are summarised.

Since the quantitation model is based on a linear calibration, the value of its intercept shall correspond to the average peak area of the instrumental background. The value of the instrumental background, obtained by measuring a real sample with a concentration of Cd less than 0.2 mg·Kg^{-1} (Cd-K_α=187 ± 44) was found to be not significantly different from the value of the intercept of the calibration curve (152 ± 26).

The accuracy and precision of the results was evaluated by measuring three replicates of four different pellets of the certified reference material NIST-1570a. Good agreement was achieved between the certified value ([Cd]=2.89 ± 0.07 $mg \cdot Kg^{-1}$) and the results obtained ([Cd]=3.05 ± 0.39$mg \cdot Kg^{-1}$, 95% confidence level) with a relative standard deviation of 7%.

A second set of 31 vegetation samples was analysed using the above calibration. In Table 7.4 the values obtained by EDPXRF and ICP-MS are presented.

	Reference values (ICP-MS)		EDPXRF	
	[Cd] ($mg \cdot kg^{-1}$)	St.Dev.[a]	[Cd] ($mg \cdot kg^{-1}$)	St.Dev.[b]
Buddleia davidii (F)-1	<0.2		<0.6	
Buddleia davidii (F)-2	1.03	0.05	1.19	0.07
Buddleia davidii (F)-3	2.01	0.02	1.79	0.27
Buddleia davidii (L)-1	<0.2		<0.6	
Buddleia davidii (L)-2	1.24	0.01	1.44	0.05
Buddleia davidii (L)-3	1.67	0.07	2.11	0.29
Buddleia davidii (L)-4	<1		1.26	0.19
Buddleia davidii (L)-5	1.11	0.05	0.97	0.34
Betula pendula-1	<0.2		<0.6	
Betula pendula-2	2.57	0.05	3.20	0.29
Betula pendula-3	1.51	0.03	1.68	0.15
Betula pendula-4	1.17	0.03	1.45	0.16
Betula pendula-5	4.40	0.10	4.57	0.48
Betula pendula-6	3.40	0.20	5.56	0.40
Betula pendula-7	3.72	0.01	3.91	0.19
Betula pendula-8	1.15	0.01	1.43	0.15
Bromus sp.-1	0.73	0.01	0.9	0.17
Bromus sp.-2	5.35	0.03	5.14	0.37
Bromus sp.-3	0.71	0.01	<0.6	
Bromus sp.-4	1.74	0.14	1.57	0.23
Leucobryum sp.-1	0.33	0.01	<0.6	
Leucobryum sp.-2	10.88	0.70	10.93	0.31
Leucobryum sp.-3	4.00	0.40	3.66	0.13
Leucobryum sp.-4	26.50	0.80	22.74[c]	0.74
Pleurocarpus sp.-1	28.30	0.10	29.09[c]	0.60
Pleurocarpus sp.-2	30.10	0.30	29.13[c]	0.43
Citrus limonium-1	0.95	0.02	0.91	0.15
Citrus limonium-2	1.07	0.04	1.02	0.21
Salicornia sp.-1	1.00	0.01	1.19	0.28
Salicornia sp.-2	3.08	0.03	3.22	0.17
Salicornia sp.-3	0.99	0.08	1.00	0.22

[a] St.Dev.: two independent replicates measured 3 times
[b] St.Dev.: two independent replicates measured 2 times
[c] Samples diluted with pure cellulose to fit into the concentration working interval.

Table-7.4.- Comparison of reference (ICP-MS) and EDXRF values for 31 validation samples.

In general, the precision attained with the proposed method is poorer than that obtained with the ICP-MS analysis, especially for concentrations close to the

detection limit (RSD~20%). Still, for concentrations higher than 7 $mg \cdot Kg^{-1}$, a relative standard deviation of 2 % is achieved.

Comparison of data obtained with the EDPXRF method with the results from the ICP-MS was performed using robust statistics. Validation samples were statistically evaluated only if the concentrations given by the reference method were included in the calibration range of 0.9 to 10.9 $mg \cdot Kg^{-1}$ (see Table 7.5).

			$Bias = \frac{\sum_{i=1}^{n}\left(\hat{y}_i - y_i\right)}{n}$	**EDPXRF=A+B×ICP-MS**			
	N	R^2	=0	B	=1	A	=0
Cd	21	0.9902	Yes	0.9843	Yes	0.1298	Yes

R^2: Correlation coefficient; A: Intercept; B: Slope; $\hat{y}_i$: EDPXRF value; y_i : ICP-MS value.

Table-7.5.- Comparison of the obtained values using robust statistics.

