Universidade de Aveiro Departamento de Ambiente e Ordenamento
2010

SÓNIA ALEXANDRA MORAIS RODRIGUES

AVALIAÇÃO DA EXPOSIÇÃO À CONTAMINAÇÃO DO SOLO EM PORTUGAL E NA UE

CONTAMINATED SOIL EXPOSURE ASSESSMENT IN PORTUGAL AND IN THE EU

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências Aplicadas ao Ambiente, realizada sob a orientação científica do Doutor Armando da Costa Duarte, Professor Catedrático do Departamento de Química da Universidade de Aveiro e co-orientação da Doutora Maria Eduarda da Cunha Pereira, Professora Auxiliar do Departamento de Química da Universidade de Aveiro

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Aos meus pais, Alexandrina e Leandro, e à minha irmã Leandra.
Sem eles, nada disto seria possível. Sem eles, nada disto faria sentido.

o júri

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palavras-chave

elementos potencialmente tóxicos; transferência solo-planta; modelos empíricos tipo Freundlich; concentrações limite no solo; avaliação da exposição; saúde animal e humana; Política de solos.

resumo

Este estudo baseou-se na análise dos mecanismos de transferência de elementos potencialmente tóxicos (PTE’s) entre o solo, a solução do solo e as plantas como forma de realizar uma avaliação mais eficaz do risco em áreas agrícolas. Foram aplicados conceitos recentemente desenvolvidos para a avaliação da reactividade biogeoquímica de contaminantes no solo e da sua partição sólido:solução recorrendo-se a modelos empíricos (tipo Freundlich). Estes modelos permitiram analisar a transferência de PTE’s ao longo da cadeia alimentar e avaliar o impacto da contaminação do solo na qualidade da alimentação animal (forragens) e Humana (vegetais e carne) em Portugal.

Os modelos empíricos de transferência solo-planta de PTE’s foram utilizadas para obter limites críticos para estes elementos em solos agrícolas em Portugal, a partir dos seus limites legais nos alimentos para animais e teores máximos nos géneros alimentícios. Simultaneamente, modelos de exposição Humana a contaminantes do solo, desenvolvidos noutros países da UE foram analisados e foi proposto um modelo de exposição para Portugal.

Este trabalho é uma contribuição para o desenvolvimento de critérios de qualidade de solos para áreas agrícolas em Portugal, tendo em vista a protecção da saúde animal e Humana. Contribuiu também para o desenvolvimento de uma estratégia de harmonização de políticas de protecção do solo (nomeadamente no que diz respeito aos problemas de contaminação) na União Europeia.

keywords

potentially toxic elements; plant-uptake; Freundlich-type models; threshold soil concentrations; exposure assessment; animal and human health; soil policy.

abstract

This study focused on the geochemical reactivity of contaminants in soils and on the solid:solution partition of PTE's using Freundlich-type empirical models to assess the transfer of contaminants along feed and food supply chains in Portugal. The evaluation of the impact of soil contamination on feed and food quality allowed a more accurate identification of sites actually at risk. Such assessment was developed considering the specificity of Portuguese soils and it took into account soil properties. Furthermore, soil-to-plant transfer empirical models were applied in the calculation of threshold concentrations of PTE's in Portuguese agricultural soils. Threshold concentrations of PTE's in Portuguese agricultural soils - soil contaminant levels above which impacts in human health (resulting from intake of food crops and animal products and exceedance of human acceptable daily intake) and impacts on animal health (due to exceedance of animal acceptable daily intake) may occur – were back-calculated from legal standards for feedstuff and food. Finally, models to assess human exposure to soil contaminants in use in the EU were analysed and a framework for exposure assessment for Portugal was proposed.

This PhD program was a contribution for development and improvement of soil quality criteria for PTE's in agricultural areas in view of animal and human health effects as well as into the development of a contaminated soil exposure assessment strategy in Portugal and provided insight into harmonization perspectives for soil policy within the European Union.

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Preface

In 2006, a "Thematic Strategy for Soil Protection" was adopted by the European Commission. As a consequence, a Soil Framework Directive was proposed. One of the aims of this Directive was to give soil protection the same status that other environmental compartments such as water and air already have within the European Union regulatory framework.

The proposal for a Soil Framework Directive was debated during the 2842nd Council meeting (Environment) in Brussels (20 December 2007) under the auspices of the Portuguese Presidency. Unfortunately, in spite of various attempts by the Presidency to present compromise proposals in order to meet delegations' concerns, it was not possible to attain the qualified majority needed to reach political agreement on a draft directive establishing a framework for the protection of soil.

In my opinion, this is not the end of the story. On the contrary: it is the first stage in the development of a regulatory framework for soil protection in the European Union. Whether that will ever take the shape of a Directive, I don't know. But what I do know is that this is an opportunity and quite a challenge for the scientific community to demonstrate that without soil protection there will be no air or water quality, no plants, no crops, no forests, no animals, and of course…no people!

Sónia M. Rodrigues
24th November 2010
Aveiro, Portugal

Chapter 1

Introduction

1.1 Principles and concepts for soil protection

Soil quality strongly influences plant growth, biomass production, biodiversity, air and water quality conservation, as well as energy balances. Soil may influence human health both through direct (ingestion, inhalation of particles and dusts and dermal contact) and indirect routes (toxic elements absorbed by plants or leached into water) (EC, 2006a). Contact with soil during leisure practices, working and day-to-day activities (inhalation of particles and dermal contact) also constitute relevant direct routes of human exposure to soil contaminants. Run-off and soil leaching may also contribute to increase contaminants' levels in surface waters, aquifers and affect drinking water quality. It is therefore important to have a clear understanding of the soil quality status, particularly in areas where human populations may be at highest risk.

Awareness has grown that soil is an important factor in the feed and food supply chain in order to deliver safe and high-quality products (Franz et al., 2008). The role of the soil in maintaining food safety is explicitly mentioned in the EU Thematic Strategy for Soil Protection (EC, 2006a). The presence of potentially toxic elements (PTE's) like Cd, As, Hg, Pb, Co, Sb, Ba and U in soils used to produce food or feed may pose a public health risk due to the accumulation of the contaminant in food and feed and the subsequent accumulation in the human body over time. This accumulation can result in a variety of health problems, such as impairment of kidney function, in both animals and humans (Franz et al., 2008). The degree to which PTE's are available for plant uptake and further accumulation in edible plant parts as well as animal target organs (such as kidneys, liver, muscle) depends strongly on the degree of pollution and soil characteristics (de Vries et al., 2007; Franz et al., 2008). Most human exposure assessments concerning the dietary intake of PTE's are based on measured concentrations in food products. However, for specific regions, monitoring data are often not available.

Information on levels of risks to the environment and human health is necessary for decision making regarding management of contaminated areas. Nowadays it is well accepted by the scientific community that data on total contents of PTE's in soils is not sufficient to assess risks associated to contaminated land since total contaminant concentrations are not necessarily indicative of actually occurring adverse effects (Sauvé et al., 2000; Meers et al., 2007a; Peijenburg et al., 2007). Elevated levels of soil elements may in certain cases occur naturally (background elemental concentrations) and be present in soils in forms that are not likely to be mobilised. Therefore, the accuracy of risk assessments and the analysis of the probability of occurrence of adverse health effects are constrained by our ability to determine the fraction of the contaminant that is reactive in soils and that may become mobile and/ or available to plants (Peijenburg et al., 2007).

1.2 Current tools for soil protection in the EU

Although the EU has no specific contaminated land legislation, in its Communication "COM (2002) 179 final" the European Commission established a timetable to produce a strategy for soil protection within the EU. Five working groups (WG), including a WG on soil contamination, have been set up and in 2006 an EU Strategy for Soil Protection was launched (EC, 2006a). A legislative proposal designed to achieve the protection and sustainable use of soil - A Soil Framework Directive – is presently under discussion. The introduction of measures to prevent soil contamination as well as requirements to Member States towards the development of inventories of contaminated sites through soil investigations and risk assessments and the definition of targets and prioritization of actions for ensuring remediation of contaminated sites are key aspects of this discussion.

Specific research needs to support an EU policy for soil protection have been identified by Blum et al. (2004). These include a requirement for comprehensive research on the analysis of processes related to threats to soil (such as contamination) and the development and harmonization of methods for risk assessment in Europe. Studies on soil quality have been undertaken but there is a specific need for the harmonization of methodologies to the selection of indicators and suitable criteria, the selection of sampling and analytical methodologies, the definition of risk assessment procedures, the identification of contaminated sites and the selection of appropriate remediation techniques.

During the last three decades, certain European countries have developed national policies for the management of contaminated sites or specific legislation regulating investigation and clean-up of contaminated land. Reviews of national soil policies from different perspectives can be found in literature (Ferguson, 1999; De Sousa, 2001; Van Veen, 2002; Prokop, 2005; Provoost et al, 2006; Smith et al, 2006; D'Aprile et al, 2007; Thornton et al., 2007; Bergius and Oberg, 2007; Bouma and Droogers, 2007; Carlon, 2007). These authors analyzed soil policies from different countries in an international context, discussing issues such as legal frameworks, financial incentives, risk assessment and soil clean-up standards.

1.3 Existing risk assessment tools for contaminated soils in the EU

Models to assess human exposure to soil contaminantion are in widespread use within the EU. An example of implicit use of such models is the comparison of measured contaminant concentrations with soil and groundwater quality standards derived from these exposure models (Swartjes, 2002). These models can also be used in decision making based on (site-specific) exposure calculations (Swartjes, 2002).

Examples of contaminated soil exposure models are from EU countries (Swartjes, 2002):

- CETOX-human (Denmark);
- CLEA (UK);
- CSOIL (the Netherlands);
- ROME (Italy);
- Vlier-humaan (Flanders, Belgium);
- an unnamed model from Sweden;
- an unnamed model from France

According to Swartjes (2002), uncertainties about model concepts and input parameters are the main sources of limitations in calculations of human exposure.

1.4 Need for an approach for contaminated soil exposure assessment for Portugal

Despite high change rates during the last two decades, overall land cover of Portugal is largely dominated by forest and agricultural areas. In 2000, agricultural areas occupied around 48% of the national territory (Rodrigues et al., 2009a). Since the traditional agri-environment in Portugal is often associated to small-scale agriculture, only in the last two decades with the increase of urban pressure, soil contamination issues started gaining attention (Rodrigues et al., 2009a).

In Portugal, although several studies have focused on agricultural soils there is still a considerable lack of information on the actual extent and nature of contaminated land problems as well as on risks to animal and human health associated to them (Rodrigues et al., 2009a). Information on regional differences in PTE's levels and soil characteristics which may lead to differences in PTE's levels in feed and food crops and eventually to differences in human dietary exposure to these contaminants is also scarce (Rodrigues et al., 2009a).

No National contaminated land management strategy has been implemented in Portugal to date. In the absence of such strategy, the prevention and detection of arising soil contamination problems have been dealt with in the scope of the national waste management strategy and other environmental regulations.

Most relevant research needs for the development of contaminated land management practices in Portugal are those associated with the definition of a risk assessment framework and setting guidelines for the evaluation of risks posed to both humans and ecosystems (Rodrigues et al., 2009a).

Given the gap between the situation at other European countries and the state of Portuguese soil policy development, it is of merit to consider contaminated land exposure assessment models already in use in the EU and to see if these provide a suitable basis to define an approach for exposure assessment in Portugal.

1.5 Aim, scope and objectives

This study will focus on the geochemical reactivity of contaminants in soils and on the solid:solution partition of PTE's using Freundlich-type empirical models to assess the transfer of contaminants along feed and food supply chains in Portugal. The evaluation of the impact of soil contamination on feed and food quality will allow a proper identification of sites actually at risk. Such assessment will be developed considering the specificity of Portuguese soils and it will take into account soil properties. Furthermore, soil-to-plant transfer functions will be derived and will be applied in the calculation of threshold concentrations of PTE's in Portuguese agricultural soils considering legal standards for feedstuff and food. Threshold concentrations of PTE's in agricultural soils are soil contaminant levels above which impacts in human health (resulting from intake of food crops and animal products and exceedance of human acceptable daily intake) and impacts on animal health (due to exceedance of animal acceptable daily intake) may occur. This PhD program will provide insight into the development and improvement of soil quality criteria for PTE's in agricultural areas in view of animal and human health effects as well as into the development of a contaminated soil exposure assessment strategy for Portugal.

In summary, the main aims of this investigation are:

- to derive improved threshold concentrations of PTE's in agricultural soils which take into account soil properties;
- to estimate human and animal dietary exposure to PTE's on a regional level, using a chain modelling approach taking into account the supply chain from soil to the consumer;
- to propose a contaminated soil exposure assessment strategy for agricultural areas in Portugal.

Specific objectives of this work are:

- to perform a critical review of contaminated soil assessment and management strategies in place in the EU and in European countries;
- the assessment of soil characteristics and of the distribution of PTE's in soil and crop samples from agricultural areas in Portugal;
- to perform an assessment of reactivity and availability of PTE's in Portuguese soils and the derivation of solid:solution partition relationships as a function of soil properties;
- to calibrate soil-to-plant transfer functions that take into account the variability of soil characteristics;
- to estimate animal (cows and sheep) and human dietary exposure to PTE's to evaluate impacts of soil contamination in feed and food supply chains in Portugal;
- to calculate threshold soil concentration of PTE's in Portuguese agricultural soils in view of animal and human health protection; these thresholds will be back-calculated taking into account soil characteristics from:
    - quality criteria in animal feed crops (undesirable substances in animal feed);
    - quality criteria for animal products in view of animal health protection;
    - criteria for animal products (organs and meat) in view of food safety and human health protection;
- to propose a contaminated soil exposure assessment framework for Portuguese agricultural soils.

This study will consider a large array of PTE's (As, Hg, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo) with particular emphasis on less studied contaminants. Both soil and crop samples will be collected. Since it would be impossible to collect samples in the entire country within the scope of such PhD program, target areas geographically distributed around the country will be selected. These areas will include both agricultural fields that are not expected to be affected by known pollution sources and soils that are known to be impacted by industrial and mining activities. The PhD program is designed in such a way that the approach developed can also be applied to other regions in the future, using region-specific data. The results obtained can also serve as a conceptual basis for future risk assessment studies to be conducted in contaminated sites in Portugal and elsewhere.

The development of this study will require the development of expertise in the areas of geochemical characterisation of soils; plant uptake processes; biochemistry and toxicology; risk assessment; environmental management and soil policy.

1.6 Tasks and milestones of this investigation

The PhD research plan will include seven main tasks:

- **Task 1** – Review of Soil Policy and Soil Protection Strategies in Europe
- **Task 2** – Review of Soil Policy and Soil Protection Strategy in Portugal
- **Task 3** – Assessment of total, reactive and available pools of PTE's in Portuguese soils
- **Task 4** – Derivation of soil:soil solution partition relationships
- **Task 5** – Assessment of concentrations of PTE's in Portuguese crops and derivation of soil to plant transfer functions
- **Task 6** - Impacts of soil contamination in feed and food supply chains in Portugal
- **Task 7** – Analysis of European human exposure models and suggestions for model development in Portugal

The interaction between the different tasks is described by Figure 1.1.

The most important milestones of this PhD program are:

- **M1** - Review of contaminated land management strategies from Portugal and other EU countries. (month 12)
- **M2** - Collection of soils and plant samples from agricultural fields from different areas in Portugal. (month 12)
- **M3** - Analysis of soil and plant samples: chemical characterisation of all samples collected; results will be used in the derivation of soil-soil solution, soil-soil solution-plants and plant-animal relationships. (month 24)
- **M4** - Derivation of soil-soil solution, soil-soil solution-plants, and soil-plant-animal transfer functions for the different PTE's; results will be used in calculations of dietary exposure (M5) and in the derivation of threshold concentrations of PTE's (M6). (month 30)
- **M5** - Dietary exposure to PTE's for animals and humans: estimation of animal and human dietary exposure to PTEs on a regional level, using measured data (M3) and the predictive modelling approach taking into account the supply chain from soil to the consumer (M4). (month 30)
- **M6** - Threshold concentrations of PTE's in agricultural soils in Portugal. (month 36)
- **M7** - Contaminated soil exposure assessment strategy for agricultural soils in Portugal. (month 42)

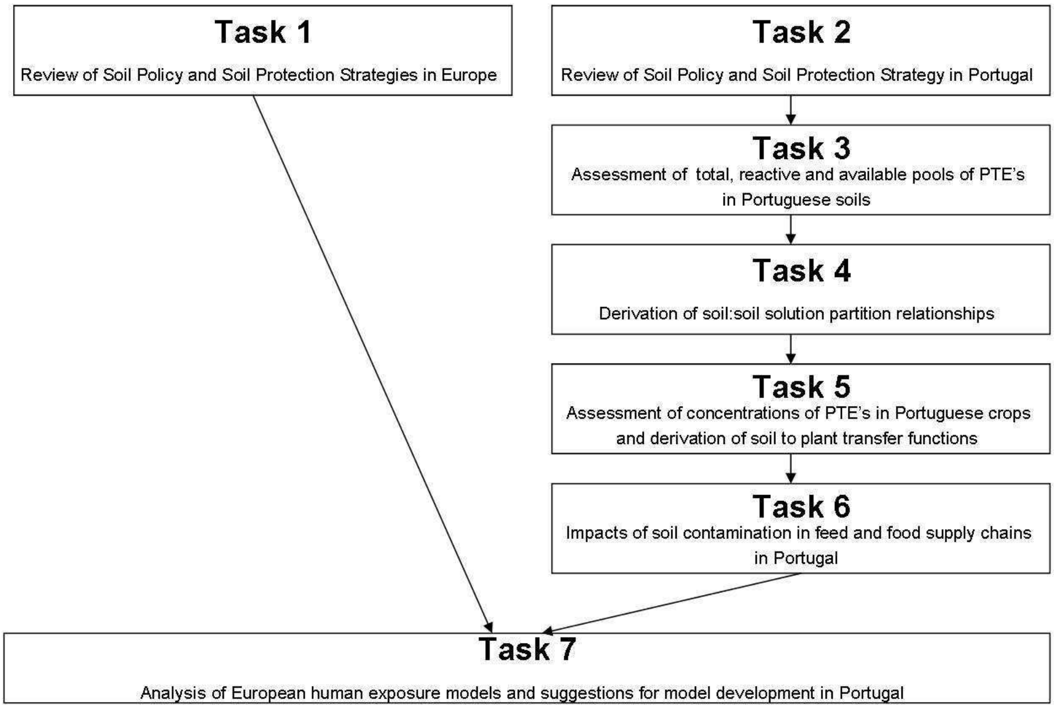

Figure 1.1: Interaction between research tasks

A timetable for the execution of the research tasks and achievement of the proposed milestones is shown in Table 1.1.

Table 1.1: Timetable for tasks and milestones

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<tr><th colspan="2">Year 1</th><th colspan="2">Year 2</th><th colspan="2">Year 3</th><th colspan="2">Year 4</th></tr>
<tr><td>6</td><td>12</td><td>18</td><td>24</td><td>30</td><td>36</td><td>42</td><td>48</td></tr>
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<tr><td></td><td>M1, M2</td><td></td><td>M3</td><td>M4, M5</td><td>M6</td><td>M7</td><td></td></tr>
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1.7 Outline of this thesis

The results obtained in the course of this investigation will be discussed in the following nine Chapters of this report:

- **Chapter 2** – Review of Soil Policy and Soil Protection Strategies in Europe
- **Chapter 3** – Review of Soil Policy and Soil Protection Strategy in Portugal
- **Chapter 4** – Contamination problems in Portugal: Experimental approach and data evaluation
- **Chapter 5** – Total, reactive and available pools of PTE's in Portuguese soils
- **Chapter 6** – Partition relationships: contribution of this study to improve the assessment of reactivity and direct availability of PTE's at contaminated sites
- **Chapter 7** – Concentrations of PTE's in Portuguese crops and derivation of soil to plant transfer functions (SPTF)
- **Chapter 8** - Impacts of soil contamination in feed and food supply chains in Portugal
- **Chapter 9** – The accumulation of soil contaminants in crops: analysis of European human exposure models and suggestions for model development in Portugal
- **Chapter 10** – General discussion and conclusions

Chapter 2

Soil Policy and Soil Protection Strategies in Europe

2.1 Introduction

Soil contamination has been identified as one of the major threats to soil function in Europe by the Communication from the European Commission "Towards a Thematic Strategy for Soil Protection" (EC, 2006a). Pressures posed over the soil resource are associated with irreversible land losses worldwide. Unsustainable development results in the production of brownfields and derelict land (Simpson, 1996). Rising degradation problems are increasingly affecting the sustainability of the soil resource and its ability to support life systems (Plant et al., 2001). The main drivers are population pressures, usually concentrating in localised areas, and changes in climate and land use.

Overall estimates from the European Environment Agency (EEA) identify metals and mineral oil as the main soil contaminants in Europe. Metals (~37%), mineral oil (~34%), PAHs (~13%), and aromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylene - BTEX, ~6%) affect almost 90%

of the European sites for which information on contaminants is available, while their relative contribution may vary greatly from country to country (EEA, 2007). Contaminated sites identified in Europe are predominantly associated with local sources deriving, in decreasing order, from industrial production and commercial services, municipal waste treatment and disposal, the oil industry (extraction and transport) and industrial waste disposal (EEA, 2007).