As it can be seen, "A" was found not to be significantly different from 0 and "B" was found not to be significantly different from 1 for the model EDPXRF=A+B×ICP-MS, leading to the conclusion that the results obtained using both techniques are in good agreement.

7.3.3. Determination of As, Pb, Fe, Cu and Zn

Typical operating instrumental parameters for the determination of As, Pb, Fe, Cu and Zn were selected based on manufacturer's recommended procedures (100 kV, 6 mA, t_{meas}=600 s).

A set of pure metal foils and pellets from compounds was used to perform the instrumental calibration and to determine the value of the geometric factor for fluorescence (G). The obtained results for both secondary targets (Zr and KBr) are shown in Figure 7.7. As far as any G is supposed to reflect only the overall effective solid angle fraction, its calculation for different elements or matrices shall give the same value. According to that, the achievement of constant values for *G* serves to prove the validity of the calibration. However, for energies less than 5 keV, the lack of accurate information on detector parameters hampers the estimation of detector intrinsic efficiency, and the calculated G values are not accurate. An alternative to solve this shortcoming is to use individual values of G for low energies. In both

calibrations, the relative inaccuracy of the values of G can be considered less than 10 %.

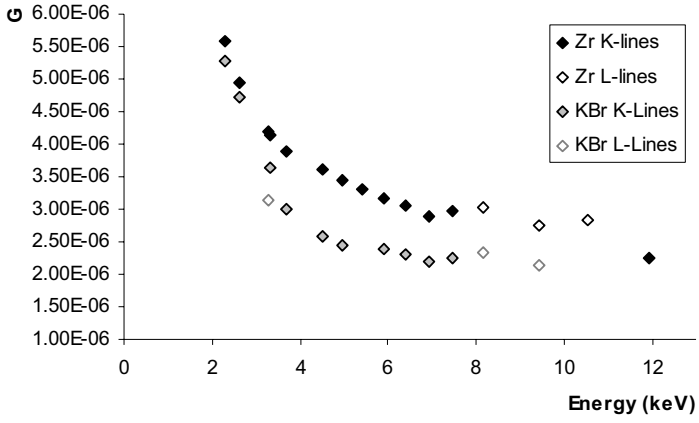

Figure-7.7.- Calibration of G factors for both excitation conditions.
(Pure metal foils were used in all cases except for Na, K, Mn and Br which pellets prepared from the following compounds: NaCl, $KMnO_4$ and KBr were employed).

The experimental calibration of the ratio of incoherent to coherent Zr-K_α scatter peaks performed to establish an equation to determine Z_{eff} for unknown samples is shown in Figure 7.8. Z_{eff} of compounds was calculated according to the following expression:

$$Z_{eff} = \frac{\sum_k w_k Z_k A_k}{\sum_k w_k A_k} \tag{7.6}$$

Where w_k is the weigh fraction, A_k is the atomic mass and Z_k is the atomic number of element k.

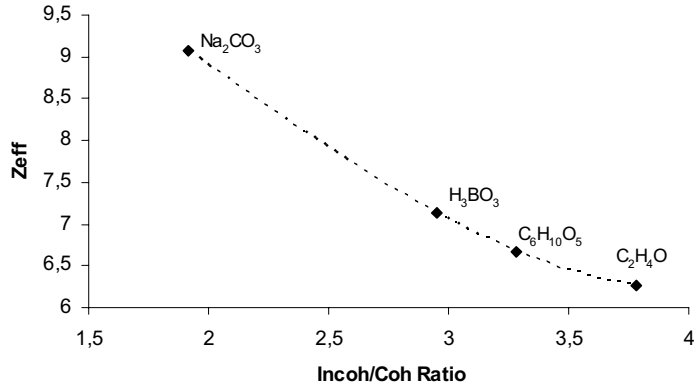

Figure-7.8.- Experimental calibration of scatter ratio.
Z_{eff}= 11.78524-0.20258 (Incoh/Coh)-0.94157(Incoh/Coh)^2+0.16114(Incoh/Coh)^3, R^2=1

Due to the high counting statistics of the scatter peak areas and the quality of the fitted model, the inaccuracy in determining the value of Z_{eff} was assessed as better than 5 %.

Analytical figures of merit for the proposed method including the relative precision (7.6), relative bias (7.7), relative uncertainty due to bias (7.8) as well as the combined uncertainty (7.9) were evaluated [7.16] by measuring 4 independent replicates of four certified reference materials. The obtained results are provided in Table 7.6.

$$U_{pre} = \frac{St.Dev}{Average} \tag{7.7}$$

$$\text{Re}\,lative\ Bias = \left(\frac{Average - Certified}{Certified} \right) \tag{7.8}$$

$$U_{bias} = St.Dev\left(\frac{x_i - Certified}{Certified} \right) \tag{7.9}$$

$$U_c = \sqrt{\left(U_{pre}\right)^2 + \left(U_{bias}\right)^2} \tag{7.10}$$

For the case of Cu and Zn, the EDPXRF determination was affected by the inaccurate fitting of the peaks (Cu-K_α = 8.047 keV, Zn-K_α = 8.638 keV) in the vicinity of an instrumental interference of W-L_α = 8.396 keV, leading to a larger relative bias in the determination of these elements. Concerning Fe, in the case of samples containing high amounts of Mn, we found also a less accurate determination.

The detection limits were calculated by using as input the values resulting from the measurement of a blank sample (cellulose with concentration values of impurities below 1 mg·Kg^{-1}). The values of the detection limits are included in the last column of Table 7.6.

Sec. target	Element	CRM	Certified ($mg \cdot kg^{-1}$)	Average ($mg \cdot kg^{-1}$)	Relative Precision (U_{pre})	Relative bias	Relative uncertainty due to bias (U_{bias})	Combined uncertainty (U_c)	DL ($mg \cdot kg^{-1}$)
KBr	**Fe**	GBW 07605	264 ± 10	205	0.01	-0.22	0.007	0.01	0.58
		GBW 07604	274 ± 10	218	0.01	-0.21	0.009	0.02	
		NIST 1570a	n.c.	302	0.03	-	-	-	
		GBW 07603	1070 ± 40	940	0.004	-0.12	0.003	0.005	
	Cu	GBW 07603	6.6 ± 0.4	9.8	0.01	0.48	0.02	0.02	0.24
		GBW 07604	9.3 ± 0.5	10.5	0.02	0.12	0.02	0.03	
		NIST 1570a	12.2 ± 0.6	17.9	0.03	0.47	0.04	0.05	
		GBW 07605	17.3 ± 1.0	17.0	0.01	-0.02	0.01	0.02	
	Zn	GBW 07605	26.3 ± 0.9	20.6	0.02	-0.22	0.01	0.02	0.18
		GBW 07604	37 ± 1	30.0	0.01	-0.19	0.01	0.02	
		GBW 07603	55 ± 2	50.4	0.02	-0.08	0.02	0.02	
		NIST 1570a	82 ± 3	82.1	0.03	0.0007	0.03	0.04	
	As	NIST 1570a	0.068 ± 0.012	<0.2	-	-	-	-	0.20
		GBW 07605	0.28 ± 0.03	0.36	0.10	0.28	0.31	0.33	
		GBW 07604	0.37 ± 0.06	0.34	0.25	-0.09	0.23	0.34	
		GBW 07603	1.25 ± 0.10	1.21	0.08	-0.03	0.08	0.11	
Zr	**Pb**	NIST 1570a	(0.20) i.v.	<0.27	-	-	-	-	0.27
		GBW 07604	1.5 ± 0.2	1.56	0.08	0.04	0.09	0.12	
		GBW 07605	4.4 ± 0.2	3.17	0.01	-0.28	0.007	0.01	
		GBW 07603	47 ± 2	47.7	0.02	0.02	0.02	0.03	

Table-7.5.- Characteristics of performance for the QXAS-Fundamental Parameter analysis of vegetation samples

7.3.4. Application to real vegetation samples

Results obtained for the analysis of the 38 actual vegetation samples comprised in this study are shown in Figure 7.9.

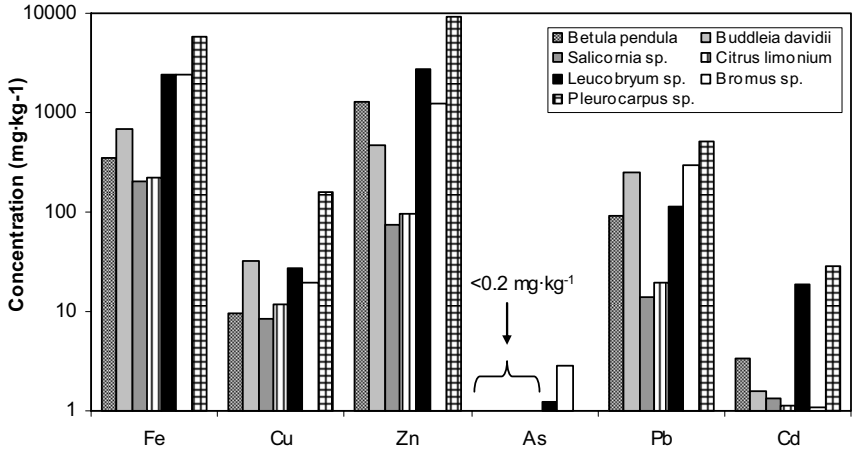

Figure-7.9.- Comparison of average metal content for all species collected at different sites around the mining areas.