Soil contamination may have important consequences in terms of soils' ability to function. Soils may fail to support vegetation and biomass production, may fail to provide valuable materials and substrate to human activities, ecological systems and biological cycling of nutrients or may be unable to act as filter and buffer, affecting the hydrosphere, compromising groundwater resources and threatening aquatic ecosystems (van Straalen, 2002; Scullion, 2006). Soil contamination particularly from historical activities, still remains a problem despite several national and international initiatives that have been established to remediate contaminated sites and to reduce the release of contaminants into the environment: licence conditions for the operation of industrial processes; control on the application of sewage sludge to land; control on the spreading of biosolids to land; and, the landfill of waste. In cases of severe contamination and where risks to human health and/ or the environment are observed, soil remediation is necessary. Although annual expenditure on clean-up in the EU Member States for the period 1999–2002 have reached € 35 per capita per year in some countries and that a substantial sum of money has already been spent on soil remediation in Europe, this is still relatively small (up to 8 %) when compared with the estimated total costs of contaminated sites remediation in Europe (EEA, 2007).

Strategies to deal with soil contamination are being developed through a variety of regulatory systems. During the last 20 to 30 years, soil protection policies have been developed and implemented in a stepwise manner, both nationally and at the EU level. Plans for the introduction of measures and requirements for EU Member States to prevent new and remediate historical soil contamination include the development of inventories of contaminated sites and the definition of targets for prioritization of remediation actions. These plans are expected to have important consequences for soil management practice and national soil policies across Europe.

2.2 Aim, scope and objectives

An analysis of the evolution of soil contamination management practices through time as well as a review of several regulatory frameworks for contaminated land management, particularly from Europe will be discussed in this Chapter. The main challenges in the development of national policies and risk assessment frameworks will also be identified.

2.3 Soil Policy and Soil Protection Strategies in Europe

2.3.1 Actions at the EU level

References to soil protection can be found scattered throughout the European Community regulatory structure, establishing a number of instruments and measures that have a direct or indirect impact on the quality of soil. A number of aspects directly or indirectly related to soil contamination and/ or remediation issues are addressed by waste, water, chemical, impact assessment, environmental liability, and air quality policies. Table 2.1 indicates the EU policy measures and instruments that explicitly (directly) address aspects of soil contamination and the legislation that may have some indirect effects on soil contamination. A distinction between local and diffuse soil contamination sources has been made to allow a better understanding of the potential impact of these instruments on the abatement of soil contamination. Most of these instruments are designed to control and/ or prevent emissions at source, reducing the influx of contaminants into the environment and in this way mitigating the impacts of the accumulation of contaminants on different environmental compartments such as the soil compartment. These measures address different types of elements and substances such as metals, acidifying and eutrophying compounds, nutrients, pesticides and other organic compounds. The Waste Framework Directive (2006/12/EC) that sets provisions for waste disposal and recovery and for regulating the recycle and re-use of contaminated wastes may indirectly contribute for a more sustainable remediation of contaminated sites and for the prevention of soil contamination. The implementation of the Water Framework Directive (2000/60/EC) towards a good status of water resources may directly lead to the recovery of contaminated areas and to the mitigation of certain soil contamination problems. Due to the strong interdependencies between groundwater and soil systems the implementation of the Groundwater Directive (Directive 2006/118/EC on protection of groundwater against pollution and deterioration (EC, 2006b) is also expected to have a direct impact on soil quality. This legislation includes provisions aimed at preventing and limiting indirect discharges (after percolation through soil or subsoil) of pollutants into groundwater. Although not dealing with historical contamination problems the Environmental Liability Directive (2004/35/EC) already includes provisions for addressing new contamination problems and for the remediation of land damage whenever there is risk associated with contaminated land and where this contamination may adversely affect human health. According to the Environmental Liability Directive, remedial measures must be function oriented and take into account harmful substances, preparations, organisms or micro-organisms, their risk and the possibility of their dispersion. Certain instruments such as the REACH regulation (Registration, Evaluation, Authorisation and Restriction of Chemical substances), the Common Agricultural Policy, Internal Market regulations on product quality and land use, urban environment, nature and biodiversity conservation policies also address in a direct or indirect way the problems related to soil contamination. So far, historically contaminated land within the EU has been dealt with mostly through market driven redevelopment or specific public driven projects.

Table 2.1: Main EU environmental policies that address soil contamination aspects

EU Environmental Policies		**diffuse soil contamination aspects**		**local soil contamination aspects**	
		addressed:			
		directly	indirectly	directly	indirectly
Waste	Waste Framework Directive (2006/12/EC, codified version of Directive 75/442/EEC as amended)				√
	Directive 91/689/EEC on Hazardous Waste, amended in 1994				√
	Directive on the Disposal of Waste Oils (75/439/EEC amended in 2000)				√
	Landfill Directive (1999/31/EC)				√
	Sewage Sludge Directive (86/278/EEC)	√			
	Directive2006/21/EC on the management of waste from the extractive industries	√		√	
Water	Water Framework Directive (2000/60/EC)	√			
	Nitrates Directive (91/676/EEC)	√			
	Urban Wastewater Treatment Directive (91/271/EEC)		√		
	Bathing Water Directive (2006/7/EC)		√		
Air	Air Quality Framework Directive (96/62/EC) and its Daughter Directives		√		
	Directive on National Emissions Ceilings (2001/81/EC)		√		
	Directive on Integrated Pollution Prevention and Control (96/61/EC)	√			
	Directive on Large Combustion Plants (LCPD) (2001/80/EC)		√		
Chemicals	Thematic strategy on the sustainable use of pesticides	√			
	Directive on Biocidal Products (98/8/EC)62	√			
	Directive 91/414/EEC on plant protection products	√			
Impact Assessment	Environmental Impact Assessment Directive (85/337/EEC amended in 1997 and 2003)	√		√	
	Strategic Environmental Assessment Directive (SEA) (2001/42/EC)	√			
Environmental Liability	Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage			√	

Although diverse, the EU instruments and measures directly addressing soil issues are somewhat fragmentary. These policies (that are not primarily oriented towards soil protection) focus mostly on diffuse rather than local contamination aspects and they are quite limited when dealing with historical contamination and site development issues.

Since knowledge of soil-related problems is increasing in the EU (Thornton et al, 2007), a Thematic Strategy on Soil Protection was launched in 2006 (EC, 2002a; EC, 2006a) which explicitly recognized the importance of preventing soil degradation. Considering that soil is a resource of common interest within the European Community, that the degradation of the soil resource may have transboundary effects and/ or affect other resources of common interest (such as water and biodiversity), that soil contamination may affect food and feed crops that are being freely traded within the internal market and therefore pose a risk to human and animal health and that the implementation of very diverging contaminated land management regimes within the EU may lead to distortions of competition within the internal market (EC, 2006c), the Commission recognized the need to enact framework legislation with the principal aim of protecting the soil resource and promoting its sustainable use. Therefore, in 2006 a new legislative proposal – a draft Soil Framework Directive, SFD (EC, 2006c) was presented by the European Commission. The draft SFD is the statutory elaboration of the Thematic Strategy which takes into account seven large-scale threats to European soils (contamination, erosion, loss of organic matter, compaction, salinization, soil sealing and landslides) and aims to prevent soil degradation, based on the following principles: integration of soil concerns into other policies, prevention of threats to soil and mitigation of their effects, preservation of soil functions within the context of sustainable use, and the remediation of degraded soils. It allows local soil and land use to be taken into account and includes the possibility to delegate the enactment of policy aims and measures to local authorities. Concerning contaminated land, the SFD (as proposed in 2006) includes a systematic inventory of contaminated sites, the definition of National Remediation Strategies and a soil status report to be made available to competent authorities whenever a site on which a potentially polluting activity has been developed, is to be sold. The Commission aims to develop grounds for a common risk-based strategy to manage historical contamination based on a step by step approach that includes the collection of the information on the full extent of site contamination problems in all Member States, on the evaluation of associated risks and on the prioritization of remediation needs.

The draft SFD demanded for a precautionary approach to be followed and defines a list of potential sources of soil contamination, such as industrial facilities, mines and waste landfills both operating and after closure, former military sites, ports and airports, dry cleaners and waste water treatment installations and considers a broad group of dangerous substances for which future soil contamination must be prevented and past contamination must be remediated (EC, 2006c). According to the draft SFD, contamination that would "hamper soil function or give rise to significant risks to human health or the environment" is to be prevented (EC, 2006c). Sites are considered contaminated and needing remediation whenever "they pose a significant risk to human health or the environment", but the mechanisms through which "significant risk" is assessed are yet to be defined. In the Impact Assessment document associated with the implementation of the Thematic Strategy the Commission estimates that 3.5 million potentially contaminated sites exist in Europe, with 0.5 million sites needing remediation (EC, 2006d). Insofar as it can be estimated the costs associated with soil contamination vary between € 2.4 and 17.3 billion per year, but other estimations indicate that these costs could amount annually to up to € 208 billion (EC, 2006d). Additional European wide data is needed to support an effective estimation of the costs associated with contamination problems, and of the costs and benefits of the implementation of the SFD in each Member State. Relevant costs for Member States may include those associated to the systematic identification of contaminated sites and their remediation, possible land values depreciation or land use restrictions. Relevant benefits are: the reduction of risks to human health for people living in the surroundings of contaminated sites or at risk of drinking potentially contaminated water; the reduction of surface and groundwater contamination; the reduction of environmental impacts and ecological risks; the reduction of losses of biodiversity and soil fertility; the benefits from recycling and re-use of materials; and the potential land value appreciation after site remediation. Moreover, given the wide range of national situations across the EU (some countries have national soil policies in place since the early 1980s, others have yet to introduce contaminated land management regulations and to start the development of a national policy), Member States are still to explore the alternatives for the implementation of the Thematic Strategy and its relation with the national approaches already developed (Bouma and Droogers, 2007). To achieve the EU soil protection objectives will require rational land use planning at national, regional and local levels that allows soil's capacity to be taken into account (Thornton et al, 2007).

The enactment of EU soil framework legislation is also associated with further harmonised research needs particularly on scientific and technical aspects of risk assessment and remediation solutions. An analysis of future research needs for Europe in support of the European Thematic Strategy for Soil Protection has been developed by Blum et al. (2004). These authors highlight the pressing need for research which combines the analysis of processes related to threats to soil (such as contamination) and the development and harmonisation of methods for soil monitoring. Despite relevant subsidiarity aspects that are crucial for the effective implementation of a flexible EU framework, the harmonisation of scientific aspects of contaminated land risk assessment also require further discussion (Carlon, 2007; Vegter, 2008). The integration of soil function analysis into site development practices as well as the definition of relationships between site clean-up and restoration of soil functions will require intensified contaminated soil research which can be rationally integrated with soil policy to allow the development of robust science-based regulatory decisions for contaminated land management. In addition, the overall effects of soil function restoration on climate and on other environmental compartments need further evaluation.

2.3.2 Other European concerted actions

Several international organizations and concerted actions have committed to the analysis of practical approaches for the prevention and remediation of contaminated soil. A few examples will be described next. The COMMON FORUM on Contaminated Land (initiated in 1994) is a network of contaminated land policy makers and advisors from national ministries in European Union Member States and European Free Trade Association countries. This is a platform for exchange of knowledge with the main objective of developing strategies for the management and treatment of contaminated sites and for land recycling. The EU project CARACAS (1995-1998), Concerted Action for Risk Assessment for Contaminated Sites in Europe, focused on the evaluation of the practical state-of-the-art of contaminated land investigation and risk assessment practices in European countries. The CARACAS project closed in 1998 and its work was incorporated into CLARINET (1998-2001), the Contaminated Land Rehabilitation Network for Environmental Technologies in Europe (http://www.clarinet.at), which developed the concept of Risk Based Land Management (RBLM) as a step forward towards an integration of sustainable soil quality, protection of water and land use management in environmental policy. Another example of stakeholders' initiatives is the network NICOLE (Network for Industrially Contaminated Land in Europe, http://www.nicole.org) that is an independently funded European forum set up in 1995 where industry, service providers and academia cooperate to drive forward practical issues to contaminated land management. The link between contaminated land policies and spatial planning also provided opportunities to use (re)-development processes as drivers for improving soil quality and to developing approaches for sustainable brownfield redevelopment and revitalisation. These aspects have been dealt with for example by CABERNET, the Concerted Action on Brownfield and Economic Regeneration Network (http://www.cabernet.org.uk) that aims to enhance the rehabilitation of brownfield sites by addressing the complex multi-stakeholder issues that are raised by brownfileld regeneration. CABERNET was established in January 2002 and builds on the work of the previous network CLARINET. The development of public-private partnerships and the re-use of marketing concepts in the context of brownfield regeneration have been analysed by the project REVIT (2003-2007), Revitalising Industrial Sites (http://www.revit-nweurope.org). The HERACLES (Human and Ecological Risk Assessment for Contaminated Land in Europe) expert network is an initiative of the European Commission, DG Joint Research Centre (*JRC*) is a long term research framework for the collaboration of the *JRC* with other European institutes (research institutes and other interested bodies) in developing common references for risk assessment of contaminated land in Europe (Swartjes and Carlon, 2008). In the framework of the HERACLES network, a review of derivation methods for soil screening values in Europe ("Derivation methods of soil screening values in Europe: a review and evaluation of national procedures towards harmonisation") has been published (Carlon, 2007). This review analyses the basis of screening values used in EU Member States and initiates a discussion on the reasons for their differences and on the scope for harmonisation.

In addition, INTERREG projects (funded under the European Regional Development Fund) and projects funded by R&D EU programmes have focused on integration aspects of contaminated land management and soil protection (Prokop, 2005). These actions have provided an important opportunity for the exchange of knowledge on contaminated soil management and a relevant underpinning of international cooperation on scientific and technical issues. International discussions on risk assessment practices have been useful to increase the flexibility of contaminated land management national regulations in Europe and for the development and implementation of site-specific risk assessment practices. International discussions on the links between soil protection and spatial planning, on multi-stakeholders participation and on the development of innovative financing schemes for brownfields regeneration allow a common understanding to be reached. They also provide an integrated information base, drawn from experiences across Europe, to support re-development projects that create opportunities for dealing with historically contaminated sites in European urban areas in a more sustainable manner. Science-based discussions on the development of European common references for soil quality assessment are also crucial for the implementation of future EU framework soil legislation.

2.3.3 Actions at a national level

European countries

Although requirements for soil protection are generally included in several national legislative acts (e.g. environmental framework legislation; water, waste and mining regulations), some countries have already developed national policies for the management of contaminated sites or specific legislation regulating investigation and clean-up of contaminated land. Reviews of national soil policies from different perspectives can be found in literature (Ferguson, 1999; De Sousa, 2001; Van Veen, 2002; Prokop, 2005; Provoost et al, 2006; Smith et al, 2006; D'Aprile et al, 2007; Thornton et al., 2007; Bergius and Oberg, 2007; Bouma and Droogers, 2007; Carlon, 2007). These authors have analysed soil policies from different countries in an international context, discussing issues including legal frameworks, financial incentives, risk assessment and soil clean-up standards. Figure 2.1 shows an overview of main national policies and regulations for the management of contaminated soils introduced by several EU Member States, Norway and Switzerland during the last 30 years and that resulted from particular national interests. A detailed analysis of each situation is not included here although a number of relevant examples will be discussed next.

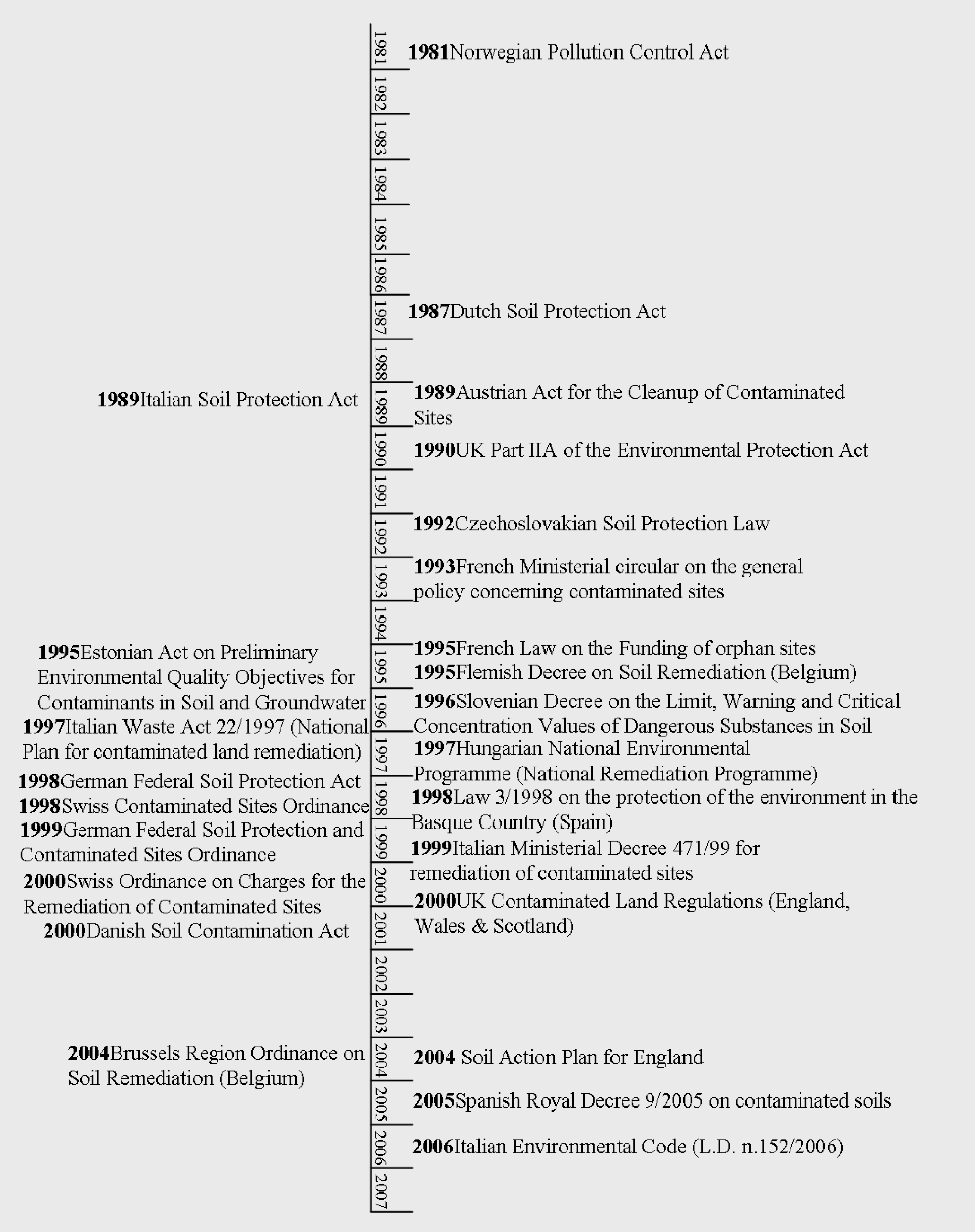


Figure 2.1: Overview of national policies and regulations for management of contaminated soils in Europe

Early in the 1980's Norway defined very specific provisions related to soil pollution, by introducing the "Pollution Control Act" (based on the "polluter-pays" principle) and by assigning responsibilities for the regulation of contaminated sites (Ferguson, 1999). Ever since, several Guidelines for soil investigations, management of contaminated sites and risk assessment procedures have been introduced in this country. In Denmark, potential problems with contaminated sites (particularly deriving from landfills of chemical waste) were identified in the early 70's leading to the revision of waste regulations to deal with soil contamination arising from waste management and twenty years later, to the development of a broader "Soil Contamination Act", more able to deal with liability issues (Ferguson, 1999). Lekkerkerk, a town in the Netherlands, gained national notoriety in 1980 with the discovery of a large-scale soil contamination problem while a housing project was under construction. This problem contributed to the set-off soil remediation policy established by the Netherlands around 30 years ago. The Netherlands was one of the pioneering EU Member States to establish specific legislation on soil protection. Soil remediation was given legal status in 1983, and later, in 1987 the Dutch "Soil Protection Act" came into force (Ferguson, 1999; Wesselink et al., 2006). The first steps of the Dutch soil policy included the definition of legal norms (intervention values) to regulate soil clean-up as part of a multifunctional remediation approach. The high costs associated to this multifunctional approach lead to the transition to a function-oriented remediation strategy in the 90's (Wesselink et al., 2006). Soil policy developments in the last 20 years in the Netherlands also included: the revision of remediation criteria; developments on soil quality objectives and risk assessment procedures; increase in flexibility for local authorities in regulating contaminated land; encouragement of local participation in the decision making process; a distinction between mobile and immobile cases of soil contamination; and the stimulation of private funding for soil remediation (Ferguson, 1999; Wesselink et al, 2006). A new framework of soil quality standards has been developed in the scope the Dutch Soil Quality Decree than entered into force in January 2008. This framework includes broad National standards derived for ten different soil functions (and simplified in three broad functions: nature/ agriculture; residential areas; and industry) on the basis of human health risks, ecological risks and agricultural production. It also includes a system to develop local standards (Pruijn and Walthaus, 2008; Wintersen and Posthuma, 2008). In brief the new system of standards comprises: target values (based on Dutch background values), intervention values (based on serious risk levels, determines the remediation urgency), and National soil use values (to determine remediation targets based on specific soil use related risks levels) (Walthaus and Wezenbeek, 2008). The National Soil Use Values are general soil quality standards to determine sustainable fit for a specific type of soil use, although local authorities may choose to develop their own Local Soil Use Values. National Soil Use Values were derived on the basis of ecotoxicological data, risk levels for human exposure (MPR, maximum permissible risks levels for humans) and the human exposure model CSOIL (Walthaus and Wezenbeek, 2008). If soil concentration values at a defined site surpass the intervention values, a stepwise risk assessment system (Soil Remediation Criterion) is applied to define the urgency of remediation (Walthaus and Wezenbeek, 2008). The newly developed Dutch soil regulatory framework also includes a risk toolbox, an instrument to support site-specific management of soil quality and soil use (Wintersen and Posthuma, 2008).

In the UK, the first institutional mechanism to address contaminated land issues was the Inter-departmental Committee on the Redevelopment of Contaminated Land (ICRCL) which was set up in 1976 with the role of developing and co-ordinating advice and guidance on human health hazards arising from the re-use of contaminated land and co-ordinating advice on remedial measures. The ICRCL published the Guidance Note 59/83 (the 2nd edition, dated July 1987) to guide practitioners dealing with the many hazards and different types of historical contamination that defined Trigger values (threshold and action values) for three main groups of contaminants and for different planned land uses. These Trigger values were formally withdrawn in 2002 by DEFRA (Department for Environment, Food and Rural Affairs). Currently, in the UK, contaminated land is identified on the basis of risk assessment. In England, Scotland and Wales the contaminated land regime is implemented through The Contaminated Land Regulations (2000, 2001 in Wales) which enforces the Part IIa of the Environment Protection Act (1990). The section 57 of Part IIa was introduced into the Environment Protection Act 1990 by the Environment Act 1995 and was implemented in April 2000 in England, in July 2000 in Scotland and in July 2001 in Wales. Part IIa introduced a new statutory regime for the identification, assessment and remediation of contaminated land in the UK and in response to this the DEFRA and the Environment Agency have developed risk-based procedures for assessing harm from contaminated sites to ecosystems (including surface waters) and human receptors. Comprehensive packages of technical guidance relevant to the assessment of human health risks arising from long-term exposure to contaminants in soil has been published by DEFRA and by the Environment Agency (DEFRA and EA 2002a; DEFRA and EA 2002b; DEFRA, 2006). In the contaminated land management revised approach, the UK has chosen to develop guideline values rather than standards, for the assessment of risks within the overall policy context of ensuring that land is ‘suitable’ for its actual or intended use. A multi-tiered approach was developed for the assessment of risks to both humans and ecosystems. The first requirement (Tier 1) for a human health risk assessment is the identification of linkages between contaminant, receptor and pathway in a properly justified conceptual model. The source-pathway-receptor (S-P-R) pollutant linkage concept is fundamental in defining the UK contaminated land regime and is described by Nathanail et al. (2005). The Tier 2 is a Generic Quantitative Risk Assessment evaluation and Tier 3 is a Detailed Quantitative Risk Assessment (Smith, 2006; Carlon, 2007). Soil Guideline Values (SGVs) were calculated to be used in Tier 2 assessment through the Contaminated Land Exposure Assessment (CLEA) model. These SGVs are in fact intervention values that when exceeded may trigger further assessment or remedial action. The CLEA model is partially probabilistic and overall exposure needs to be calculated using the probability distribution functions of exposure parameters for each receptor (Carlon, 2007). It should be emphasised that this approach is advocated to allow prioritisation of sites for further investigation and subsequent "determination" of the significance of potential exposure on a contaminated site (requiring remediation within a defined period). The involvement of the local community in the decision making process from the earliest stages of the implementation of risk management is strongly encouraged by the UK contaminated land management system. Moreover, within the UK, soil remediation is closely linked to the planning regime and land development process. The "Part IIa" regulations essentially relate to land which would not be subject to development control.

In Belgium different soil policy formulations exist in Flanders and Walloon Region. Flanders adopted its Soil Remediation Decree in 1995 that contains an obligation to carry out an investigation at every transfer of land on which a "risk activity" is or has been developed. More recently (at the end of 2006) the Flemish Parliament adopted a new decree that entered into force during 2008, although the basic principles of soil remediation criteria remain the same from 1995 (Dries et al., 2008). A distinction between "historical contamination" and "new contamination" is made and remediation which is primarily triggered by land transfer processes follows rules appropriate to each case. Soil clean-up standards follow a risk-based approach and are used to indicate a level of contamination that if exceeded could cause significant harm for human health. Five classes of land use have been defined and the Vlier-Humaan model is used to characterise pre-defined exposure scenarios and perform exposure calculations (Carlon, 2007). The legal framework for contaminated land management in the Walloon region is constituted by the Law of the Walloon government for the cleaning of contaminated sites and rehabilitation of brownfields (from 2004) and three kinds of risk-based standards have been developed for soil and groundwater quality assessment on contaminated sites: reference values, trigger values and intervention values.

Following a series of provisions related to remediation of contaminated land that were included in waste management policies, a Ministerial Decree concerning soil contamination (M.D. n°471/99) came into force in Italy in 1999. More recently, in 2006, provisions for the management of contaminated sites have been included in the Legislative Decree n°152/06 (revised by the Legislative Decree n°04/08) which include the development of human-health site specific risk assessment whenever defined screening levels for soil, subsoil and groundwater are exceeded (D'Aprile et al., 2008).

The German Federal Soil Protection Act came into force in 1998, and the accompanying sublegal regulations in 1999 and integrates aspects of soil protection, remediation and pollution prevention (Carlon, 2007). The Act includes three types of risk-based standards: trigger values (that consider soil-to-human, soil-to-plant, and soil-to-groundwater pathways), action values (that consider soil-to-human and soil-to-plant pathways) and precaution values (to prevent new soil pollution). Whenever possible, considerations on the contaminants bioavailability are to be included in exposure assessments (Ferguson, 1999).

A Spanish regulation on contaminated soils was published in January 2005 (Royal Decree, RD 9/2005) and has been recently explained by Tarazona et al. (2005). This regulation is supported by the previous Spanish Waste Law (Ministerio de Presidencia 1998), and encompasses exclusively soils polluted by industrial activities. The RD 9/2005 defines a regulatory framework to establish those industrial activities that may result in soil contamination, defines a flexible and tiered system that includes risk-based Generic Values of Reference (GVRs) for sixty priority pollutants (and a methodology to derive these GVRs) and considers the possibility of further site-specific risk assessment. This regulation is risk-based and considers the protection of human and ecological receptors combining chemical and biological tools. The human health risk assessment is based on the analysis of relevant exposure routes for three land uses (industrial, residential and natural soil). The environmental risk assessment includes chemical analysis and direct toxicity testing, and covers three main ecological receptors: soil organisms, associated aquatic systems and terrestrial vertebrates. The inclusion of direct toxicity testing as a legal method for classifying a soil as contaminated is considered a key element of the Spanish approach.

Table 2.2 provides an overview of general practices for the identification and characterisation of contaminated sites in twenty three European countries. The overriding aspect of all these measures is that risk-based soil quality objectives (particularly risks posed to human health and the environment) are guiding the process. In some cases, risk-based national guideline values or norms have been developed for an effective and comparable classification of contaminated soils. These thresholds also indicate contamination levels above which soil remediation is needed/ mandatory. In other countries, quality objectives and remediation targets are defined through site-specific risk analysis, and specific guidelines for the development of risk assessments are available. Furthermore, some European countries apply multi-tiered approaches that combine both the use of screening guideline values for the preliminary identification of contaminated sites, and site-specific risk assessments for more detailed investigations.

Table 2.2: Overview of general practices for the identification and characterisation of contaminated sites in twenty three European countries (Based on data from: Ferguson, 1999; CLARINET, 2002; CLARINET, Contaminated Land Approaches in 16 European Countries, Online on the internet http://www.clarinet.at/policy/, accessed: December 27, 2007)

Country	**Most common approach for the classification of contaminated sites and definition of clean-up criteria**	**Specifc contaminated land policy**
Austria	Site-specific risk assessment	yes
Belgium (Flanders)	Site-specific risk assessment (exposure assessment)	yes
Bulgaria	Norms of Maximum Admissible Contents of Hazardous Substances in the soil	no
Czech Republic	"ABC" limit values: A - Background values; B - Possible adverse effects; C - Significant risk to human health and the environment. Risk assessment approach for state B criterion.	no
Denmark	Risk-based guideline values	yes
Estonia	Target Values and Guidance Values (based on risk for human health)	preliminary
Finland	Risk-based guideline values	no
France	Site-specific risk assessment (Tiered approach: Preliminary site investigation; Simplified Risk Assessment; Detailed Risk Assessment)	no
Germany	Risk-based soil screening values (trigger values) and action values	yes
Hungary	Limit values for soil and groundwater: A: Background values; B: Threshold values of contamination; C: Threshold values of measures; D: Target values. (based on Dutch, German, US EPA and Canadian guidelines)	preliminary
Italy	Original ‘limit value’ approach has been included into a ‘risk-based’ multi-tier approach: Tier 1 - screening values or contamination threshold values; Tier 2 - site-specific target levels or risk thershold values	yes
Latvia	Threshold values (Dutch threshold values used as reference)	no
Lithuania	Standards for contaminated soil and groundwater drafted (in line with with Dutch threshold values). Site-specific simplified risk assessment.	no
Norway	Tiered approach: Tier 1 - Generic Target Values ("TVs" based on existing Dutch and Danish guidelines); Tier 2 - Site specific risk assessment (when TVs are exceeded); Tier 3 - Detailed investigation	Part of “Pollution Control Act” and several specific Guidelines
Poland	Standards for environmental protection are generally based on fixed regulatory limits, but still no generic values for contaminated land. US EPA methods often used in site-specific risk assessments.	no
Portugal	Guideline values - Ontario (Canada) guideline values used as reference	no (under development)
Slovakia	Target values or permissible levels (former Dutch threshold values list was adapted in 1994)	yes
Slovenia	Limit, Warning and Critical Concentration Values of Dangerous Substances in Soil	yes
Spain	Screening/ guideline values and site-specific risk assessment	yes
Sweden	Site-specific risk assessment (exposure assessment). The Swedish EPA defined guideline values for levels in polluted soils, for the most sensitive types of land-uses	no
Switzerland	Site-specific risk analysis. Intervention values for leachate and gaseous phase.	yes
Netherlands	Risk-based criteria: *background values*, *maximum values (residence)*, *maximum values (industry)* and *intervention values*. CSOIL model is used to quantify exposure to contaminated terrestrial soils.	yes
United Kingdom	Site-specific risk assessment based on Source-Pathway-Receptor approach and on the definition of "pollutant linkages". Soil Guideline Values have been derived using the Contaminated Land Exposure Assessment (CLEA) model for three land uses.	yes

Issues related to the assessment of significant risk to human health and ecosystems from contaminated land originated most relevant discussions, particularly during the last decade, and the level of uncertainties associated to the risk assessment process is still considerably high. Nathanail (2006) presented a discussion on the appropriateness of generic and site specific criteria and on the advantages and drawbacks of each strategy concluding that there is a need for higher consistency on terms definition, their application and interpretation. Other authors (El-Ghonemy, 2005; Evans et al, 2006; Rothstein et al, 2006) presented reviews of the gaps and risks of risk-based regulations which are most relevant for the on-going discussion – the authors describe how issues such as imprecision, uncertainties, operation and normative challenges carry significant implications for achieving regulatory targets.

In addition to the discussion on the generic vs. site-specific risk assessment approaches it is equally important to refer that the identification of receptors during the risk assessment process and whether these are humans and/or ecological systems has different implications for the development of the process and provide a most relevant discussion as well. Faber (2006) and Smith et al (2006) describe the state-of-the-art of site-specific ecological risk assessments in terrestrial ecosystems in Europe and the different frameworks used in different countries. According to Smith et al (2006), most countries use tiered approaches and generic guidelines for a first screening of ecological risk and are still at the stage of developing suitable frameworks. The needs for research on the derivation of robust and suitable ecological parameters, assessment criteria and guidance on measuring harm in relation to ecological functions are strongly emphasised (Faber, 2006; Smith et al, 2006). Immediate questions arise when we consider whether soil functions can be quantified, and how robust procedures to measure harm in relation to function can be effectively developed. The experience from this diverse range of “common” approaches highlights the need for tests of significance in relation to appropriate receptors. The prioritisation of

S-P-R linkages using common screening approaches is often undermined by natural variability of the environment coupled with differences in outcome for given land uses.

Other countries

Worldwide, as for example in the USA and Canada, specific regulatory measures have been implemented for management and remediation of contaminated sites over the last few decades. The awareness of human health problems associated to soil contamination in the USA (as for example at the "Love Canal" area) lead to the development of the Comprehensive Environmental Response and Liabilities Act (CERCLA) in 1980, also known as "Superfund" as this act introduced specific provisions for setting a fund for the remediation of contaminated sites. The Risk Assessment Guidance for Superfund (RAGS) was published in 1989 and has been a major impetus to the application of risk assessment to the management of contaminated land at the USA. The practice of human and ecological risk assessment became the primary decision making tool to the management of contaminated sites, following the publication of the Risk Based Corrective Action (RBCA) standard by the American Society of Testing Materials (ASTM) in 1995 (Salhotra, 2008). Other landmark publications such as the US EPA's RAGS (Part D) Preliminary Remediation Goals (1994), the Brownfields Action Agenda (1996), the US EPA's Draft Vapour Intrusion Guidance Document (2002), state-specific RBCA programs and voluntary clean-up programmes, define the general framework for contaminated sites management at the USA (de Sousa, 2001; Salhotra, 2008). All levels of government provide some type of funding and/ or incentives for site remediation and re-development (de Sousa, 2001). Nowadays, the RBCA (or risk based decision making, RBDM, or risk informed decision making, RIDM) generic approach has been customised according to regulations and public policy of different States and are applied to sites with different sizes and complexities. The application of these processes includes four main elements: risk based site characterization (that involves the collection of site specific data, the identification of exposure pathways and the quantification of risk for each pathways); risk assessment (integrated and multidisciplinary analysis of risks); and risk management and communication (involves measures to risk reduction and post risk management) (Salhotra, 2008).

In Canada, environmental regulatory issues including contaminated sites are shared among the different levels of government. Relevant legislation and administrative policies at the federal level include the "Canadian Environmental Protection Act" from 1998, the "Guidance Manual for Developing Site-specific Soil Quality Remediation Objectives for Contaminated Sites in Canada", 1996 and the "Recommended Canadian Soil Quality Guidelines" from 1997 (de Sousa, 2001). The two types of criteria, risk-based guideline values and site-specific risk assessment, are used for the investigation of contaminated sites and the definition of clean-up goals in Canada (de Sousa, 2001). National guidelines comprise both generic soil quality criteria and guidance for developing site-specific criteria. Each Canadian province and territory is responsible for the development of their own remediation criteria, guidelines for use at contaminated sites and procedures for the implementation of site-specific risk assessments (de Sousa, 2001).

Further than Europe and North-America, other countries as for example China (Luo et al, 2009), South Korea (the Korean Soil Protection Act was established in 1995 and amended in 2002 and 2005 (Jeong et al., 2008)) and Japan (in Japan the Soil Contamination Countermeasures Law was enforced in 2003, as described by Ogata and Murakawa (2008)) are also currently involved in the development and implementation of regulatory decisions for risk-based management of contaminated land.

2.4 Comparability of national contaminated land management regimes

Some similarities but also some differences can be found in national contaminated land regimes and associated risk management approaches across Europe and worldwide. Particularly in Europe, this poses important challenges regarding the implementation of an EU regulatory framework and the development of a concerted approach to deal with common problems.

In general, most relevant elements that are common to the various national programmes dealing with soil contamination (although sometimes differently dealt with) are:

- Liability and funding issues: in general the "polluter-pays" principle is applied as far as possible but assigning liability on soil contamination cases is not always an easy task; several countries have defined specific approaches to assign legal responsibilities, to deal with orphan sites, and to combine private with public funding for soil remediation (e.g. base public funding on specific taxes). In the UK, for example, the private sector drives and funds the majority of land development and remediation projects (CLARINET, 2002). In some countries (e.g. Netherlands) there is a hierarchy in terms of liability – polluter, land owner, government and there are specific mechanisms for the protection of innocent land owners (de Sousa, 2001).
- Level of intervention: to achieve soil quality objectives in each country, action is required at different levels and falls under different jurisdictions (national, regional and local). European countries have different administrative structures and in some cases, regulatory measures to manage contaminated sites vary even within the country (e.g. Belgium) or are adapted concerning regional or local specificities (e.g. Spain). In some countries as for example the UK, local authorities have responsibility for dealing with effects on public health from land contamination, and development on or near contaminated sites (Ferguson, 1999).

- Historical contamination: many sites in Europe have been severely contaminated by a wide range of activities in the past. There is a legacy of historical soil contamination (with levels and types that vary from country to country) deriving mostly from chemical industry and waste landfills (metals and organic compounds), oil industry and petrol stations (mostly organic compounds), mining activities (metals), agriculture (pesticides, fertilisers and metals), service stations and dry cleaners (solvents) and/ or abandoned military sites (shooting ranges, airbases, fuel stocks, harbours, storing facilities, etc) contaminated with metals, organic compounds and explosives. The existing EU policies that tackle soil protection issues do not apply to contamination which occurred prior to its entry into force. Historical contamination is expected to be addressed by the proposed EU SFD. Nevertheless, several countries (e.g. Netherlands, France, Spain, and Hungary) have already introduced integrated nation-wide programmes that often include national inventories of contaminated sites and remediation strategies, setting remediation targets and the definition of implementation, financing and progress reporting structures.
- Multifunctional vs. function-oriented approach: the general tendency even in cases where national policies first tackled a multifunctional remediation approach (e.g. Netherlands) it is to move towards "fitness-for-use" remediation objectives in all countries. In some cases where generic criteria have been developed, these relate to specific land uses. Site-specific risk assessments are generally conducted considering present/ future land use of the site under investigation.
- Use of limit-values and/ or site-specific risk assessments: a variety of approaches have been applied across European countries during the past few years to develop the quality objectives for contaminated sites and to define soil clean-up criteria.

In countries where a framework for contaminated land management is in place, the national land use and spatial planning systems play an important role in the remediation and clean-up of contamination. Their action is generally a response to the needs associated to land transfer, site development and re-development processes. Although the planning and contaminated land regimes are generally two distinct systems, there is a degree of interaction between them. Moreover, as it is common that both national and local authorities play a role in the process, the intervention of national authorities is mostly commonly required when:

- dealing with funding issues;
- remediating historically contaminated sites where no liable party can be identified (the so-called "orphan sites");
- defining strategies for soil pollution prevention and control; and,
- defining national soil quality monitoring and protection strategies.

National interventions are particularly devoted to the definition and management of areas of potential risk at national level and the prevention of future risks, while regional/ local interventions are much more focused on the assessment of actual risks at regional/ local level and their minimisation. Table 2.3 shows a contaminated land management matrix that includes an overview of the main features which are commonly characteristics of interventions by national and regional/ local authorities in contaminated land management. There are many opportunities for sharing information and for the integration of both levels of intervention. Therefore, an important challenge in the production of robust decisions for contaminated land management is data integration, particularly when dealing with different spatial scales of intervention. Integrated layered responses have to be developed to deal with different levels of action, analysing the situation at different scales and incorporating uncertainties from different sources.

Table 2.3: Contaminated Land Management Matrix for two levels of intervention

	National Level of intervention	**Regional/ Local level of intervention**
Nature	Normative: definition of a national soil policy framework and regulatory strategy	Site specific
	Relates to the national land use planning system and to other national policies and strategies such as environmental and public health protection, agriculture, industry, mining, oil and gas	Relates to local land development plans
	Relates to EU policies and international conventions and agreements and reports to international bodies such as EEA	Reports to local authorities, land owners, insurance companies
	Qualitative, conceptual and dynamic	Quantitative, deterministic/ probabilistic
General framework	Driver-Pressure-State-Impact-Response (D-P-S-I-R)	Source-Pathway-Receptor (S-P-R)
Scope	Strong focus on soil pollution prevention and control	Strong focus on clean up of polluted sites
	General guidance: definition of national environmental protection objectives and remediation targets	Action oriented: Site clean up criteria and site-specific action values
	Deals with funding, liability and enforcement strategies	Deals with transfer of land, site development and re-development issues
	Historical soil contamination	Present contamination
	National monitoring	Site assessment
	Focus on soil polluting sectors and activities	Focus on soil polluting projects
	Main focus on risk prevention and management	Main focus on risk assessment and reduction
Data and Information sources	National databases, metadata, historical records and desk studies	City departments information, field data and detailed site investigations
	Background concentrations of trace elements at National level	Regional/ Local background values

Vegter (2008) has described three generations in soil policy making which, according to the author, may be found in Europe: Generation 1 (Command and Control regulations by national authorities – early policies that arose in the 1980s that included systematic inventories of contaminated sites and the classification and prioritization of sites according to numerical standards); Generation 2 (Flexibility in national regulations, room for local specific decisions –flexible, risk based land management and fitness-for-use decision making that considers spatial planning priorities and includes public-private partnership financing); and Generation 3 (Regulations are used to create opportunities and to remove barriers for remediation by private parties – has just started in some countries, focus on the economic and social viability of the redevelopment of a site and aims at managing liabilities and increasing voluntary remediation by private parties). Therefore it is most needed and challenging to implement a flexible EU framework legislation that sets common grounds for contaminated land management throughout Europe and ensures the improvement of soil conditions in both countries where the extent of site contamination problems is still unknown and in countries which have dealt with contaminated land for thirty years and where political attention has shifted from Generation 1 for Generation 2 or 3. Harmonisation of approaches for the definition of significance of identified risks and their prioritisation, require further discussion at an EU level as they are fundamental to allow regulators from each Member State to proceed and to enable site remediation to take place. A most relevant work on this field is currently being developed by the European HERACLES expert network (Swartjes and Carlon, 2008).