It can be observed that the higher concentrations found in all the species studied correspond to Zn, Pb and Fe, which were the main metals extracted from the ore vein of both mining districts. Besides, significant concentration of minor metals such as Cd and As are also present in the vegetation specimens, being the largest in the case of moss species (*Leucobryum sp., Pleurocarpus sp.*) and grasses (*Bromus sp.*) with values up to 28 $mg \cdot Kg^{-1}$ and 3 $mg \cdot Kg^{-1}$ for Cd and As, respectively.

Table 7.6 summarizes the values obtained for *Betula pendula* at different sampling spots.

	Non-affected area	Mine	Pb-Zn concentrate remains	Pb-Zn landfill
Fe	42.4	394	334	356
Cu	2.4	11.3	10.9	5.6
Zn	368.0	1390	1390	1030
As	<0.2	1.1	<0.2	0.3
Pb	0.6	5.7	118	55.2
Cd	<0.6	1.5	2.7	6.3

Table-7.6.- Metal content ($mg \cdot Kg^{-1}$) in *Betula pendula* species at the different sampling spots.

High concentrations for all the studied metals were found in the samples corresponding to sites affected by mining activities, corroborating the metal accumulation process occurring in vegetation. These results prove the risk that abandoned mining districts represent for the biota in these areas.

7.4. CONCLUSIONS

The results of this study highlight the suitability of high-energy EDPXRF analysis for the determination of several trace elements (i.e. Cd, As and Pb) in vegetation species. The combination of selective excitation and the reduction of spectrum continuum leads to a significant improvement in the achieved sensitivity and limits of detection. Besides, the use of a gadolinium anode X-ray tube and a Ge semiconductor detector overcomes the problems of reduced sensitivity and spectral interferences inherent to the choice of L_α lines for the determination of heavy metals such as cadmium, commonly used in conventional XRF instrumentation. The K_α line-based analysis of this element is feasible in the low $mg \cdot kg^{-1}$ range.

On the other hand, the use of a standard-less fundamental parameter approach (IAEA QXAS) allowed the determination of other trace metals in the absence of suitable certified reference materials and to accurately compensate for self-attenuation effects in the sample. The obtained accuracy and precision are in most cases acceptable for environmental studies.

The simple sample preparation, versatility and relatively fast determinations make EDPXRF an attractive alternative for the routine analysis of vegetation samples in environmental studies.

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CONCLUDING REMARKS

This thesis has given a brief overview of different analytical methodologies based on X-ray fluorescence spectrometry and inductively coupled plasma atomic spectroscopy for the assessment of metal dispersal around mining environments.

Despite that the detailed conclusions from the present research have been included in the related chapter, the main conclusions and an outlook on the most remarkable issues for future research will be discussed in the following section.

In Table C.1, a comparison between the outcome in vegetation sample analysis performed using the diverse configuration of XRF spectrometers studied in the course of this work (Chapters 5-7) is presented, and the most relevant conclusions achieved are discussed as follows:

- The achievement of quantitative results by means of XRF technique is possible if appropriate quantification models are applied. In the case of vegetation specimens' analysis, the use of synthetic cellulose calibrators together with a correction method based on the use of influence coefficients prove to be suitable for quantitation purposes (Chapter 6). In a similar way, the fitness of standard-less fundamental parameter quantification model for low-Z matrices has also been demonstrated (Chapter 7).

- Data obtained in Chapter 5 and Chapter 7 showed up the benefits of using polarised X-rays sources (primary beam scattered by a secondary target) to reduce the characteristic high degree of scattering of the X-ray source by vegetation samples leading to an improvement of the limits of detection (especially for heavier elements) compared to conventional WDXRF instrumentation (Chapter 6).

- The use of suitable secondary targets (ST) for excitation increases X-ray production due to the choice of quasi-monochromatic radiation with energy close to the photo-electric absorption edge of a given element of interest. Therefore, compared to other conventional XRF techniques, it has the advantages of increased sensitivity and specificity for trace element analysis (Chapter 7).