2.5 The challenges in the development of national soil policies and in the development of national remediation programs

A driver-pressure-state-impact-response (D-P-S-I-R) framework (EEA, 2000) can be used as a methodological approach to support interventions on contaminated land management at a national level, focusing on policy and strategic planning issues. A D-P-S-I-R structure defining processes in time affecting soil and allowing a better understanding of dynamic processes underlying soil functions and strategies with the potential to reduce threats has already been used as an operational framework for the EU Thematic Strategy for soil protection by the Technical Working Groups (TWGs) involved in its development (Blum et al., 2004; Bouma and Droogers, 2007). Key aspects of this framework are the characterisation of processes leading to changes in soil quality and associated impacts that may occur in three particular domains: risk to human health, ecological risk, and alteration of soils' ability to function. While an extensive body of scientific literature exists for the first two domains, the characterisation of different soil functions and the identification of respective indicators is still an area with critical research needs.

The most relevant data requirements for the development of a country specific D-P-S-I-R framework are:

- Drivers: Identification of most relevant sources of soil contamination at national level and possible transboundary contamination sources;
- Pressures: Identification of most relevant contaminants (both threshold and non-threshold contaminants) associated to country specific key soil contamination problems;
- State: Development of information on natural background concentrations of relevant contaminants, on national soil types and variability of relevant soil properties, and on the preliminary identification of potentially contaminated sites;
- Impact: Development of country specific risk assessment approach – this should include the definition of a risk assessment conceptual model.

The definition of a conceptual risk assessment model is a crucial step in the assessment of the "Impact" of soil contamination. This model needs to:

- Identify relevant receptors (conceptual models already in place have defined objectives for the protection of specific receptors such as: human health, terrestrial and aquatic ecosystems, groundwater, surface water, and agricultural production);

- Identify relevant exposure pathways (and associated exposure variables and parameters) – conceptual models already in place are based on the consideration of several exposure pathways such as soil outdoor and indoor pathways, soil derived diet exposure, soil-groundwater pathways, soil-surface water pathways, soil-plant pathways; and
- Select toxicological data to be used.

The Response (from the D-P-S-I-R framework) that is expected from the regulatory agencies is in this case the development of a Contaminated Land Legal Framework (CLLF). This CLLF must consider:

- The selection of sensitive land uses (several soil functions have been selected in EU countries such as: nature, agriculture, public green areas, residential with or without garden, and industrial;
- The derivation of soil screening values (or the definition of a methodology to derive them) and soil clean-up criteria;
- The development of site-specific investigation guidelines;
- The definition of a risk management strategy and national remediation targets;
- The integration of the CLLF with other national environmental policies.

The development and the implementation of a CLLF require concerted action from several stakeholders: policy and decision makers; private sector operators; Research and Development (R&D) community; and citizens in general.

Furthermore, the definition of a CLLF implies several decisions from decision makers. Some of these decisions are political choices, some are regulatory or management decisions and some are technical or science-based decisions. Examples of political choices include, as described by Salhotra (2008): the selection of protected receptors; the definition of acceptable levels of health risk and the consideration of acute and/ or chronic risks; the choice of fate and transport models; the selection of chemicals of concern; the implementation of institutional controls as a risk management strategy; the consideration of the additive risk related to the presence of multiple chemicals of concern and multiple exposure pathways; emphasis on resource protection vs. risk reduction.

Based on an assessment of the history of contaminated land regimes already implemented in several countries, the key legal and management decisions for countries to establish soil specific legislation are as described next:

a) Organisation of government and non-government administrative structures (from national to local level) to deal with soil policy development and implementation aspects, including efficiency and impact assessment mechanisms;

b) Integration of soil policy with other national environmental and planning policies and environmental protection practices and mechanisms;

c) Definition of a hierarchical structure for issues of liability and specific funding schemes (e.g. development of public funding programmes for high risks sites or "orphan" sites that may be supported by specific environmental taxes);

d) Development of incentive mechanisms for increasing private funding of site remediation projects and voluntary plans, to extend the market driven process, active in many countries;

e) Development of well equipped national operational research programmes - these programmes should consider research on the assessment of impacts of site contamination on soil function, on the integration of soil function analysis into site development and on the definition, on the quantification and assessment of significance of risks posed by contaminated sites, and on the development of cost-effective remediation solutions;

f) Raising public awareness and convincing several stakeholders of the importance soil protection issues;

g) Development of highly effective communication and stakeholder engagement systems.

Science-based decisions are those associated to scientific aspects of the risk characterisation and assessment processes and are those expected to offer more possibilities for the harmonisation of methodologies among different countries. Political and regulatory or management choices are by nature, country specific.

2.6 Summary and Conclusions

At the heart of the process for managing contaminated land there are issues of human and animal health protection; ecosystems and biodiversity conservation; water and air quality; and crop and food safety that are common among all European countries. Although certain EU Member States have already contaminated land regimes in place, many others still don’t. Moreover, considering that certain contaminated areas have transboundary nature and that there are soil contamination problems which are common to several EU countries (in some cases associated to huge costs for society) there is a need for a concerted action within the EU. There are technical aspects of site characterisation, risk assessment and remediation that can be harmonised at the same time that there are trans-scientific aspects of these processes that require political choices and that need to be customized by EU Member States. In addition, it is important to be aware that pollution does not recognize geographic boundaries and that the problem of soil contamination is also being addressed by other countries outside EU. Therefore the results and the sustainability of the implementation of current practices should be further analysed from a global perspective.

The analysis of almost three decades of national and international experiences in dealing with soil contamination issues has also highlighted relevant policy issues. An effective contaminated land regime can be derived from lessons from past actions and practices in several countries. A contaminated land management strategy must consider the key stages: dealing with historically contaminated sites, managing present contamination and preventing future contamination of land. Where land is statutorily defined as contaminated land, and particularly where it is proposed to build on a contaminated site, an effective remediation option has to be selected on the basis of a national remediation strategy and on the basis of national provisions on risk assessment. For the process to be robust and effective, a proper interaction between the national level of intervention and local authorities – or in other words, the interaction between the planning and the contaminated land regimes has to be effective. A D-P-S-I-R framework can be used to supporting the development of effective country specific regulatory decisions for contaminated land management at the various levels of intervention.

Chapter 3

Soil Policy and Soil Protection Strategy in Portugal

3.1 Introduction

As described in Chapter 2, soil protection policies including the prevention and remediation of contaminated sites in Europe have been developed during the last 30 years, and implemented both nationally (particularly in the UK, Denmark, The Netherlands, Germany, Austria, Belgium, Italy and Spain) and at the EU level. Other countries, such as Portugal, a relative late-comer to the management of contaminated land, have yet to implement a national soil policy.

Plans for the introduction of measures and requirements for EU Member States to prevent and remediate soil contamination, particularly through the development of inventories of contaminated sites and the definition of targets for prioritization of remediation actions, are expected to have important consequences for soil management practice and national soil policies across Europe. For many Member States, for example in the case of

Portugal, there are potentially significant and costly obligations to meet EU demands.

3.2 Aim, scope and objectives

The Portuguese situation regarding soil contamination problems will be analysed in this Chapter. The main challenges and demands for the future development of a regulatory framework for contaminated land management in Portugal based on the analysis of the temporal evolution and specific aspects of soil management practices in the UK, the Netherlands and Spain will also be discussed here.

3.3 The history of contaminated land management in Portugal

3.3.1 Overview of background environmental and socio-economic aspects

Despite high change rates during the last two decades, overall land cover of Portugal is largely dominated by forest and agricultural areas. In 2000, forest areas occupied 38 % of the national territory, natural vegetation covered 9% of the country and agricultural areas occupied around 48% of the country (IA, 2005a). Artificial surfaces (which include the urban fabric, industrial, commercial, transport, mines, dumps, ports, airports, and construction sites) and other land covers (wetlands and water bodies) occupied around 3 % and 2 % of the national territory, respectively (IA, 2005a; Freire and Caetano, 2000). A study on the changes of land cover and land use in Portugal between 1985 and 2000 (IA, 2005a; Freire and Caetano, 2000) showed that: the percentage of artificial surface in the country expanded by 41 % between 1985 and 2000 (from around 170x10^3 to around 240x10^3 ha) particularly due to an increase in areas of: the urban fabric (from 134 x10^3 ha to 175 x10^3 ha); industrial, commercial and transport units (from 21 x10^3 ha to 37 x10^3 ha); mines, dumps and construction sites (from 9 x10^3 ha to 17 x10^3 ha); and artificial non-agricultural vegetated sites (from around 6 x10^3ha to 9 x10^3 ha). On the other hand the percentage of total agricultural area and natural vegetation cover decreased in about 5 % and 9 %, respectively, between 1985 and 2000 (IA, 2005a). The highest increase in development rates from 1985 to 2000 was observed between the kilometre 6 and 12 from the coastline, particularly in the metropolitan areas of Lisbon and Oporto, in the Setúbal area and in Algarve. It should be noticed that, in Portugal, 50 % of the artificial surfaces are located within 15 km of the Atlantic Ocean (in an area representing less than 13 % of the surface of Continental area) indicating and increasing urban pressure on the coastline associated to high development rates and to an expansion of the urban fabric as well as the appearance of new commercial and industrial areas (Freire and Caetano, 2000). The largest industrial facilities as well as chemical products and fuel storage parks in Portugal are also located in coastal areas, and most particularly close to river estuaries in Oporto, Aveiro, Lisbon, Setúbal and Sines (IA, 2005b; APA, 2007).

In very general terms, industrial organization in Portugal in the last decades reflected three major ownership patterns: private domestic firms mostly concentrated in traditional, light industries and in the clothing and construction sectors; public enterprises which dominated mining and major heavy industries in the past, mainly iron and steel, petrochemicals, shipbuilding, petroleum refining, and electricity; and subsidiaries of multinational corporations, particularly electronics, automotive, pharmaceutical, and electrical machinery industries. Foreign investments were also important in the pulp and paper, chemical, food products, and clothing industries. Several major state-owned industrial enterprises were privatized during the 1990s.

Estimates of the quantity of industrial wastes produced in Continental Portugal indicated a global production value of 29×10^6 tons of industrial wastes in the year 2001, from which 254×10^3 tons (0.9 % from the total) were considered dangerous industrial wastes (IA, 2003; IR, 2003). It has also been estimated that the manufacture of metallic and chemical products and the electricity sector accounted for around half of the quantity of dangerous wastes produced in 2001 and that: 48 % of the 254 x10^3 tons of dangerous industrial wastes were used oils; around 17 % were organic and inorganic chemicals; and 11 % were solvents (IA, 2003; IR, 2003).

There is also a most relevant history of extraction of mineral resources in Portugal. Tungsten, tin, uranium, copper, iron, manganese, zinc, gold, alloy minerals, coal and non-metallic minerals were extracted in commercial quantities at hundreds of mining sites across the country, and for several decades (IGEO, 2008). The highest volume of mineral resources extracted was observed in the late 1950s. The sector of minerals extraction in Portugal has experienced significant changes during the last 50 years and most of mining installations were closed during that period (IGEO, 2008).

In Continental Portugal about 32 % of the drinking water supply has groundwater origin while in Azores and Madeira, over 96 % of the drinking water derives from groundwater sources (INAG, 2005).

3.3.2 Policy framework and regulatory structure

Since the traditional agri-environment in Portugal is often associated to small-scale agriculture, only in the last two decades with the increase of urban pressure particularly in coastal areas, soil contamination issues started gaining particular attention. Several circumstances may have contributed for this late start. The absence of pressure towards redevelopment in historically contaminated areas and the inexistence of a national contaminated land management strategy may have prevented contaminated sites to be located and assessed. Where these could be found, the costs associated with site remediation and redevelopment processes, the absence of guidance for risk assessment and management, together with difficulties in assigning legal responsibility or defining a hierarchy of liability may sometimes have prevented site remediation to occur. The first projects on soil contamination assessment and remediation started in 1994

(APA, 2007). One of these projects was the study on “Methodologies for the Remediation of Contaminated Groundwater and Soils” developed in the Chemical Complex of Estarreja (Central Portugal) in 1994. The outcome of this study was a set of pollution control measures. Moreover, a large-scale remediation project was developed in the area of the World Exhibition, the EXPO ’98 (Lisbon). An oil refinery, storage tanks, a sulphuric acid plant, a thermal cracking unit and a landfill previously occupied the site allocated to the EXPO ’98. Therefore, a large-scale remedial action (which involved mainly excavation and landfill of contaminated soils) was started in 1994 (APA, 2007). Other examples of site contamination assessment and remediation projects developed in the country since 1994 are related to specific interventions in oil refinery areas (and respective storage parks), energy production facilities, dumping sites, and industrial sites (Decree-Law n.º 89/2002 in D.R. 1ª Série nº 83, 09-04-2002, pp. 3375-3377, online on the internet http://dre.pt/pdf1sdip/2002/04/083A00/33503382.PDF, Downloaded December 27, 2007). These actions have been prompted either by direct intervention of public authorities in the scope of the national waste management strategy, the Environmental Framework Law (Law nº11/87) and in specific cases of detection of risks to human health or by initiatives of private parties to comply with waste management regulations, environmental regulations (such as pollution prevention and control, and environmental impact assessment) and specific sustainable business practices (e.g. in the case of multinational corporations). Moreover, a few, specific aspects of historical soil contamination gained attention during the last years. The legacy of several decades of extraction of mineral resources in Portugal, as well as the recognition of potential risks to human health and the environment deriving from former mining activities, led the Government to regulate the recovery and environmental monitoring of degraded mining areas (IA, 2005b). The environmental rehabilitation of degraded mining areas is subject of Government contracting since 2001. The Decree-Law nº198-A/2001 from the July 2001, defines the legal framework and the concession terms for the environmental recovery of degraded mining areas, particularly abandoned mining sites (specially in cases where private liability cannot be assigned) and mining sites considered of public interest such as those for extraction of radioactive minerals. Over 160 mining sites for potential intervention were identified and in many of these sites rehabilitation projects are planned or are currently being developed (IA, 2005b). While preparing this report, no published statistical information or nation-wide data on remediation of historically contaminated sites implemented in the scope of redevelopment and/ or processes of sale and transfer of land could be found.

So far, remediation projects have been implemented without the support of a national contaminated land management strategy. As no specific guidance for site assessment and remediation has been developed in Portugal, the approaches defined by the Canadian Environmental Quality Criteria for Contaminated Sites (particularly from Ontario) as well as methodologies proposed by USEPA have been used as a basis for contamination assessment and for the definition of clean-up criteria (Ferguson, 1999; CLARINET, 2002). In the absence of a national contaminated land management strategy in Portugal, the prevention and detection of arising soil contamination problems have been dealt with in the scope of the national waste management strategy and soil contamination and remediation provisions have been included in environmental and waste management regulations. At the moment, provisions regarding soil protection and soil decontamination are found in different legislative instruments, particularly:

- The Decree-Law nº 118/2006 that regulates the use of sewage sludge in agriculture to prevent harmful effects on soil, plants and humans (in D.R. 1ª Série nº118, 21-06-2006), amended in 2009 by the Decree-Law nº 276/2009 (in D.R. 1ª Série nº192, 2-10-2009);
- The Portuguese Environmental Framework Law ("Lei de Bases do Ambiente", Law n.º 11/87, in D.R. 1ª Série nº 81, 07-04-1987);

- The Decree-Law nº516/99 that defines the Portuguese Strategic Plan on Industrial Wastes (PESGRI'99) (in D.R. n.º 280, Série I-A, 1999-12-02)
- The Decree-Law nº178/2006 on waste management, which sets the legal framework for decontamination of contaminated sites and assigns the responsibility for licensing decontamination projects to regional authorities of waste management (in D.R. Série I n.º 171, de 2006-09-05); and,
- Other national regulations on water, integrated pollution prevention and control and environmental impact assessment.

An earlier study (Ferguson, 1999) on the assessment of risks from contaminated sites in 16 European countries reported that at that time Portugal had no specific programmes for funding the investigation, identification and clean-up of historically contaminated soils. According to the Portuguese Environmental Framework Law (Law nº11/87) the costs associated to the recovery of degraded areas must be supported primarily by the polluter although it is a fundamental duty of the Portuguese State to ensure that degraded areas are rehabilitated. In 2002, a review of decision support tools for contaminated land management by the Contaminated Land Rehabilitation Network for Environmental Technologies (CLARINET, 2002) reported that by then there was already a plan to produce national legislation on contaminated land and a risk assessment decision support system for Portugal (CLARINET, 2002).

Ferguson (1999) stated that although there was no compiled data on contaminated sites in 1999, there was already sufficient information to make a preliminary identification and characterisation of many sites, namely those related to existing industrial areas and uncontrolled waste deposits. The Portuguese Strategic Plan on Industrial Wastes (PESGRI'99) adopted in 1999 and reviewed in 2001 (PESGRI'2001) included a preliminary analysis of potentially contaminated sites in Portugal and most relevant contamination sources in the country. PESGRI'2001 identified local sources such as industrial activities (chemical, extractive and steel industry); mining sites; and former waste dumping sites (in 2000, 335 sites were catalogued, these are presently closed) that may be potentially associated to site contamination in Portugal. Diffuse sources such as agricultural practices; road traffic, railways (in areas up to 100 m from roads and railways, soils may be contaminated with metals); old buried fuel deposits (with no protection against corrosion) and leaching from wastewater systems were also identified as potential contributors to soil contamination. Moreover, a preliminary inventory of contaminated sites prepared in 2001 within the scope of the national strategy for waste management (Decreto-Lei n.º 89/2002 *in* D.R. 1ª Série nº 83, 09-04-2002, pp. 3375-3377, *online on the internet* http://dre.pt/pdf1sdip/2002/04/083A00/33503382.PDF, *Downloaded* December 27, 2007), which was developed based on the identification of activity sectors potentially associated to the production of dangerous wastes as on consultations to several public and private authorities, has identified 3,256 sites for further priority interventions (1,765 petrol station areas; 1,491 industrial areas - petroleum refineries, chemical and steel/ metal industry). A total of 6,315 sites were identified as second priority, and included mainly industries of electronics, components and explosives. Further 450 sites for potential intervention were identified. Potential contamination at these sites relates to both metals and hydrocarbons. According to the same document, a preliminary chemical characterisation of soil samples was performed at only 19 sites and insofar as it could be estimated the remediation costs for 12 of those sites are expected to be around €5 million. In 1998, an inventory of the Portuguese National Laboratory of Civil Engineering (LNEC) has identified over 2000 potentially contaminated sites: 1800 industrial wastes disposal sites; 302 dump sites; 107 mining sites; and 96 other sites (such as landfills, controlled dumps, storage facilities, airports, and composting areas) (DGA, 2000).

The Portuguese Environmental Agency (“APA”) was created in October 2006, and since then has the responsibility for the development and enforcement of several environmental protection policies in Portugal, including the introduction of provisions to rehabilitate contaminated sites. Before that, whenever a contaminated site was found and there was a need for intervention from the parties involved, the evaluation of risks and soil decontamination were usually covered by advice from the Waste Institute.

In addition, the Portuguese Planning System has several instruments for spatial planning and land use management in Portugal which are regulated by the Directorate-General for Spatial Planning and Urban Development, under the auspices of the Ministry for Environment, Spatial Planning and Regional Development: at a local scale (municipal and intermunicipal plans for spatial planning and urban development); at a regional scale (regional plans of spatial planning); and at a national scale (special plans for spatial planning, sectoral plans and recently, a National Program of the Physical Planning Politics). The National Program of the Physical Planning Politics was introduced in September 2007 (Law n.º 58/2007, “Programa Nacional da Política de Ordenamento do Território (PNPOT)” *in* D.R. 1ª Série, nº170, 04-09-2007). This Program defines several strategic and specific national objectives that comprise the development of information systems on natural resources including soil geochemical mapping and soil quality monitoring towards the prevention of soil degradation (2007-2013), the development of a National Integrated Strategy on Risk Prevention and Reduction (2007-2008) and foresees the implementation of a National Strategy on Soil Protection (2007-2013) integrated with the EU Thematic Strategy on Soil Protection, with the principal aims of supporting the conservation and sustainable use of soils by promoting the implementation of the Codes of Good Agricultural and Forestry Practices, implementing measures against desertification and promoting the conservation of national geological resources (Law n.º 58/2007, “Programa Nacional da Política de Ordenamento do Território (PNPOT)” *in* D.R. 1ª Série, nº170, 04-09-2007).

3.3.3 Soil studies and data availability

Some insights into the status of soil systems in Portugal emerge particularly from the soil science domain. Soil quality studies developed in Portugal, particularly during the last two decades have focused on wide-ranging objectives and approaches. Studies on agricultural systems (such as Abreu et al., 1993; Fernandes et al., 2000; Otieno et al., 2006; da Silva and Silva, 2006; Monteiro and Lopes, 2007; Ferreira and Gonçalves, 2007; Cameira et al., 2007) have generally comprised the analysis of the productivity of the soil system. These studies included the analysis of water balances, soil tillage practices, soil organic matter and/ or crop growth, often focusing on nutrients availability and evolving through time into more comprehensive soil productivity investigations. Specific studies on infiltration, water storage capacity, soil hydrophobicity, water repellency and soil erosion (e.g. Doerr et al., 1998; Thomas et al., 1999; Keizer et al., 2005; da Silva et al., 2004) have contributed to better understand soil systems functioning. These, have focused on the analysis of soil filter and buffer capacity and its ability to support plant growth in a country that has Mediterranean-type climate with dry, hot summers and wet, cool winters and spatially variable vegetation patterns.

Soil contamination investigations developed in Portugal during the last decades encompassed site investigations to define the levels and distribution of contamination mainly arising from industrial activities (Barradas et al., 1992; Inacio et al., 1998; Costa and Jesus-Rydin, 2001; Morgado et al., 2001; Pereira et al., 2005); agricultural practices (Azevedo et al., 2002; Gonçalves and Alpendurada, 2005; Gonçalves et al., 2006); and mining activities (Reis et al., 2004; Ferreira da Silva et al., 2004; Ferreira da Silva et al., 2005; Batista et al., 2007). Comprehensive soil contamination and pollution assessments in Portugal have been developed particularly at mining sites, with important research into risk assessment, including assessments of elements uptake, bioavailability and mobilisation (Henriques and Fernandes, 1991; Farago et al., 1992; Alvarenga et al., 2004; Patinha et al., 2004); and more recently, soil biology, ecotoxicology and toxicity studies (Pereira et al., 2004; Loureiro et al., 2005; Pereira et al., 2006). Studies have also been undertaken here on the effectiveness of plant based remediation technologies, bioremediation and revegetation of historical contamination (Mench et al., 2003; Costa and Duarte, 2005; Lazaro et al., 2006). A more limited number of studies have focused on diffuse and line sources of contamination such as the impacts of urban development on soil systems (Rodrigues et al., 2006) and the study of metal contamination deriving from highway runoff (Barbosa and Hvitved-Jacobsen, 1999). Geographically broader investigations, covering the whole territory of Portugal and including both biomonitoring (Figueira et al., 2002; Ventura et al., 2005) and geochemical mapping (Ferreira et al., 2001; Inacio et al., 2008) contribute to a better understanding of elemental background concentrations and to the identification of most relevant anthropogenic sources of metal contamination across the country.

Existing national databases, directories and information sources that can be useful for the definition of science-based regulatory decisions for contaminated land management include:

- Background concentrations of trace elements: “Soil Geochemical Atlas of Continental Portugal”, Universidade de Aveiro, 2003 (Inacio et al., 2008)
- Geology and Mining Installations: e-Geo – National System on Geoscientific Information, INETI, 2006
- Industry: EPER – European Pollutant Emission Register
- Waste: National Inventory on Industrial Waste, Instituto dos Resíduos, 2003
- Environmental Data: “Atlas do Ambiente”, Instituto do Ambiente, 2003

Although a considerable lack of information on the actual extent and nature of contaminated land problems in Portugal as well as on risks associated to them appears to exist, the country can now benefit from the experience on contaminated land management in other countries to develop and implement a national soil policy.

3.4 Lessons learned for soil policy formulation in Portugal

The EU Thematic Strategy on Soil Protection launched in 2006 (EC, 2002a; EC, 2006a) explicitly recognised the importance of preventing soil degradation and the need for enacting framework EU soil legislation with the principle aim of protecting soil and promoting its sustainable use. This Strategy puts therefore a clear emphasis on the need for all EU countries to develop National soil policies that are concerted with EU action on soil protection issues. Portugal is no exception.

The main features of soil policies from the different EU countries as well as the comparability of their respective contaminated land management regimes have been discussed in Chapter 2. From that discussion, there is a number of aspects of the contaminated land management regimes from the UK, the Netherlands and Spain that can be most relevant for soil policy formulation in Portugal and will here be subject of further consideration. Table 3.1 presents an overview of the history of the implementation of soil-related regulatory decisions at the UK, the Netherlands and Spain in comparison with the Portuguese environmental policy framework. The UK and the Netherlands have been two of the pioneer EU Member States to implement National contaminated land management regimes although in relatively different contexts.

Table 3.1: Overview of the history of the implementation of soil-related regulatory decisions at the UK, the Netherlands and Spain in comparison with the Portuguese situation

UK	the Netherlands	Spain	Portugal
1974- Control of Pollution Act	**1983-** Interim Soil Remediation Act	**1986-** Toxic and Dangerous Wastes Law	**1987-** Law nº11/87 - Environmental Framework Law
1976- Set up of the Inter-departmental Committee on the Redevelopment of Contaminated Land (ICRCL)	**1987-** Soil Protection Act		
	1993- Landfill (Soil Protection) Decree	**1989-** Industrial Wastes Plan	**2000, 2001-** Portuguese Strategic Plan on Industrial Wastes
	1994-1998- Circulares for Intervention values for soil remediation	**1995-** National Plan for contaminated sites remediation	
1957-1984- Occupiers Liability Act			
1989- Control of Pollution Act	**1996-** Soil Protection Act (revised)	**1998-** Wastes Law (10/1998)	**2002-** Decree-Law nº 152/2002" on Landfills
1989- Water Act	**1997-** Circular on the remediation deadline for cases of serious contamination for which remediation is urgent	**1998-** Law 3/1998 for the Protection of the Environment (Basque Country)	
1990- Town and Country Planning Act			**2006-** Decree-Law nº118/2006 that regulates the use of sewage sludge in agriculture to prevent harmfull effects on soils, plants and humans
1990- Environmental Protection Act			
1991- Water Resources Act	**1998-** Circular on Remediation Regulations Soil Protection Act	**2005-** Royal Decree (RD 9/2005) by which the list of potentially soil polluting activities, the criteria and the standards for the declaration of polluted sites are established	
1991- Controlled Wastes Regulations			
1995- Environment Act (Part IIa)	**1998-** Storage in Underground Tanks Decree		
1996- Landfill Tax (Contaminated Land) Order			**2006-** Decree-Law nº178/2006 on waste management
	1999- The Buildings Materials Decree		
2000, 2001- The Contaminated Land Regulations (England, Scotland and Wales)	**2000-** Circular on Target Values and Intervention Values for Soil Remediation		
		2005- Law 1/2005 for prevention and remediation of soil contamination (Basque Country)	**2007-** Law nº 58/2007 - National Program of the Physical Planning Politics
	2006- Soil Protection Act (revised)		
	2008- Soil Quality Decree		

At the UK the long and varied history of heavy industrial sectors associated to an equally long history of land recycling, of a very active property market, and of a rapid turnover of ownership in comparison to other countries, have shaped the type of arising contamination problems and the approach adopted to deal with them (Lowe and Lowe, 2001). Although contaminated land aspects have been included in several environmental management regulatory decisions since the 1970s (Table 3.1) the need for consistency of the approaches taken by different authorities to deal with soil contamination problems as well as the need for a more tailored regulatory mechanism better able to include liability rules and to reflect the complexity of the problems have prompted the introduction of a specific contaminated land policy in 1995 (Part IIa, introduced in the 1990 Environmental Protection Act by the 1995 Environment Act). The Part IIa, together with the Contaminated Land Regulations and the Statutory Guidance issued by DEFRA (DEFRA, 2006) constitute the UK risk-based contaminated land regime which is comprehensively described by Lowe and Lowe (2001). Several lessons that can be learned from Part IIa regulatory system may be most useful for soil policy formulation in Portugal. First, the UK policy approach has three main essential components (Lowe and Lowe, 2001): preventing the creation of new contamination; promoting the remediation of the existing legacy of soil contamination problems through the redevelopment process; and intervening through a regulatory process to deal with existing problems where necessary. In order to achieve these objectives, potential risks associated to land contamination are evaluated on the basis of a system of incentives and controls and through a "suitable for use" approach (which means of the basis of the particular use of land and its environmental settings). Wherever necessary damaged land is brought back into beneficial use in a way that the costs burdens faced by individuals, companies and society as a whole are proportionate manageable and economically sustainable. The issue of liabilities were crucial to the introduction of Part IIa in the UK, particularly since stakeholders became sensitised to liability aspects of contaminated land in the early 1990s. This will also be an important aspect for soil policy formulation in countries with no history of people and/ or companies being held liable for land damage, such as Portugal. And although the Environmental Liability Directive (2004/35/EC) has already set the framework for many cases of liability definition in the context of environmental damage, the contribution of the UK's regulatory system can be most useful in these matters: this system includes statutory guidance on allocating liabilities between different liable persons and comprises six exclusion tests to focus liability on those who are most responsible for the risk. Moreover, although Part IIa is essentially an "enforcement regime" this is also aimed at underpinning voluntary remediation. At the UK, the private sector drives and funds the majority of land development and remediation projects (CLARINET, 2002). The regulatory system provides several opportunities for voluntary action to replace formal enforcement action in the context of redevelopment, particularly by reducing the uncertainties surrounding liability allocation (Lowe and Lowe, 2001). Such an approach may be helpful for countries to reduce the costs burden of soil policy implementation. Another aspect of the UK contaminated land regime that can provide a suitable basis to the formulation of regulatory decisions in Portugal is the degree of interaction between the land use planning and the contaminated land management systems. The current situation in Portugal is that contaminated land aspects are being addressed both by waste management regulations and the spatial planning program (Table 3.1). At the UK, the majority of site remediation projects are implemented through the land use planning system when sites are developed and redeveloped. The main regulatory role under Part IIa fall to local authorities that have planning, development and urban regeneration control and that are expected to play a more significant role in communication of risk to local communities. Additional funding has been provided to local authorities to reflect their new duties under the Part IIa regime (Lowe and Lowe, 2001). The Environment Agency (of England and Wales) has in the case of contaminated land management a central role in the production of technical best practices, and in providing information and guidance to local authorities. The EA is the enforcing authority only in the cases designated by "special sites" in Part IIa. Recently, the UK has undertaken a review of soil management and in particular functional aspects within which contaminated land is considered. The devolved administrations of Scotland, England, Wales and Northern Ireland, have slightly different environmental administrations and legal regulation differences exist between Scotland and the rest of the UK. As a consequence of this two consultation exercises have been undertaken on a Soil Strategy for England (DEFRA, 2008) and the Scottish Soil Framework (Scottish Government, 2008). The aims of these activities are to prepare future land use policy which promote the sustainable management and protection of soils consistent with the economic, social and environmental needs of the regions: raising awareness; integrating soil protection in other environmental policies; identifying knowledge gaps and research needs; and improving the accessibility to information on soils particularly by supporting the development of a soil monitoring network. These Strategies take into account specific pressures on soils which are threats across all soil functions (climate change, soil sealing, soil erosion, loss of biodiversity, compaction, organic matter loss, atmospheric deposition, contamination); it is based on the analysis of soils and land use in the UK and on the principles of preservation of soil functions in a context of climate change; it is supported by a series of policy levers (that include, among others, the Contaminated Land Regime under Part IIa); and it identifies a series of activities that will contribute to specific soil outcomes (associated to the protection of the soil resource and its functions).

In the Netherlands the development of a soil policy is intrinsically associated with the increased awareness of human health hazards associated with contaminated land in the 1970s and early 1980s, when soil pollution emerged as a national problem and the needs to promote a sustainable reuse of soils and sediment become obvious (Pruijn and Walthaus, 2008). As it can be seen in Table 3.1, this country has a long history policy-making, implementation, evaluation and improvement surrounding soil protection and contaminated land remediation. These aspects have been broadly discussed by Rodrigues et al. (2009a) but the most relevant issue to support soil policy formulation in Portugal is that by implementing local soil policies and of soil policy legislation in the Netherlands led to improvement of national soil legislation, particular to the improvement of the risk assessment approach; the improvement of the workability of soil legislation; the reduction of costs for parties operating in the soil market; and policy enforcement (Pruijn and Walthaus, 2008). Particularly useful aspects for Portuguese decision makers are the decision to make local authorities part of the legislative process, and the improvement of standards of soil quality through time in the Netherlands. These standards can now be set by local authorities and formulate targets for the quality of soil in specific areas on the basis of existing soil quality, of a particular land use and of measures for local sustainable development. Moreover, the improvement of scientific and technical understanding from decades of experience on the development of soil remediation projects in the Netherlands, are important lessons for other countries.

The Spanish situation was included for comparison in Table 3.1 due to its geographical proximity to Portugal and because Spain has recently (in 2005) published comprehensive legislation on contaminated soils which has derived from the national waste management regulatory framework. The Spanish Wastes Law (10/98) in 1998 defined that soil contamination should be assessed on the basis of risks to human health and the environment. Moreover, before introducing specific legislation on contaminated soils, the Spanish Central Government in co-ordination with the Autonomous Regional Governments, developed a National Inventory of potentially contaminated sites in Spain (Ferguson, 1999). Subsequently, the Spanish Royal Decree 9/2005 (RD 9/2005) on contaminated soils was introduced in 2005. This is supported by the Spanish Waste Law covering soils polluted by 101 industrial activities, and facilities handling significant amounts of hazardous chemicals (over 10,000 kg per year) or fuel (300,000 L fuel per year or storing 50,000 L fuel at any time) (Tarazona et al., 2005). Following the implementation of the RD 9/2005, these parties must present a preliminary report on waste handling and chemicals manipulation. According to RD 9/2005, whenever there is an alteration of land use, the owners of land where activities that could potentially cause soil contamination have been developed must report that situation. The Spanish regulation on contaminated soils is risk-oriented and has emerged from available information on industrial wastes and on the description on how chemicals are handled within industrial facilities, including the identification of the possibilities for soil contamination due to current handling (Tarazona et al., 2005). Following a similar approach, the provisions regarding contaminated land management and remediation in Portugal have been so far included in waste management legislation and associated to the national waste management strategy (Table 3.1). Information on industrial wastes production is available, particularly from the Portuguese Strategic Plan on Industrial Wastes (2000, 2001) and most relevant provisions regarding waste handling, risk reduction and environmental liability have already been included in the Decree-Law nº178/2006 on waste management.

3.5 Lessons learned for the development of a risk management strategy in Portugal

From a scientific perspective, the development of a national contaminated land management strategy is associated to most relevant decisions and research needs, particularly for the development of an approach for the identification, evaluation and management of risks arising from soil contamination.

Different countries have defined different approaches for the classification of contaminated sites, risk assessment and definition of clean-up criteria. These differences have been broadly described by Rodrigues et al., (2009b).

Nevertheless, there are specific aspects of the UK, the Dutch and Spanish national risk management strategies that can be most useful for the formulation of regulatory decisions on these matters, in Portugal. A very general overview of these aspects is presented in Table 3.2. It should be noticed that currently, the three countries consider the derivation of generic soil screening values but in addition local/ site specific risk assessments underpin national risk management strategies.

Table 3.2: Generic overview of risk management strategies adopted by the UK, the Netherlands and Spain (DEFRA and EA, 2002a; Swartjes, 1999; Tarazona et al., 2005; Carlon, 2007; Walthaus and Wezenbeek, 2008)

	UK	**the Netherlands**	**Spain**
Types of soil screening values	Generic Soil Guideline Values (SGVs) for human health. SGVs are "intervention values" that indicate that soil concentrations may pose unacceptable risks. SGVs are trigger values for further investigation and site specific risk assessment.	Target Values of negligible risk (based on Dutch soil background values); Intervention values (based on serious risk levels, determine the remediation urgency); National soil use reference values (determine remediation targets for specific soil uses); Local soil use reference values (can derived locally, the lower limit are target values and the upper limit are the intervention values). Screening values are derived on the basis of human health and ecosystem protection.	Generic Reference Levels (GRLs): trigger site-specific risk assessment. GRLs have been derived for organic contaminants, regarding human health and ecosystems protection, in separate.
Land uses considered for the derivation of generic soil screening values	Residential with garden and allotments (including home grown vegetables); Residential with children playing fields; Industrial or commercial	Nature and agriculture; Residential areas; Industry (that can be further divided in ten soil functions)	Residential areas (where industrial activities are also permitted); Industry; soils where all potential uses are allowed (including recreational and agricultural activities)
Protected receptors	Human Health; Ecosystem; Groundwater (is taken into account regarding the ecosystems protection (aquatic organisms))	Human Health; Ecosystems; Groundwater (considers both the protection of ecological receptors and groundwater for drinking uses).	Human health; Ecosystems; Groundwater and surface water (both considered regarding the protection of ecological receptors)
Approaches used for the derivation of soil screening values and exposure assessements	The probabilistic CLEA (Contaminated Land Exposure Assessment) is used for the derivation of human health SGVs and for human exposure assessments (DEFRA and EA, 2002b). Ecotoxicological and ecological assessments are based on the Ecological Risk Assessment (ERA) framework (Smith et al, 2006).	Human health and ecotoxicological risk limits were used in the derivation of integrated Intervention values. The deterministic CSOIL model is used for the derivation of human health risk limits and human exposure assessments (Brand et al., 2007). The ecotoxicological risk limit is defined as the HC50 for receptors and to assess actual ecological risks the TRIAD approach is used (Rutgers et al, 2000; Posthuma et al., 2005). Other models, the SEDISOIL and the VOLASOIL may also be used to enable assessment of exposure to sediments and grounwater contamination with volatile contaminants (Carlon, 2007).	The RD 9/2005 does not include a description of the methodology used to derive human health GRLs. The overall approach is a combination of European (SSC, 2003; EC, 2003) and North-American (USEPA 1989; USEPA 1998) methodologies. Ecological screening values are based on the INIA (2001) approach. Direct toxicity testing is a formal requirement for site-specific ecological assessments.

Different types of screening levels are derived in the UK, the Netherlands and Spain, in the same way that different approaches are used to derive these values and to support the development of exposure assessments (Table 3.2). It should be added that the source-pathway-receptor (pollutant linkage) concept is fundamental in defining the UK risk management approach and that the CLEA model used in the UK is the only probabilistic approach to exposure assessment currently in use in the EU (Carlon, 2007). No national generic reference levels (GRLs) for metals are defined in the Spanish RD/2005 on soil contamination. In Spain, the responsibility to derive GRLs for metals has been transferred to the regional governments.

From the above, seems clear that a national tiered national risk management strategy in view of land use must be developed in Portugal and specific guidance for risk assessment must be provided. From the analysis of current experience in other EU countries, the most relevant regulatory decisions and requirements for the derivation of such an approach can be clearly defined and are shown in Table 3.3.

Table 3.3: Regulatory decisions and requirements for the development of a national risk management strategy in Portugal

	Requirements/ Actions Needed	
Regulatory Decisions	Contaminated Land Legal Framework (CLLF)	Selection of soil functions and sensitive land-use applications: examples from other countries are nature, agriculture, urban, residential with or without garden, recreational, public green areas, commercial and industrial
		Selection of chemicals of concern
		Derivation of Soil Screening Values and the of the scope for their application, and/ or definition of a methodology to derive them
		Development of Guidelines for Site-specific Investigations
		Definition of a Risk Management Strategy and associated institutional controls
		Derivation of soil clean-up criteria and soil use values and/ or definition of a methodology to derive them
		Definition of National remediation targets
		Integration of the CLLF with other environmental policies
	Exposure modelling (Conceptual model)	Identification of relevant receptors: examples from other countries are human health, terrestrial and aquatic organisms, groundwater drinking, surface water, and agriculture production
		Selection of fate and transport models
		Definition of relevant exposure pathways and exposure parameters
		Definition of acceptable levels of health risk and selection of toxicological data
		Decision on the inclusion of biavailability and bioaccessibility information
		Decision on consideration of chronic (lifelong) risks in addition to acute risks
		Decision on consideration of additive risks related to the presence of multiple chemicals of concern and multiple exposure pathways

The development of a Contaminated Land Legal Framework (CLLF) for Portugal implies several political, regulatory and management and scientific-based decisions that include particularly the selection of sensitive land uses, the identification of protected receptors and relevant exposure pathways, the selection (and or generation of information) on exposure parameters and toxicological data and the definition of limits of acceptable risk (Table 3.3). Socio-economic and scientific aspects as well as environmental and human health protection needs should underpin the derivation of Portuguese soil screening values, the development of guidelines for site-specific investigations and the definition of a risk management strategy, soil clean-up criteria and National remediation targets. According to the HERACLES report on the derivation of soil screening values in Europe (Carlon, 2007), 11 European countries have developed national risk assessment models (such as the CLEA model in the UK and the CSOIL model in the Netherlands) while 5 other countries adopted (and adapted) models available from countries rather than developing a new modelling exercise.