- The combined use of a gadolinium anode X-ray tube and a high-energy Ge semiconductor detector (Chapter 7) allow performing EDXRF analysis using K-lines of high atomic number elements. In this way, the problems of reduced sensitivity and spectral interferences inherent to the choice of L_α lines for the determination of heavy metals (commonly used in conventional XRF instrumentation) are overcome and the determination of some important pollutant elements such as Cd is feasible in the low $mg \cdot Kg^{-1}$ range.

- The accuracy and precision of the results obtained by the proposed XRF methodologies are in most cases acceptable for environmental studies and comparable to those achieved by atomic sepctroscopic thecniques unless the concentrations are close to the detection limits.

In view of the above points it could be concluded that new high-energy polarised-beam EDXRF instrumentation offers the advantage of higher versatility in its configuration compared to conventional XRF, since an instrument can be designed to generate a range of excitation and polarisation conditions optimised for different groups of analytes. Thus, it could be a good analytical tool for trace metal analysis of vegetation samples as an alternative to classical destructive analytical methods (Chapter 5) given that it provides accuracy and precision levels fulfilling the requirements for environmental studies.

It is expected that future improvements in the XRF instrumentation could increase, even more, the instrumental sensitivity and thus, X-Ray fluorescence spectrometry could offer new possibilities in the environmental field in the upcoming years.

Table-C.1.- Summary of the performance of XRF analysis of vegetation samples.

XRF Configuration	Elements	Sample preparation	Instrument characteristics	Excitation	Acquisition time (s)
EDPXRF ***(Chapter 5)***	K, Ca, Mn, Fe, Cu, Sr, Pb, Zn	▪ Powdered material ▪ P=10T, t=60s ▪ ∅=2cm ▪ Thickness=0.1mm	▪ X-Ray tube - Anode: W - Power: 3kW - kV/mA: 50/20 ▪ Detector - Type: Si(Li)detector - Resolution:130eV at 5.9 keV	▪ Primary X-ray beam scattered by a secondary target (Mo) ▪ Polarised x-ray beam (Cartesian geometry)	1000s (total)
WDXRF ***(Chapter 6)***	Na, Mg, Al, P, S, K, Ca, Mn, Fe, Co, Zn, As, Sr, Pb	▪ 2 g powdered material ▪ P=20T, t=60s ▪ ∅=4 cm	▪ X-Ray tube - Anode: Rh - Power: 1kW - kV/mA: 20/50 (Na, Mg, Al, P, S), 50/20 (Ca, Mn, Fe, Co, Zn, As, Sr, Pb),40/25 (K). ▪ Crystal: OVO-55 (Na,Mg), PET (Al,P.S), LIF200 (K,Ca,Mn,Fe,Co,Zn,As,Sr,Pb) ▪ Collimator (º): 2 (P), 0.46 (Na,Mg,Al,S,K,Ca), 0.23 (Mn,Fe,Co,Zn,As,Sr,Pb) ▪ Detector - Type: FPC (Na, Mg, Al, P, S, K, Ca), SC (Mn, Fe, Co, Zn, As, Sr, Pb) - Resolution:40eV at 8.047 keV	▪ Primary X-ray beam	100s (Co, Zn,Pb) 30s (others)
High-energy EDPXRF ***(Chapter 7)***	Cd, Pb,As, Cu,Fe,Zn	▪ 2 g powdered material ▪ P=20T, t=60s ▪ ∅=3.2cm	▪ X-Ray tube - Anode: Gd - Power: 600W kV/mA: 100/5 (Cd), 100/6 (others) ▪ Detector - Type: High-energy Ge semiconductor - Resolution <140eV at 5.9 keV	▪ Primary X-ray beam scattered by changeable secondary targets (Cd: Al_2O_3, Pb: Zr, Others: KBr) ▪ Polarised x-ray beam (Cartesian geometry)	1000s (Cd) 600s (others)

Notes:

- Crystals: OVO-55 (W/Si multilayer) PET (Pentaerythrite), LiF200 (Lithium fluoride).
- Detectors: FPC (flow proportional counter), SC: Scintillation counter.

Table-C.1 (Continued). - Summary of the performance of XRF analysis of vegetation samples.