The selection and adaptation of a Risk Assessment Conceptual Model for Portugal, or the development of a country specific one is urgently needed. The model must cope with country specific soil types, soil properties and background concentrations of different chemical elements and compounds. Conceptual models for human exposure from other countries are based on the consideration various types of exposure pathways. Carlon (2007) mentions the following:

- Soil outdoor pathways;
- Soil indoor pathways;
- Soil derived diet exposure;
- Soil-groundwater pathways; and
- Soil-surface water pathways.

Different routes of exposure (inhalation, dermal contact, and ingestion - direct and indirect) are considered in the modelling of these pathways. In most countries the application of exposure and toxicological modelling are used for the derivation of soil screening values. The process of the establishment of soil screening values and/ or the definition of a methodology to derive them (e.g. to be derived by local authorities to be used at the site-specific level) should also be initiated in Portugal as soon as possible. Both generic and site-specific approaches have advantages and drawbacks that have been thoroughly described by Nathanail (2006). Decision support for these matters is currently most needed. Moreover, only recently, ecological risk screening values have been introduced by a few European countries in their regulatory framework and in many countries these are now under development (Carlon, 2007). Faber (2006) describes the current state of the development of site-specific ecological risk assessments in Europe and may provide useful insight for the derivation of an approach to ecological risk assessment in Portugal.

The group of chemicals of concern for which soil screening values are to be derived also needs further discussion. Other European countries usually consider the following: metals and metalloids (As, Cd, Cr, Cu, Hg, Pb, Ni, Zn), aromatic hydrocarbons (e.g., benzene, ethyl benzene, toluene), polycyclic aromatic hydrocarbons (e.g., naphthalene, anthracene, benzo(a)anthracene, benzo(ghi)perylene, benzo(a)pyrene), chlorinated aliphatic hydrocarbons (dichloromethane, trichloroethylene, tetrachloromethane), chlorinated aromatic hydrocarbons (chlorobenzene, hexachlorobenzene), pesticides (atrazine, DDT), dioxins and dioxin like PCBs (Carlon, 2007). The approach selected for the derivation of soil screening values in Portugal as well as a CLLF should be flexible enough to allocate screening values for different receptors, and to allow future revisions and updates, particularly to consider changes in toxicity information, emerging substances and possibly, to take into account the effects of mixtures of contaminants.

It is important that a national strategy risk management strategy for Portugal:

- is defined in a consistent and sound manner and it is integrated into a broader national soil protection strategy;
- it is adapted to the distinctiveness of Portuguese soils and of the Portuguese socio-economic and cultural settings;
- allows the achievement of national human health and environmental protection targets;
- allows a realistic risk evaluation; and
- it is able to increase stakeholder confidence in the process.

The discussion on the development of a risk management strategy should involve the research community, regulators and decision makers, regional and local public authorities as well as private sector stakeholders. Most relevant research needs are those associated to the methodology to be followed in problem definition and assessment, which requires the establishment of links between sources of contaminations and concentrations of contaminants in soils with pathways of exposure and defined receptors (both humans and ecological receptors) while developing realistic scenarios of exposure (Source-Pathway-Receptor framework). If it is not possible to define these linkages at the site level, then contamination is not generally regarded as posing risk (Petts, 1997; Nathanail, 2006). A risk assessment framework to be defined in Portugal should also support the implementation of any future EU Thematic Strategy for Soil Protection and integration with other regulatory regimes and the enactment of EU framework soil legislations and whilst they must at the same time be comparable with other European approaches, embracing national concerns. From a scientific perspective, one innovative aspect is that this risk assessment framework should take into account the analysis of the impacts of soil contamination on the different soil functions at both site and regional levels. Those functions to be considered under investigation should be those defined in the EU Thematic Strategy for Soil Protection (EC, 2006a) and for this analysis to be effective, the development of function indicators are most needed.

3.6 Summary and Conclusions

An important message from this study is that the development of regulatory decisions for contaminated land management for Portugal is necessary to prevent soil degradation and to support the implementation of the EU Thematic Strategy for Soil Protection. A second message is that the formulation of soil protection regulations in EU Member States that still lack these approaches can be effectively supported by the experience from other European countries that have already introduced and improved national soil policies during the last three decades. These experiences should however be carefully adapted to the distinctiveness of each country rather than just transferred into their regulatory frameworks. Moreover, other countries such as the USA and Canada have also long-term experience in the implementation of contaminated land regimes and their contribution for the development of improved soil policies should be further analysed.

Soil quality research as well as preliminary lists of potentially contaminated sites developed mostly during the last decade showed that there are soil degradation problems in Portugal. Soil studies have also shown that risks to human health and ecosystems may arise at some of these contaminated sites. Although relevant regulatory decisions regarding the recovery of degraded mining areas have already been implemented and certain provisions concerning soil decontamination have been included in waste management regulations, the current Portuguese environmental regulatory framework is still insufficient to:

- detect and address problems associated with historical soil contamination (other than those deriving from mining practices);
- and provide guidance and decision support for the development of effective risk assessment practices and site remediation projects.

There are indications from APA that a national regulatory framework for soil decontamination will be implemented in Portugal. Nevertheless, the author considers that a comprehensive national contaminated land management strategy should be developed in the short term. This strategy should be part of a broader national soil protection policy to support the implementation of the EU Thematic Strategy on Soil Protection in Portugal that would take into account other threats to soil function in Portugal besides contamination, such as soil erosion and soil organic matter loss.

A national contaminated land management strategy for Portugal should:

1. allow to locate and prioritise interventions at historically contaminated sites, particularly those derived from former industrial, chemicals storage, energy production and waste disposal practices and to develop a restoration strategy for those sites (including guidance on risk assessment, remediation, funding and liability aspects) – for example, by developing a more comprehensive inventory of contaminated sites and by introducing an obligation to land owners to report on previous land uses and past potential polluting activities whenever an alteration of the soil function is foreseen;
2. allow dealing with soil contamination problems currently deriving from polluting activities by providing guidance for: risk assessment; prioritisation of sites for further investigation and subsequent "determination" of the significance of potential exposure; and for defining remediation urgency;
3. allow the prevention of future soil contamination problems by identifying potential soil polluting activities and implementing control measures.

Such a strategy should combine interventions at both national level (particularly from APA) and at regional and local levels. A D-P-S-I-R and Source-Pathway-Receptor framework has been proposed by Rodrigues et al. (2009a) to support the articulation of these interventions. Moreover, a risk-based decision support framework and a risk management strategy are required to deal with problems at site level. As seen in other countries, to be effective, the definition of guidelines for the investigation and remediation of contaminated sites requires concerted action between several stakeholders.

A nation-specific, multi-layered approach combining both generic risk-based guidelines and detailed site-specific risk assessments appears to be the most effective approach but further investigation is needed. A wealth of experience from other countries, particularly from the UK (where the source-pathway-receptor pollutant linkage concept is used to guide risk assessment) and the Netherlands, is most useful to support the development of a Risk Assessment Conceptual Model for Portugal. Political and management decisions for the development of such a conceptual model should consider specifically the selection of sensitive receptors. Given the importance of groundwater resources for drinking water supply in Portugal and the relevance of agricultural production for the country's economy the following receptors should be subject of consideration: human health, terrestrial and aquatic ecosystems, groundwater drinking and agricultural production. The risk management strategy must also be function-specific and relevant land uses such as agricultural, natural areas, residential with and without vegetable production, public recreational areas, commercial and industrial should be considered. Risk-based decisions should reflect the specificities of Portuguese soils and take into account background concentrations of chemical elements and local-specific soil properties. Specific technical guidance for the implementation of risk management practices will have to be provided and certain aspects of the risk assessment process should be subject of further research: linkages and cause-effect relationships between emissions of contaminants and effects on human health and ecosystems; bioavailabilty and bioaccessibilty of soil contaminants; better understanding of soil capacities and of soil function indicators; development of technologies and tools for cost-effective site characterisation and remediation in the Portuguese context.

It should be emphasised that planning control can also be used as an important contaminated land management strategy. Although APA and regional environmental authorities implement and enforce environmental regulatory decisions in Portugal, their interventions in the scope of the national contaminated land management strategy proposed here would relate mostly to land that is out of the development control (with the exception of cases where changes in land use are foreseen). Therefore, the implementation of a national regime for contaminated land management must be concerted with the national spatial planning system and regional and local planning authorities.

Finally, the polluter-pays principle and the provisions of the EU Environmental Liability Directive should be observed in the implementation of a national contaminated land management regime. Nevertheless, a system for assigning liability and to deal with site remediation funding issues in Portugal, and particularly at the so-called orphan sites, should be considered. Since remediation and re-use of contaminated land are frequently hindered by financial constraints, economic and legal incentives should be used to encourage sustainable redevelopment at contaminated sites and reduce the demand of greenfields. Examples of such incentives can be found at the UK. Given the increasing urban pressure along the coastline in Portugal, these incentives would be particularly important for a more sustainable development at coastal areas.

In order to provide scientific support for future developments of a contaminated land management strategy in Portugal, soil contamination problems in the country as well as associated risks to the terrestrial food chain will now be analysed in detail and will be discussed in Chapters 4-8.

Chapter 4

Portugal as a Case-Study:

Experimental approach and data evaluation

4.1 Introduction

In contaminated areas, potentially toxic elements (PTE's) may pose significant risks to ecosystems and human health. Leaching of PTE's to ground and surface water as well as uptake of contaminants by arable crops are examples of processes that can be associated to such risks. To assess the level ok risks posed by soil contamination it is essential to understand the source of contamination as well as the pathways through which contaminants may reach sensitive receptors such as humans (Petts et al., 1997; Römkens et al., 2004). The transfer of contaminants from soils to receptors is controlled by several physical, geochemical, biochemical and biological processes which determine the reactivity of PTE's in soils and their availability to living organisms (Römkens et al., 2004). Hence, to characterise pathways of exposure to soil PTE's it is crucial to develop an approach to assess processes controlling the distribution of the contaminants between the different soil phases as well as between different environmental compartments including air, water and biota.

Although several soil contamination studies have been develop in Portugal, particularly in the last two decades (as described in Chapter 3) there is still a need to better understand the source, distribution and extent of soil contamination problems within the country as well as a need for tools to effectively characterise the processes and pathways of exposure to soil contaminants that may lead to risks to sensitive receptors.

4.2 Aim, scope and objectives

In this Chapter, the methodology and experimental approach followed in this study to characterise soil contamination problems in Portugal and pathways of exposure to soil contaminants for grazing animals and humans will be described. Details on types of samples collected, on the sampling approach as well as on analytical methodologies and respective quality control will be provided.

4.3 Selection of sampling areas

4.3.1 Origin of soil samples

Areas selected for this investigation comprise fields used mainly for agriculture (crop production) and pasture. Five main criteria and requirements were considered for the selection of sampling sites and collection of soil samples:

- collect samples in areas with different geological, mineralogical and geochemical backgrounds across the country;
- collect samples both in areas where significant anthropogenic contamination is not expected to occur and in areas where relevant sources of contamination are known to exist;
- select areas with different origins and types of contamination;

- select vegetated areas (crops or pasture fields) for soil sampling;
- collect a considerable number of soil samples in each sampling area to allow a comprehensive statistical analysis of the results obtained.

Different areas have been sampled across Portugal and a total of 136 soil samples were collected at:

- Esposende (coastal area, North-West of Portugal): 30 soil samples from agricultural fields located away from known relevant point contamination sources);
- Estarreja (coastal area, North/Central-West Portugal): 39 soil samples from agricultural fields surrounding an industrial area (Estarreja Chemical Complex);
- Lousal, Caveira and Aljustrel (South-West of Portugal): 67 soil samples from agricultural fields surrounding three mining areas (mostly used for sulphides, pyrites, and Cu extraction).

The location of these sites is shown on Figure 4.1. The coordinates of all sampling sites as well as respective land uses is shown in Annex I (Table 1).

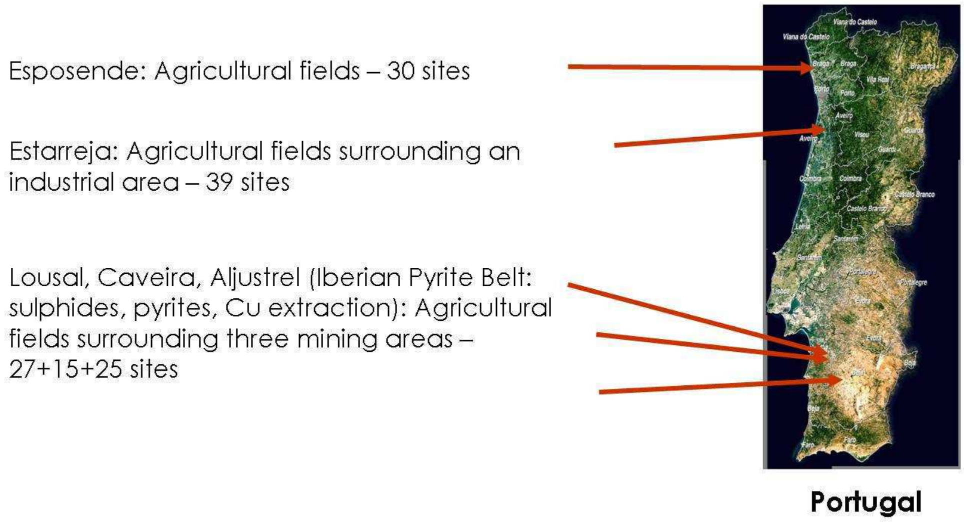


Figure 4.1: Location of sampling areas

The geology and soil types occurring at sampling areas considerably vary. According to Inacio et al. (2008), in geological terms, Portugal can be divided in two major units: the Hesperian Massif and the Epi-Hercynian cover. The Hesperian Massif is dominated by granitoids and a flysh-type series of schists and graywackes. It is of Precambrian and Palaeozoic age and it occupies more than half the country. In the Epi-Hercynian cover limestones, marls, shales, sandstones and conglomerates are the common lithological units. It occupies the western and southern Meso-cenozoic margins and the basins of Tejo and Sado rivers.

The lithology of soils from the North-West of Portugal including the Esposende study area includes mostly granitoids, and the soil types are predominantly Arenosols and Cambisols (Inacio et al., 2008). The Estarreja sampling area is located close to the North-Eastern edge of the Aveiro sedimentary basin (Costa and Jesus-Rydin, 2001). In this area, the Precambrian schistose bedrock is covered by 12 m thick Quaternary deposits, composed of inter-stratified conglomerates, sandstone, shale and marlstone (Costa and Jesus-Rydin, 2001). The dominant soil types in the Estarreja area are Podzols and Cambisols (Inacio et al., 2008). The three mining areas that were selected for this investigation are located in massive sulphide deposits of the Iberian Pyrite Belt. Both Lousal and Caveira are placed in a lineament of the Volcano-Sedimentary Complex (VSC) of this VHMS (Volcanic Hosted Massive Sulphides) province. Soil types in the Lousal, Caveira, and Aljustrel areas include Podzols and Leptosols (Inacio et al., 2008).

Esposende study area

Esposende is a small town of ~9,100 inhabitants located in the north-western coast of Portugal (41° 32′ 0″ N, 8° 47′ W″). The entire county has a population of around 35,000 inhabitants. Main activities in this region include agriculture, fisheries, tourism and small textile industry. The sampling areas include agricultural fields located away from any known point source of contamination as shown in Figure 4.2. In total, 30 soil samples were collected from 7 agricultural fields located at the following areas of the Esposende County: Curvos, Palmeira de Faro, Gandra and Esposende.

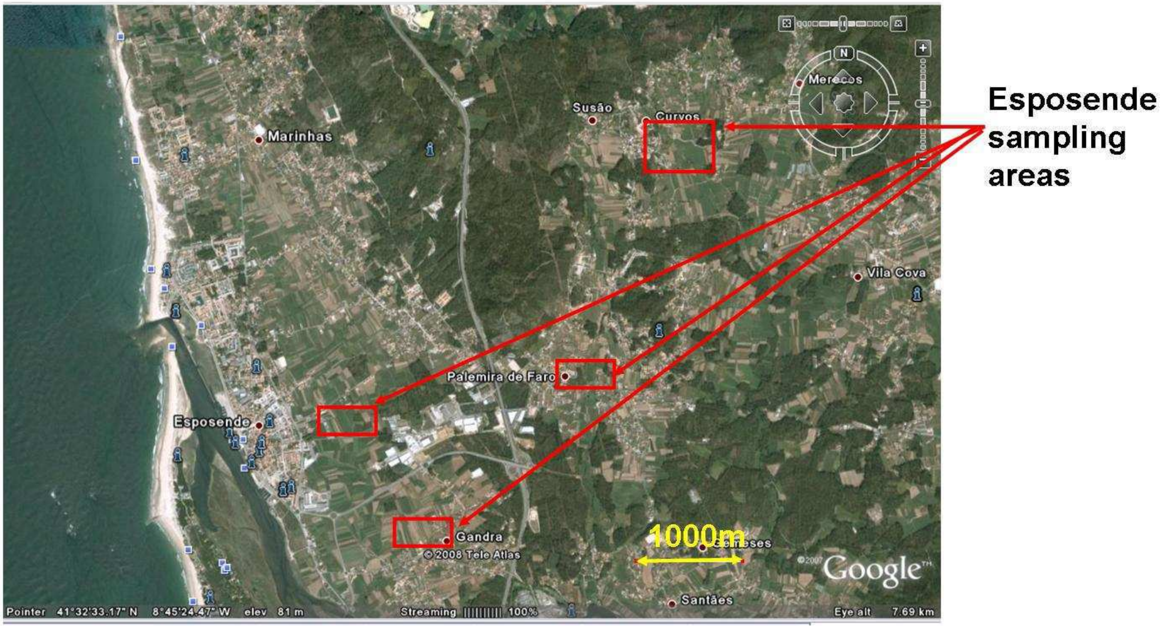


Figure 4.2: Aerial view of sampling areas at Esposende

Estarreja study area

The Estarreja Chemical Complex (North-West coastal area of Portugal) dates back to the 1950s. This industrial area is located 1 Km away from Estarreja town centre and it has an area of around 2 Km^2. Most relevant industries that are part of the Estarreja Chemical Complex include (Costa and Jesus-Rydin, 2001): *Quimigal* - it produced ammonium sulphate from sulphuric acid and ammonia (1952-1990s), nitric acid and ammonium nitrate (1974-1990s) ; since 1978 and until nowadays it produces nitric acid, aniline and nitrobenzene in an industrial unit named *Anilina Portugal*; *Cires* – it was started in 1963 and it produces synthetic resins such as polyvinyl chloride, PVC, from vinyl chloride monomer, VCM (VCM was also produced at this unit until 1986); *Dow Portugal* – it produces isocyanide polymers of aromatic base since 1978; *Uniteca* – a chlor alkali plant installed in 1956, it produces sodium and chlorine compounds from rock salt and used electrolytic cells with Hg cathodes for several decades. The *Uniteca* chlor alkali plant started to change the production process in 1994 and completely ceased the use of Hg cathodes in 2002. According to Costa and Jesus-Rydin (2001) several tons of solid wastes generated by the industrial processes were stored within the area of the Estarreja Chemical Complex: 150,000 ton of pyrite waste including ashes, dust and sludge from gaseous effluent treatment (containing As and several metals); 60,000 ton of sludge containing Hg; 300,000 ton of calcium hydroxide sludge. Until 1975, three man-made effluent streams were used to transport liquid effluents from the Estarreja Chemical Complex (Vala de S. Filipe, Vala da Breja and Vala do Canedo). These man-made streams, and particularly Vala de S. Filipe, run through agricultural fields and were used to transfer liquid effluents containing aniline, benzene, monochlorobenzene, mononitrobenzene, As, Hg, Zn, Pb and other metals into the "Esteiro de Estarreja", which is a river branch of a nearby lagoon ("Ria de Aveiro) (Costa and Jesus-Rydin, 2001).

Since the 80's, several studies carried out in the area reported the occurrence of high concentrations of metals, particularly Hg, in sediments and aquatic ecosystems from "Ria de Aveiro" (Pereira et al., 1997; Ramalhosa et al., 2005; Monterroso et al., 2007; Coelho et al., 2007; Coelho et al., 2008; Válega et al., 2008; Válega et al., 2009). Enhanced levels of Hg were also found in soils from the vicinity of the Estarreja Chemical Complex and Estarreja urban area (Inacio et al., 1998; Cachada et al., 2009; Reis et al., 2009).

An aerial view of the sampling areas is shown in Figure 4.3. The sampling areas in Estarreja included agricultural fields surrounding the Vala de S. Filipe (former effluent stream) and located in the southern area of the Estarreja Chemical Complex. In addition, soil samples were also collected at agricultural fields located in the centre of the town, about 2-3 Km south from the Chemical Complex. A total of 39 soil samples were collected.