XRF Configuration	Quantitative analysis	Limits of detection ($mg \cdot kg^{-1}$)	Relative precision (%)	Relative bias (%)	Observations
EDPXRF *(Chapter 5)*	▪ FP	-K=10.0 -Ca=20.0 -Mn=4.0 -Fe=3.1 -Cu=1.2 -Zn=1.2 -Sr=1.1 -Pb=1.1	*(NIST SRM 1571)* -K=20.0 -Ca=14.3 -Mn=5.2 -Fe=3.4 -Cu=21.4 -Zn=1.2 -Sr=5.7 -Pb=0.7	*(NIST SRM 1571)* - K=2.0 - Ca=0.5 - Mn=5.5 - Fe=-1.0 - Cu=16.7 - Zn=0 - Sr=-5.4 - Pb=2.2	
WDXRF *(Chapter 6)*	▪ ECC (synthetic cellulose standards)	-Na=80 -Mg=50 -Al=30 -P=30 -S=40 -K=400 -Ca=200 -Mn=6 -Fe=20 -Co=0.5 -Zn=5 -As=1 -Sr=0.7 -Pb=6	*(CBW 07602)* -Na=8.2 -Mg=2.9 -Al=5.0 -P=3.4 -S=3.2 -K=3.2 -Ca=2.2 -Mn=5.6 -Fe=5.8 -Co(<LD) -Zn=4.5 -As(<LD) -Sr=3.2 -Pb=8.9	*(CBW 07602)* -Na=0 -Mg=-5.2 -Al=-6.5 -P=42.4 -S=-3.1 -K=10.6 -Ca=2.3 -Mn=-6.9 -Fe=1.0 -Co(<LD) -Zn=6.8 -As(<LD) -Sr=-10.1 -Pb=11.3	
High-energy EDPXRF *(Chapter 7)*	▪ ECC (Cd) ▪ FP (IAEA-QXAS) (others)	-Pb=0.27 -Zn=0.18 -Fe=0.58 -As=0.20 -Cu=0.24 -Cd=0.7	*(CBW 07602)* -Pb=2.0 -Zn=2.0 -Fe=0.4 -As=8 -Cu=1 *(NIST SRM 1570a)* -Cd=7.2	*(CBW 07602)* -Pb=2.0 -Zn=-8.0 -Fe=-12 -As=-3 -Cu=48 *(NIST SRM 1570a)* -Cd=5.5	▪ Cd (Kα line) ▪ Interference of As-Kα and Pb-Lα was solved by employing selective excitation with targets of different materials

Notes:

- ECC: Empirical calibration curve, FP: Fundamental parameters approach (standard-less)
- Relative precision = (St.Dev/Average)*100
- Relative bias= ((Average-certified)/certified)*100
- NIST SRM 157: [K]=1.47±0.03 %, [Ca]=2.09±0.03 %, [Mn]=91±4$mg \cdot kg^{-1}$, [Fe]=300±20 $mg \cdot kg^{-1}$, [Cu]=12±1 $mg \cdot kg^{-1}$, [Zn]=25±3 $mg \cdot kg^{-1}$, [Sr]=37±1 $mg \cdot kg^{-1}$, [Pb]=45±3 $mg \cdot kg^{-1}$.
- CBW07602: [Na]=1.10±0.06 %, [Mg]=0.287±0.011 %, [Al]=0.214±0.018 %, [P]=0.083±0.003 %, [S]=0.32±0.02 %, [K]=0.85±0.03 %, [Ca]=2.22±0.07 %, [Mn]=58±3 $mg \cdot kg^{-1}$, [Fe]=1020±40 $mg \cdot kg^{-1}$, [Co]=0.39±0.03 $mg \cdot kg^{-1}$, [Zn]=20.6±1 $mg \cdot kg^{-1}$, [As]=0.95±0.08 $mg \cdot kg^{-1}$, [Sr]=345±7 $mg \cdot kg^{-1}$, [Pb]=7.1±0.7 $mg \cdot kg^{-1}$, [Cu]=6.6±0.4 $mg \cdot kg^{-1}$.
- NIST SRM 1570a: [Cd]=2.89±0.07 $mg \cdot kg^{-1}$.
- Limits of detection: (see Chapter 5, Chapter 6 and Chapter 7 for calculation details)

The application of single and sequential extraction procedures in the laboratory prove to be a suitable approach to predict metal mobility and bio-availability of metals in the mining wastes (Chapter 2 and Chapter 3).

On the other hand, the quadrupole based ICP-MS technique proposed for isotopic analysis of vegetation samples and mining tailings provides sufficient precision (RSD~0.1% except in the case of isotope ratios involving ^{204}Pb, RSD~0.2-0.3%) to distinguish between the more probable sources of lead contamination in the plant specimens studied (Chapter 4).

In Table C.2, a brief summary of the main conclusions derived from the application of the methodologies named above to the samples collected in the mining districts of concern is presented.

From the data attained it could be concluded the risk of metal dispersal around theses areas coming from the indiscriminately dumped mining wastes. It is supposed that the information obtained from the present research served as a good starting point for further evaluation and remediation of these old disused areas in order to protect public health and environment quality.

Issue	Related chapter	Analytical procedures	Conclusions
Metal mobility in mining wastes	Chapter 2	Mining waste samples: ▪ Sequential extraction procedure (BCR scheme) ▪ Leaching tests (TCLP and DIN 38414-S4).	▪ The "pseudototal" metal content in mining wastes is up to 20 000 $mg \cdot Kg^{-1}$ for Pb, 145000 $mg \cdot Kg^{-1}$ for Zn and 250 $mg \cdot Kg^{-1}$ for Cd, respectively. ▪ More easily mobilised forms were predominant for Cd and Zn (up to 70 % and 25%, respectively). ▪ Largest amount of Pb was associated with the iron and manganese oxide fractions (from 36 to 90%). ▪ All the mining wastes studied could be considered as hazardous according to specified regulations (leaching tests). ▪ pH of mining wastes is one of the main parameters affecting metal mobility
Metal bioavailability to vegetation species	Chapter 3	Mining waste samples: ▪ Single extraction procedure (DTPA) ▪ Step-1 and step-2 BCR scheme ▪ XRD Vegetation specimens (*Betula pendula*): ▪ EDPXRF	▪ The main sulphides of the ore vein (PbS and ZnS) have been converted into other species (mostly $PbSO_4$ and $ZnSO_4$) with a higher potential mobility. ▪ Bioavailability studies based on the use of DTPA seems to be useful for the determination of the metal fraction that a plant can possibly taken up if pH values of mining tailing are not too low (pH<5). ▪ Extractable metal fractions determined by employing the step-1 and step-2 of BCR scheme are a poor indicator of metal bioavailability. ▪ *Betula pendula* species seems to be useful as lead pollution indicator.
Metal content in vegetation species	Chapter 3 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Vegetation samples (*Betula pendula*, *Buddleia davidii*, *Leucobryum sp.*, *Broumus sp.*, *Pleurocarpus sp.*, *Salicornia sp.*, *Citrus limonium*): ▪ XRF (EDPXRF, WDXRF, high-energy EDPXRF)	▪ In general, metal contents of vegetation specimens collected in the mining districts are substantially higher than those from a specimen sampled at a clean region, above all concerning to Pb and Zn. ▪ The presence of high amounts of metals in the specimens from the mining areas seems to be directly associated with a decrease in nutritional elements such as K. ▪ Higher amounts of Cd were found in the case of moss species (*Leucobryum sp.*, *Pleurocarpus sp.*) and grasses (*Bromus sp.*).
Source of the accumulation of lead in vegetation species	Chapter 4	Vegetation samples (*Buddleia davidii*), mining wastes, soils: ▪ ICP-QMS	▪ From isotopic analysis of vegetation samples and mining wastes it was shown that the lead in the leaves and flowering tops of *Buddleia davidii* specimens from the mining area did not have the same isotopic composition as a specimen from a clean region but was isotopically identical to the mining wastes collected. This trend suggest that the lead accumulation in plants is primarily derived from the mining operations rather than other lead sources such as the past leaded-petrol emissions.

Notes:

- XRD: X-Ray diffraction spectrometry, XRF: X-ray fluorescence spectrometry, EDPXRF: Polarised-beam energy dispersive X-ray fluorescence spectrometry, WDXRF: wavelength dispersive X-ray fluorescence spectrometry, ICP-QMS: Quadrupole-based inductively coupled plasma mass spectrometry.

Table-C.2. - Summary of the main conclusions derived from the analysis of mining wastes and vegetation samples collected at the mining districts studied.

AGRAÏMENTS

Una vegada algú em va dir que el món de la recerca era "vocacional". Després d'aquests quatre anys treballant en aquest camp, realment crec que sabia el que es deia...

Passat aquest temps me n'adono de les moltes coses positives que aquesta experiència m'ha aportat a nivell professional, però també en l'àmbit personal. Per aquest motiu, en aquestes línies voldria donar el més sincer agraïment a totes aquelles persones que d'una manera o altra han col·laborat en la realització d'aquest treball i han fet possible que avui aquesta tesi sigui una realitat.

En primer lloc, voldria agrair de forma especial als meus directors de tesi, a la Dra. Manuela Hidalgo i al Dr. Ignasi Queralt, el seu suport incondicional durant tots aquests anys. Nela, gràcies per donar-me l' oportunitat d'introduir-me en el món de la recerca i per confiar en mi. Ignasi, gràcies per a donar-me l'oportunitat d'introduir-me en el món de la Fluorescència de Raigs-X (una tècnica una mica oblidada per molts químics...) i també pels teus valuosos consells. Ha estat un plaer treballar amb vosaltres!