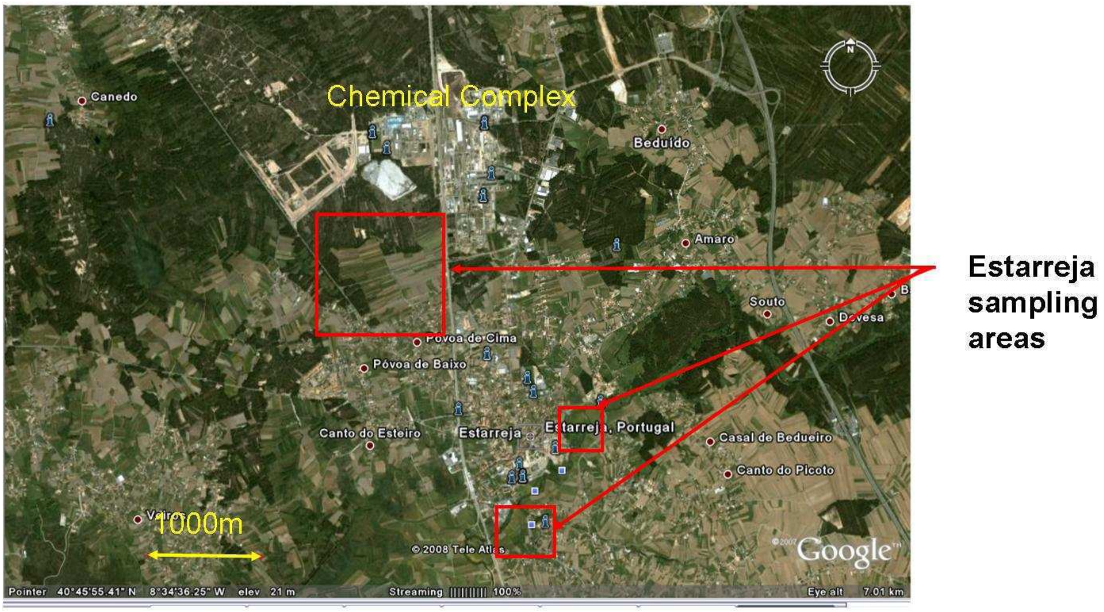


Figure 4.3: Aerial view of sampling areas at Estarreja

Lousal, Caveira and Aljustrel study areas

Between 1900 and 1988 the activities at Lousal mine (Iberian Pyrite Belt, South-West Portugal) included mostly surface and underground (to a depth of around 500 m) extraction of pyrites (FeS_2). The ore was stored and removed elsewhere for chemical treatment. The coarse-grained pyrites were sent to the Estarreja Chemical Complex to produce sulphuric acid (Ferreira da Silva, 2006). Potential environmental risks at Lousal area are related with tailings deposited at the mining site. These tailings are mostly barren material mixed with the leftovers of the fractionation of extracted materials. The volume stored on-site is higher than 1 Mt (Reis et al. 2005; Ferreira da Silva et al. 2005).

During the present investigation, a total of 27 soil samples were collected from 6 agricultural/ pasture fields located at three distinct areas surrounding the Lousal mine (Figure 4.4).

Past mining activities at the Caveira sulphide mine (Iberian Pyrite Belt, South-West Portugal) included pyrites and Cu extraction. From 1936 until the 1970´s Caveira massive sulphides were exploited for sulphur. Although the mine is already closed metal contaminated soils and acid mine drainage pose severe environmental problems at the site. Large volumes of waste were produced by the mining activities and various types of tailings are still deposited in the area (the amount of waste stored on the site is estimated to be higher than 2 Mt). Rainwater circulates and percolates easily over and through these tailing materials causing significant erosion and transport of tailings debris to areas nearby and downstream.

During the present study, a total of 15 soil samples were collected from 5 distinct fields surrounding the Caveira mine (Figure 4.5).

The third mining area selected for this study is Aljustrel (South-West Portugal) which is also part of the Iberian Pyrite Belt. The mineralisation is characterised by the dominance of pyrite, associated with other ore minerals (sulphides), the most important of which are chalcopyrite ($CuFeS_2$), spharelite (ZnS) and galena (PbS). Mining activities at Aljustrel were suspended in 1993 and large amounts of waste materials including tailings were left untreated (Alvarenga et al., 2004). Mining operations, particularly the extraction of Cu, Pb and Zn at Aljustrel re-started in 2008.

A total of 25 soil samples were collected at Aljustrel, from 9 agricultural/ pasture fields located either in the vicinity of the mine and/ or in the vicinity of potentially contaminated water streams (Figure 4.6).

While the sampling areas from Lousal and Caveira were located within a 1-2 km distance from the respective mining installations, in the case of Aljustrel samples were collected within a 10 km radius from the mine. This is due to the fact that several water streams flow in the surroundings of

Aljustrel mining area. Sites located close to these streams were included in this investigation.

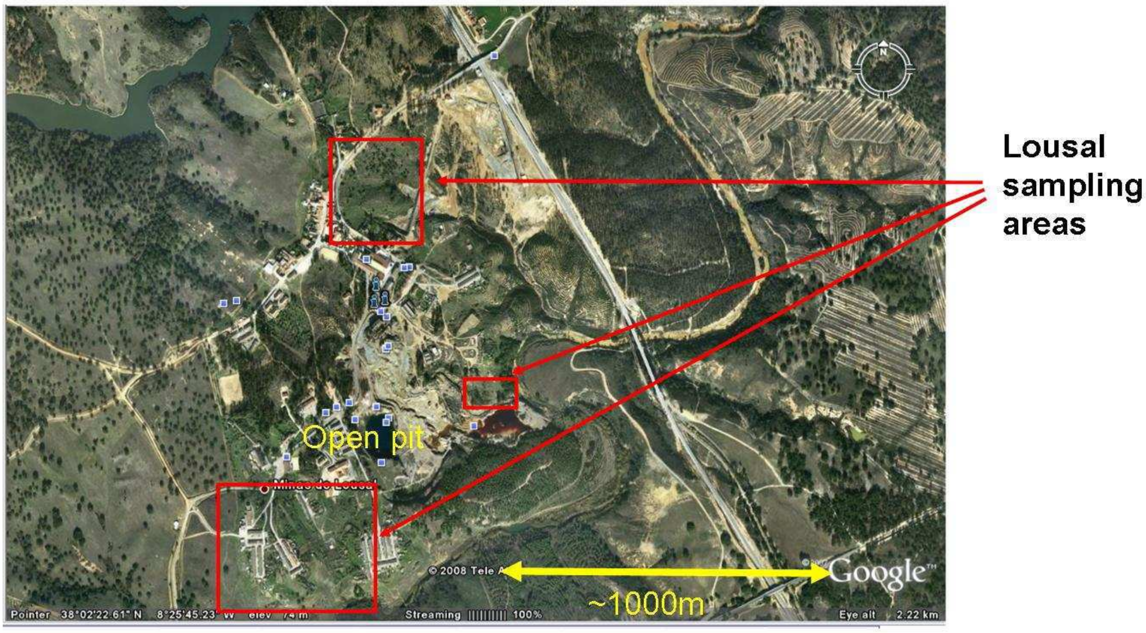


Figure 4.4: Aerial view of sampling areas at Lousal

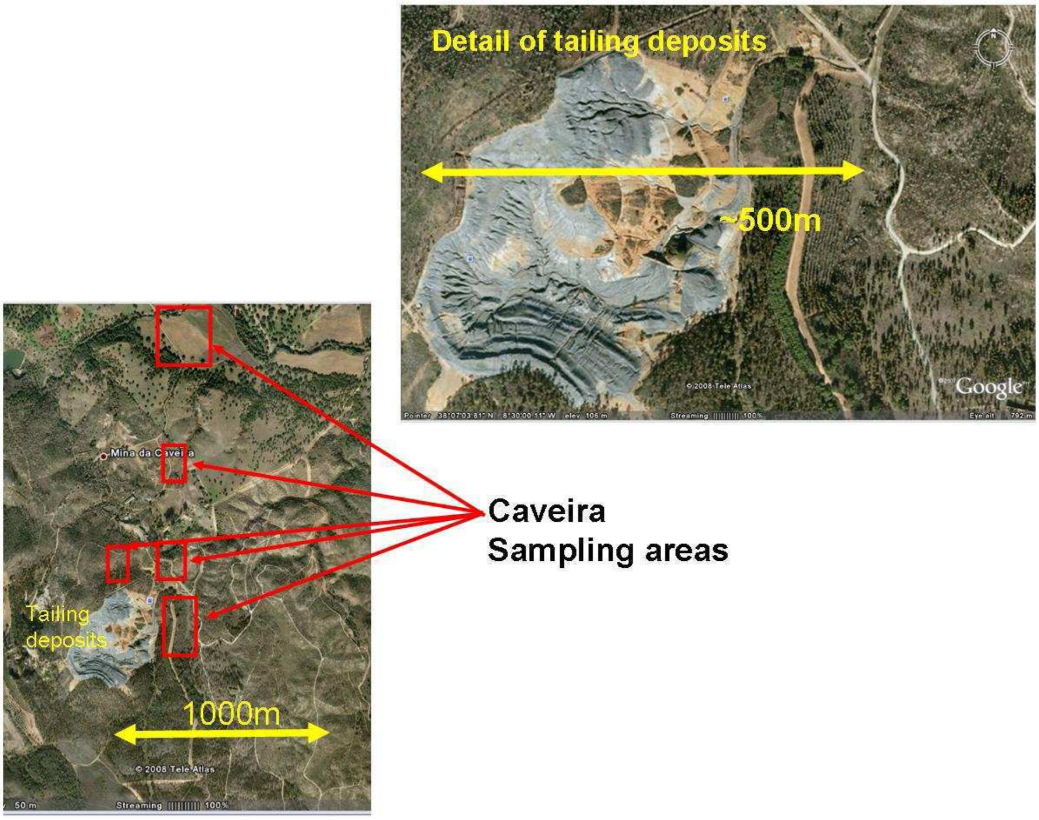


Figure 4.5: Aerial view of sampling areas at Caveira

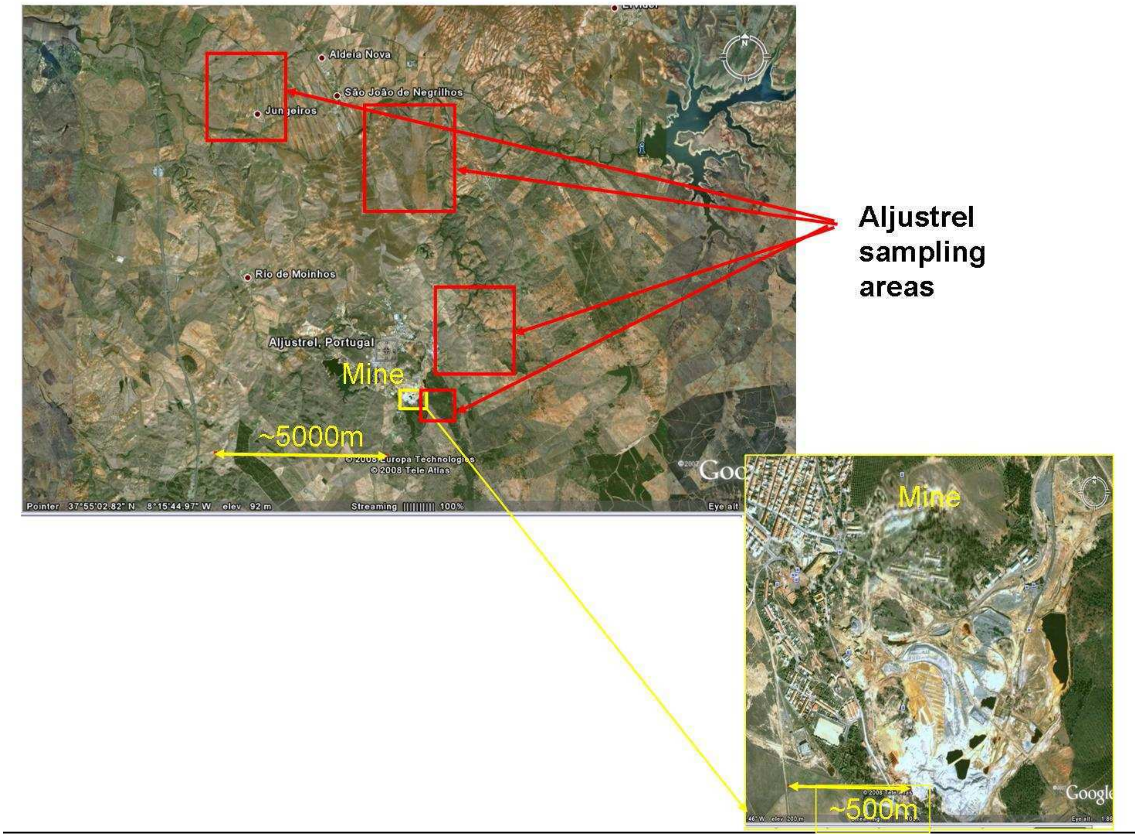


Figure 4.6: Aerial view of sampling areas at Aljustrel

4.3.2 Origin of plant samples

After selecting soil sampling areas, plant species sampled included those growing at those sites. At each sampling point, plants were sampled together with soils. A total of 128 plants were sampled. The following vegetation samples were collected at the time of harvest (mature crops):

- Ryegrass (*Lolium perenne*), n=73
- Italian ryegrass (*Lolium multiflorum*), n=9
- Orchard grass (*Dactilis glomerata*), n=12
- Collard greens, “couve-galega” (*Brassica oleracea*), n=23
- Rye (*Secale cereale*), n=11

An overview of the type of plant collect at each sampling site is shown in Annex I (Table 1).

4.3.3 Soil and plant sampling procedures

At each sampling site, plants were sampled first. In the cases of grasses and rye, 10-100 plants (including both root and aerial parts) were collected into plastic bags. In the case of collard greens one entire plant (root, stem and several leaves) was collected into a plastic bag at each sampling point. After the collection of plants, surface soils underneath were sampled using a plastic spade.

Soil samples were taken from the surface layer of the soils (0 – 15 cm) using a plastic spade. At each site five soil samples were collected within 1 m^2 and combined to obtain a single bulk composite sample of 1 to 1.5 kg. After collection, samples were stored in plastic bags and immediately transported to the laboratory. The coordinates of each sampling site were recorded using a GPS.

4.3.4 Soil and plant samples pre-treatment and storage

The pre-treatment of soil samples was performed following the ISO 11464:1994 procedure. This International Standard specifies the pre-treatment required for soils that are to be subjected to physical-chemical analysis. The soil samples were spread in plastic trays, in layers thinner than 15 mm and were dried in the air, at room temperature for at least three days and until constant weight. Direct sunlight was avoided. Big stones were separated while spreading the material, by hand picking. Samples were crushed while still damp and friable and again after drying, using a plastic hammer. The soil was sieved (using a Nylon® sieve, aperture of 2 mm, Bioblock Tamis Nylon® DIN 4195) and the fraction smaller than 2 mm was stored in plastic bags, at room temperature and in the absence of light, for further analysis.

Immediately upon arrival to the laboratory, plant samples were thoroughly cleaned with tap water and a brush to remove all traces of dust and soil from the roots and leaves and rinsed with distilled water. Samples were dried at room temperature for 12 h. After this time, roots were separated from the aerial parts of plants. All materials were weighted using an analytical balance, put on plastic trays and dried in the oven at 40 °C until constant weight. The dried samples were ground and stored in plastic bags and in the absence of light, at room temperature, for further analysis.

4.4 Soil and plant analysis

In dried and sieved soils the following parameters were measured:

- pH_{CaCl2}
- total and organic carbon (Org C) (%)
- particle size distribution (% of sand, silt and clay)
- Fe and Al in an ammonium oxalate-oxalic acid extract (amorphous oxides, $Fe_{am\text{-}ox}$ and $Al_{am\text{-}ox}$, mmol kg^{-1}) (also phosphorous, P_{ox}, was measured)
- total Hg concentration (mg kg^{-1}) (total Hg pool)
- concentration of As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo after *aqua regia* ($HCl:HNO_3$ 3:1) extraction (mg kg^{-1}) (total element pool)
- concentration of Hg, As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo after extraction with 0.43 M HNO_3 (mg kg^{-1}) (reactive element pool)
- concentration of Hg, As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo after extraction with 0.01 M $CaCl_2$ (mg kg^{-1}) (available element pool)

Chemical analysis of soil major components (SiO_2, Al_2O_3, Fe_2O_3, MnO, MgO, CaO, Na_2O, K_2O, TiO_2, P_2O_5) using X-Ray fluorescence (XRF) and flame-spectroscopy methods (Rocha and Gomes, 1995) were performed in a selected group of 50 soil samples at the Department of Geosciences, University of Aveiro.

In dried and ground plant samples the following parameters were measured:

- total Hg concentration (mg kg^{-1})
- concentration of As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo after pulverisation at 450 °C and *aqua regia* digestion (mg kg^{-1})

A detailed description of the analytical methodologies will be presented next.

4.4.1 Soil pH$_{CaCl2}$

The pH of the soil samples was determined following the ISO 10390:1994 procedure and using a 1:5 (V/V) suspension of soil in a solution of 0.01 M calcium chloride ($CaCl_2$). The fraction <2 mm of air-dried, sieved soil samples were used for this purpose.

A representative portion of the soil sample was taken to the sample bottle and five times its volume of 0.01 M $CaCl_2$ solution was added. After a vigorously mechanical mixing during 5 min, the suspension was left to settle. The pH-meter was calibrated using buffer solutions with pH 4.00 and 7.00. The pH determinations were performed twice, after 2 h and 20 h, using a glass electrode (WTW, Sentix 41). Just before the determinations of pH, the sample bottles were shaken and the value was measured in the settling suspension. The pH value was read after stabilization (value over a period of 5s does not vary more than a 0.02 unit). All suspensions were prepared in duplicate, according to the procedure. The accepted variation of the results in the separately prepared suspensions was 0.15 for pH<7 and 0.20 for pH>7.

4.4.2 Determination of total and organic carbon (elemental analysis) in soils

The total carbon content in air-dried, sieved (<2 mm) soil samples was determined after dry combustion, according to the ISO 10694:1995 procedure. The Org C content was also measured after the removal of carbonates following the same procedure.

The total carbon was determined in soil sub-samples of around 100 mg using an instrument for CNHS microanalysis (LECO, CHNS-932). In this system, the carbon present in the soil is oxidized to carbon dioxide (CO_2) by heating the soil to at least 900 °C in a flow of oxygen-containing gas that is free from carbon dioxide. The amount of carbon dioxide released is the measured using an infrared detection method. Calibration of the instrument was performed by elemental analysis of standard substances supplied by the instrument's manufacturer for this purpose. Three replicate measurements were performed in each sample. Reference materials (Eurovector E11036 and Eurovector E11037) were also analyzed (with recovery percentages of 99-114 %).

For the determination of organic carbon content, an excess of solution of 4 M of hydrochloric acid (HCl) was added to a crucible containing a weighed quantity of soil. The crucibles were left to stand for 4 h and then were dried for 16 h at 60-70 °C. The analysis of carbon content after the removal of carbonates (organic carbon) was performed using the same procedure of total carbon determinations.

4.4.3 Soil particle size distribution

The percentages of sand, silt and clay fractions in soil samples were determined by granulometric analysis which was performed using a Coulter LS230 laser diffraction particle size analyzer, based on Fraunhofer principle. The equipment allows the determination of particles sizes in the range of 0.04 µm-2000 µm.

In order to improve the accuracy of measurements, sub-samples of around 20 g of the air-dried, <2 mm fraction of soil samples were weighted using an analytical balance and sieved to <1 mm, using a Retsch® stainless steel Test Sieve. The fraction of soil <1 mm was then weighted again and used for analysis of particle sizes by laser diffraction. About ~0.40 g of each soil sample (<1 mm) was added to ~40 mL of water. After sonication (ultrasound) during 10 min, these samples were analysed using the Coulter LS230 equipment

The classification of soils followed the USDA Texture Classes: sand fraction (0.050<%<2mm), silt fraction (0.002<%<0.050mm), and clay fraction (%<0.002mm). Classification of samples was achieved by using the *Talwin 42*® classification software program.