La resta de companys de l'Àrea de Química Analítica de la Universitat de Girona han tingut també un paper important en la realització d'aquesta tesi. Gràcies per la vostra ajuda i per totes les estones que hem compartit, tant en els bons moments com en els no tant bons... Gràcies Victòria, Juanma, Enriqueta, Clàudia i Roberto, pels vostres consells tot i no estar directament relacionats amb el meu tema de recerca. Mònica, gràcies per fer-me de guia en el món de l'ICP-MS i per haver volgut perdre amb mi llargues estones intentat donar sentit als resultats obtinguts. I espero que ningú s'enfadi si destaco a les meves "ex-companyes" i "companyes" de despatx i de laboratori. Han estat moltes hores de convivència i de "rialles": Gemma, Carolina, Marta i Sònia, doctores i futures doctores, us desitjo el millor pel futur!!! A tu Sara, què t'he de dir... Qui ho havia de dir que finalment arribaria aquest moment??? Gràcies per ser una bona amiga i companya de "batalletes" durant aquests anys. Molta sort en el camp del suro! I a vosaltres Marta, Mercè, Mireia, Raquel i Chantal us animo a seguir endavant i espero que tingueu molta sort en les

vostres tesis! Ànims!!! ...No et pensis que m'oblido de tu Sílvia. Gràcies per la teva eficiència en el lab i també per les bones estones que hem compartit. Molta sort!

També voldria agrair el suport rebut dels Serveis Tècnics de Recerca de la Universitat de Girona, i en especial a la Dra. Lluïsa Matas, per la seva dedicació i ajuda amb l'ICP-MS i el microones.

A significant part of the experimental work presented in this thesis has been developed in the "Departamento de Física, Linha Experimental do Centro de Física Atómica Universidade de Lisboa (Portugal)" and in the "Micro and Trace Analysis Centre (MiTAC), Department of Chemistry, University of Antwerpen (Belgium)". Therefore, I want to give my deepest gratitude to Maria Luisa De Carvalho Leonardo, porfessora do Centro de Física Atómica da Universidade de Lisboa, and René Van Grieken, director of MiTAC, for their hospitality and generosity in offering me the possibility to work in their group, meant much more to me than just a professional challenge. Thank you!!!

I also want to thank some people who helped me to feel more at home in Antwerpen. These three months were an unforgettable journey and a self-discovery experience. I want to show my appreciation to all the members of the MiTAC for the support received during my stage there, especially to my "office matters" Katleen and Veli. It was really nice to share with you a lot of "coffee cups". I hope we can keep our friendship for years (and I hope to see you in Girona in a near future!).

Un agradecimiento muy especial a Román Padilla, miembro del CEADEN (Departamento de Física), por compartir conmigo un poquito de su sabiduría en XRF y por sus consejos y sugerencias durante mi etapa de investigación en Antwerpen. Román, sin duda eres una de las mejores personas que he conocido. Gracias por todo!

També vull agrair el suport rebut pels membres del consell General d'Aran, en especial a en Jordi Gavaldà i a en Joan Capdevila, en les campanyes de mostreig al districte miner de la Val d'Aran.

En un altre terreny que no és el professional, també hi ha molta gent que ha fet molt per mi i a qui voldria donar el meu agraïment.

Gràcies a les companyes de pis que he tingut durant aquests últims anys a Girona, en especial a tu Elena que has estat per a mi la germana que mai he tingut. També voldria agrair als amics de Riudaura i Olot l' interès i suport que han mostrat sempre per la meva trajectòria a la Universitat i per les bones estones que hem passat junts. Un petó molt fort als més petits, que em fan somriure fins i tot quan no en tinc ganes.

Un bloc d'agraïments molt gran per a la meva família. Gràcies pares per haver-me ajudat en els moments que més ho necessitava i per haver-me animat i donat suport en tot allò que m'he proposat, sense vosaltres no ho hagués aconseguit!!! Gràcies també als meus avis per la seva preocupació constant per saber com em van les coses per Girona.

... I finalment només em resta agrair a la persona amb la qual he compartit gairebé mitja vida, el seu recolzament constant. Gràcies David per estimar-me tal com sóc, per confiar en les meves possibilitats i pels bons moments que hem passat junts i els que espero que podrem passar durant molts anys més en aquest nou camí que hem iniciat.

De tot cor, gràcies a tots!