4.4.4 Amorphous Fe and Al oxides

Amorphous Fe oxides (Fe_{am_ox}) and amorphous Al oxides (Al_{am_ox}) were determined by the extraction of 2.5 g of soil with 50 mL of a 0.1 M oxalic acid solution (buffered to pH 3 by ammonium oxalate) and shaken mechanically in the dark for 2 h. After shaking, the samples were immediately filtered through vacuum driven filtration using a Millipore® filter unit and a fine filter paper (0.45 µm pore). Aluminium and Fe contents in the filtered extracts were analysed by inductively coupled plasma mass spectrometry (ICP-MS) according to ISO 17294-1:2005 and ISO 17294-2:2003, using a Thermo X-Series quadrupole ICP-MS (Thermo Scientific) equipped with a Burgener nebulizer (1.0 mL min^{-1}), nickel cones, and a peristaltic sample delivery pump. Operating and instrumental conditions include the use of ^{115}In as internal

4.4.5 Contents of potentially toxic elements in soil and plant samples

Total Hg in soil (<2 mm) and plant samples (roots and aerial parts)

The determination of total Hg concentrations in soil (air-dried, sieved <2 mm) and both aerial parts and roots of plants (oven-dried at 40 ºC and grinded) samples was carried out by thermal decomposition atomic absorption spectrometry with gold amalgamation (LECO model AMA-254), a rapid total Hg determination method described by Costley et al. (2000). The use of this technique complies with EPA Method 7473 (Hg in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry). The method is based on the thermal decomposition of the solid sample in a combustion tube at 750 °C, in an oxygen atmosphere, collection of elemental Hg vapour in a gold amalgamator and detection by atomic absorption spectrometry (AAS). The determinations were performed directly on solid samples without sample pre-treatment or digestion, avoiding Hg losses and contamination during digestion. The detection limit of this technique for total Hg was 21 ng kg^{-1}. The technique is free from matrix interferences and it allows obtaining robust, reproducible and comparable results for total Hg concentrations in different environmental matrices (Pereira et al., 2008). Before the analysis, each sample bag was manually shaken prior to opening in order to re-homogenise the content, and then it was allowed to settle for 10 min. Intakes of solid samples for analysis varied between 2.5 and 400 mg. At least three replicate measurements were carried out in each soil/ plant sample analysis. Possible micro-heterogeneity effects derived from small sample intakes (<10 mg) were compensated by a higher number of replicates (acceptable relative standard deviation among replicates: <10 %). Several blanks were run before and between the analyses of samples. Certified reference materials (BCR 141R - Calcareous Loam Soil, BCR 142R - Light Sandy Soil, and BCR 060 - Aquatic plant (*Lagarosiphon major*)) were analysed every day prior to the beginning of the analysis. The measurement of reference materials was again repeated at the end of the day. The percentages of recovery of total Hg in the certified reference materials varied between 82.8 % and 119.9 %.

Aqua Regia extraction of soil samples (<2 mm fraction)

The extraction of potentially toxic elements soluble in *aqua regia* was performed following the ISO 11466:1995 procedure. The air-dried, sieved (<2 mm) soil samples were extracted with a hydrochloric/nitric acid 3:1 mixture by standing for 16 h at room temperature, followed by boiling under reflux for 2 h (Figure 4.7).

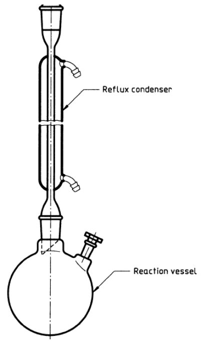


Figure 4.7: Reaction vessel and reflux condenser for *aqua regia* extraction of soil samples

3.00 g of each soil sample was weighted into a 250 mL reaction vessel and it was moistened with 1 mL of water. 21 mL of HCl (HCl, 37 %, ρ=1.19 g cm^{-3}) were added while mixing, followed by 7 mL of nitric acid (HNO_3, 65%, ρ=1.40 g cm^{-3}), added drop by drop, to reduce foaming. The reaction mixture stood for 16 h at the room temperature for slow oxidation of organic matter to occur. After 16 h, the temperature was raised until reflux conditions were reached and maintained for 2 h, ensuring that the condensation zone was lower than 1/3 of the height of condenser. After 2 h the temperature was decreased and the condenser was rinsed with 10 mL of 0.5 M HNO_3 solution. The mixture stood about 2 h for settlement of the insoluble residue. The supernatant was decanted onto a filter paper; the filtrate was collected into a 100 mL volumetric flask and diluted to volume with 0.5 M HNO_3 solution. The insoluble residue onto the filter and the residue of the flask were washed with 10 mL of 0.5 M HNO_3 solution and this filtrate was collected with the first. The concentrations of trace elements in filtrates were analysed by ICP-MS (as described in section 4.4.4). The detection limits for the different elements are shown in Table 4.1.

Table 4.1: Detection limits of different elements in ICP-MS analysis

^{7}Li	^{9}Be	^{11}B	^{23}Na	^{24}Mg	^{27}Al	^{29}Si	^{31}P	^{39}K	^{44}Ca	^{52}Cr	^{55}Mn	^{56}Fe	^{59}Co
µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
<0.1	<1	<5			<10	<250	<10	<10		<0.05	<2	<5	<0.1
^{60}Ni	^{65}Cu	^{66}Zn	^{75}As	^{82}Se	^{85}Rb	^{88}Sr	^{95}Mo	^{111}Cd	^{118}Sn	^{121}Sb	^{137}Ba	^{208}Pb	^{238}U
µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
<0.5	<0.5	<5	<0.5	<1	<0.1		<1	<0.1	<5	<0.1	<0.1	<0.1	<0.1

For quality assurance of the result method blanks and replicates were used. Blank samples were run with the batch of soil samples by performing the operation on the reaction vessel containing no sample. The CMI7004 (Loam soil), BCR-142R (light sandy soil) and the BCR-143R (sewage sludge amended soil) certified reference materials were used for quality control. Average recovery percentages of the different elements were between 83 and 109%: 83 % (Be), 91 % (As), 94 % (Cu), 94 % (Zn), 95 % (Pb), 97 % (Mn), 99 % (Co), 101 % (Ni), 101 % (Ba, indicative value), 106 % (Cr) and 109 % (Cd).

Analysis of plant samples

The concentrations of the remaining metals and metalloids in all plant samples (shoots) were determined by ICP-MS after *aqua regia* digestion. Vegetation samples were pulverised at 450 °C and a 0.5 g split of each material was leached in *aqua regia* ($HCl–HNO_3–H_2O$) at 95 °C, for 60 min. After cooling the solution was diluted to a final volume of 10 mL using 5 % HCl. Solutions were aspirated into a ICP-MS (Perkin Elmer Elan 6000/9000) and 17 elements were analysed for (detection limit between brackets in mg L^{-1} unless stated otherwise): Mo (0.01), Cu (0.01), Pb (0.01), Zn (0.1), Ni (0.1), Co (0.01), Mn (1), Fe (0.001%), As (0.1), U (0.01), Cd (0.01), Sb (0.02), Cr (0.1), Ba (0.1), B (1), Al (0.01%), Se (0.1). Duplicate plant samples were digested and analysed. Six blanks and a total of eight samples of two internal reference materials were included in the samples batch for analytical quality control. Average recoveries of the different elements were within 84 and 115%.

4.4.6 Assessment of reactive and available pools of PTE's in soil samples

0.43 M HNO_3 extraction (reactive pools of PTE's in soils)

An extraction with 0.43 M nitric acid (HNO_3) at 1:10 soil:solution ratio was applied in order to estimate reactive metal contents (Römkens et al., 2004; Rodrigues et al., 2010a). This extraction gives information on the metal contents in soils that are expected to be involved in chemical reactions such as those occurring in relatively weak bound forms and in the soil solution and those absorbed on the soil's exchange complex.

Each soil sample was extracted at a 1:10 weight to volume ratio with a 0.43 M HNO_3 solution at room temperature. 5.00 g of the air-dried, sieved (<2 mm) soil samples were weighted into a dry plastic shaking bottle using an analytical balance. 50.0 mL of a 0.43 M HNO_3 solution (prepared by diluting 30 mL of Hg-free HNO_3, ρ=1.40g cm^{-3}, in ultra-pure water in a 1000 mL volumetric flask) were added to each shaking bottle which was then mechanically shaken for 2 h at room temperature. After 2 h shaking, the samples were immediately filtered through vacuum driven filtration using a Millipore® filter unit and a fine filter paper (0.45 µm pore). A portion of each filtrate was collected into an amber glass bottle for the analysis of Hg and the remaining filtrate was collected into plastic tubes for the analysis of other elements. All filtrates were kept at 4 ºC until further analysis. Each soil sample was extracted in triplicate. Two extraction blanks were included in each batch of 20 bottles, and were treated in the same manner.

0.01 M $CaCl_2$ extraction (available pools of PTE's in soils)

An extraction with 0.01 M calcium chloride ($CaCl_2$) at 1:10 soil:solution ratio was applied in order to estimate available metal contents (Rodrigues et al., 2010b). According to Houba et al. (2000) this extraction procedure gives a better indication of bioavailability than do total soil metal contents. From 12 different commonly used extraction procedures Meers et al. (2007a) reported the 0.01 M $CaCl_2$ extraction to be the most versatile since it provided a good indication of phytoavailability for all metals under evaluation (Zn, Cd, Cu, Pb and Ni).

Each soil sample was extracted at a 1:10 weight to volume ratio with a 0.01 M $CaCl_2$ solution at room temperature (Rodrigues et al., 2010b). 3.00 g of the air-dried, sieved (<2 mm) soil samples were weighted into a centrifuge tube using an analytical balance. 30.0 mL of a 0.01 M $CaCl_2$ solution (prepared by dissolving 1.47 g of dihydrated calcium chloride, $CaCl_2.2H_2O$ p.a., in ultra-pure water in a 1000 mL volumetric flask) were added to each centrifuge tube which was then mechanically shaken for 2 h at room temperature. After 2 h shaking, the pH was measured in the suspension and the samples were centrifuged at 3000 *g* for 20min (Sigma Laboratory Centrifuges, 4-10, N°10850, 220V, 50Hz). Extracts for analysis of PTE's were not filtered to avoid losses due to possible adsorption of the analyte into the filter, contamination or secondary reactions with soil suspensions (Houba et al., 2000). A portion of the clear centrifugate was collected into an amber glass bottle for the analysis of Hg and the remaining extract was collected into plastic tubes for the analysis of other elements. The centrifugates were acidified (pH <2) with a 1 M hydrochloric acid (HCl) solution to prevent adsorption to the recipients and growth of bacteria, and the samples were kept at 4 °C until further analysis. The analysis of Hg in the extracts was performed within 48 h after extraction. Each soil sample was extracted in triplicate. Two extraction blanks were included in each batch of 20 centrifuge tubes, and were treated in the same manner.

Dissolved organic carbon (DOC) was determined in 0.01 M $CaCl_2$ extracts. Extracts for the analysis of DOC were produced together with those for the assessment of available pools of PTE's following a similar procedure for soil extraction and centrifugation. After centrifugation, the supernatant soil mixtures for analysis of DOC were not acidified but filtered through a 0.45 μm carbon-free filter, preserved at 4 °C and analysed for DOC in solution according to the high-temperature combustion method (Standard Methods for the Examination of Water and Wastewater-Method 5310 B, http://www.standardmethods.org/).

All labware used in the extraction procedures was cleaned with DERQUIM LM02 5 % (24 h), HNO_3 25 % (24 h) and rinsed thoroughly with ultra-pure water.

Analysis of Hg in soil 0.43 M HNO_3 extracts

Total dissolved Hg concentrations in solutions of soil samples extracted with 0.43 M HNO_3 were measured by thermal decomposition atomic absorption spectrometry with gold amalgamation (LECO model AMA-254), similarly to the determination of total Hg in solid samples (Rodrigues et al., 2010a). A specific programme of temperatures for the analysis of liquid samples was applied. A 1.00 mL liquid sample intake for each analysis was used. The detection limit of this technique for total Hg was 59 ng L^{-1}.At least three replicate measurements were carried out in each analysis (acceptable relative standard deviation among replicates: <10 %).

Analysis of Hg in soil 0.01 M $CaCl_2$ extracts

Total dissolved Hg concentrations in solutions of soil samples extracted with 0.01 M $CaCl_2$ were measured by Cold Vapour Atomic Fluorescence Spectrometry (CV-AFS; PSA model Merlin 10.023 equipped with a detector PSA model 10.003) using tin(II) chloride as a reducing agent. 500 µL of a saturated solution of potassium persulfate were added to 15 mL of each extract sample which was then subject to irradiation by a UV lamp (1000 W) for 30 min (Rodrigues et al., 2010b). Following irradiation, the excess oxidant was reduced with 37.5 µL of 12 % hydroxylamine solution (w/v) prior to analysis (Mucci et al., 1995). The equipment was calibrated every day with acidified (Hg-free HNO_3) standard solutions prepared from a 1000 mg L^{-1} solution (BDH). The detection limit of the CV-AFS technique for total Hg was 2.3 ng L^{-1}. At least two duplicate analysis of each extract were performed.

Analysis of the remaining elements in soil extracts (0.43 M HNO_3 and 0.01 M $CaCl_2$)

Extracted contents of PTE's were analysed by ICP-MS (as described in section 4.4.4). The calibration solutions were prepared in the same extraction solution as the samples in order to remove the matrix effect.

Extraction blanks were always run with samples, being their contribution corrected when necessary.

4.5 Statistical analysis of data

SPSS 10.0 for Windows was used for calculation of descriptive statistics and for statistical analysis of data (factor analysis and multiple linear regression). Factor Analysis was performed to identify those underlying factors that explain the pattern of correlations within the set of observed variables. The Principal Components method with Varimax Rotation was applied as an extraction method. The regression method was used to derive factor scores. SPSS was also used for regression analysis. The stepping method criteria using a probability of F of 0.05 for entry and 0.10 for removal was applied for linear regression. The relevance of inclusion of each variable into the model was determined on the basis of the t value for its respective regression coefficient and the two-tailed significance level of t. The relative contribution of the different variables within a model was further assessed by its Beta coefficient.

4.6 Derivation of empirical models

4.6.1 The modelling approach

The analysis of transfer of PTE's between environmental compartments requires the development of a modelling approach. In general terms, in this study in particular there was a need to:

- Model the reactivity of PTE's in soils;
- Model the partition of PTE's between the soil solid-phase and the soil solution (available pools);
- Model the transfer of PTE's from soil to plants;
- Model the transfer of PTE's along the terrestrial feed and food supply chain.

Models to describe the partition of contaminants within the soil system and their distribution between soil and other environmental compartments range from very simple linear models to complex mechanistic models. The goal of this study was to develop an approach that can be applied at various scale levels with an emphasis on regional and national applications. It is also important that this approach requires a limited number of input parameters that in the future can be generated by national soil monitoring or soil information systems. Therefore it was decided to test empirical models that take into account both the contents of PTE's and key soil properties with relevant regional variability.

In recent soil studies Freundlich equations have been used to describe experimental results particularly to describe the process of sorption of inorganic elements to the solid phase (organic matter and clay) (Sauvé et al., 2000; Römkens et al., 2004). The advantage of this approach is that the concept of a Freundlich equation comes close to the nature of the sorption phenomena in soil since it allows to incorporate various soil properties that control the adsorption of elements in soils. The major limitation is that according to this conceptual approach there is no maximum adsorption capacity of the soil and therefore its application should be limited to situations where the degree of saturation of sorption sites with the elements under study is low as it is generally the case at contaminated sites (Römkens et al., 2004). It should also be mentioned that other processes such as precipitation are not accounted for by Freundlich-type empirical models.

An adaption of the "classic" Freundlich equation often used in soil sorption studies is given by:

$$C_{soil} = K_f \times C_{solution}{}^{n} \qquad \textbf{4.1}$$

Where C_{soil} is the concentration of the element of interest in the solid phase, $C_{solution}$ is the concentration of the elements in soil solution, K_f is a constant and n is the non-linearity term ($0<n<1$) (Sauvé et al., 2000; Römkens et al., 2004; Römkens et al., 2009a). The value of K_f depends soil properties and assumes competitive adsorption between H^+ and free metal ions onto binding sites as for example, organic carbon and clay:

$$K_f = K \times [OC]^a \times [clay]^b \times [H^+]^c \qquad \textbf{4.2}$$

An alternative model to predict solubility of PTE's in contaminated soils can be derived from a semi-mechanistic approach that assumes that free elements (Me^{x+}) and H^+ compete for adsorption on the soil's binding sites (Sauvé et al., 2000; Römkens et al., 2004):

$$\log[C_{solution}] = \mathrm{int}\,ercept + n \times \log[C_{soil}] + (a...i) \times \log[binding\_sites] - c \times pH \qquad \textbf{4.3}$$

Often, log-transformation of the experimental data followed by multiple linear regression analysis is performed to obtain the model coefficients. The details of the approach developed in this study will be given in Chapters 5, 6, and 7. An overview of the different model equations will presented next.

4.6.2 Modelling reactive pools of PTE's

In this study the hypothesis that the influence of soil properties on the extractability of reactive concentrations of PTE's could be analysed using a Freundlich-type approach was tested. Freundlich-type empirical models were derived on the basis of linear regression results (Römkens et al., 2004; Römkens et al., 2009a; Rodrigues et al., 2010a):

$$\log[PTE_{reactive}] = \mathrm{int}\,ercept + n \times \log[PTE_{total}] + (c + ...i) \times \log_{10}(soil\_property) \qquad \textbf{4.4}$$

Where:

[$PTE_{reactive}$]: reactive pool of each PTE in mg kg^{-1};

[PTE_{total}]: total pools of each PTE in mg kg^{-1};

a, b, c, ..,i, n: regression coefficients for the log transformed (except for pH) values of each soil property included in the model.

4.6.3 Modelling available pools of PTE's

The Freundlich-type approach was also applied to model available pools of PTE's as a function of their respective reactive pools. The multiple linear regression analysis was performed using the following equation:

$$\log[PTE_{available}] = \mathrm{int}ercept + n \times \log[PTE_{reactive}] + (c + ...i) \times \log_{10}(soil\_property) \qquad \mathbf{4.5}$$

, where $[PTE_{available}]$ and $[PTE_{reactive}]$ are the available and reactive pools of PTE's, respectively and *a* is the intercept and *c* to *n* are the coefficients for the log transformed values of each soil property relevant to the model.

4.6.4 Derivation of soil to plant transfer models

The transfer of PTE's from soil to crop often is a non-linear process that involves desorption of PTE's from the soil solid-phase, sorption onto root surfaces, uptake by roots and internal root to shoot transfer processes (Kalis et al., 2007). The overall soil to shoot transfer can be described with curvilinear functions – soil to plant transfer functions, STP (Krauss et al., 2002; Kalis et al., 2007). Here a Freundlich-type function was used to link levels of PTE in soil to those in shoots or roots (for Hg only) similar to the ones used by (Krauss et al., 2002):

$$[PTE]_{plant} = a \cdot [PTE]_{soil}^{b} \qquad \mathbf{4.6}$$

The coefficients in such Freundlich-type equation reflect both the influence of environmental and soil conditions in the uptake process (*a*) and the plants capability to regulate metal concentrations in its tissues (*b*).

Log-transformed data (Log10) was used for regression analysis due to the occurrence of non-normality situations in the distribution of soil and plant data. Since these data were generally strongly positively skewed, a log-transformation resulted in approximate normal distributions (Webster, 2001). Here we use two equations to link the availability of PTE's in soil to levels in crops. The first one is based on the direct measurement of the available pool by 0.01 M $CaCl_2$ according to (Koopmans et al., 2008; Römkens et al., 2009a):

$$\log[PTE]_{plant} = \log(a) + b \cdot \log[PTE]_{soil(available)} \qquad \mathbf{4.7}$$

The second equation uses both the reactive soil PTE content in combination with soil properties to predict the levels in plants (Koopmans et al., 2008; Römkens et al., 2009a):

$$\log[PTE]_{plant} = \log(a) + b \cdot \log[PTE]_{soil(reactive)} + (c...n) \cdot \log[soil\_properties] \qquad \mathbf{4.8}$$

Where:
PTE_{plant} : PTE content in plant in mg kg^{-1} dry weight (d.w.)
$PTEs_{oil}$: PTE in soil measured by 0.01 M $CaCl_2$ (available) or 0.43 M HNO_3 (reactive) in mg kg^{-1} d.w.
a, b, c... n: regression parameters

4.7 Transfer of PTE's along the feed and food supply chain

The analysis of the transfer of PTE's along feed and food supply chains will be performed in Chapter 8. This assessment will require the calculation of daily intakes and acceptable daily intakes of PTE's for animals, the calculation of concentrations of PTE's in animal organs as well as daily intakes of PTE's for humans. Further details on the approach followed to evaluate the impacts of soil contamination on the terrestrial foodchain are given in Chapter 8. An overview of the equations used in these calculations will be given next.

4.7.1 Calculation of acceptable daily intakes (ADI's) of PTE's for animals in view of food safety and animal health criteria for animal organs

The intake of PTE's by animals relates both to feed consumption and soil ingestion (Smith et al., 2009). The daily intake (DI) of PTE's by animals can be calculated by (de Vries et al., 2007; Smith et al., 2009):

$$DI_{animal} = [PTE]_{feed} xI_{feed} + [PTE]_{soil} xI_{soil} \qquad \textbf{4.9}$$

Where:

DI_{animal}= daily intake of a PTE for grazing animals (cow and sheep) in mg d^{-1}

$[PTE]_{feed}$ and $[PTE]_{soil}$ = concentration of the PTE in feed and soil samples, respectively, in mg kg^{-1} d.w.

I_{feed} and I_{soil} are the daily intake of feed and soil, respectively, by grazing animals (cow and sheep) in kg d^{-1} d.w.

4.7.2 Estimation of concentrations of PTE's in animal organs and calculation of animal ADI's

According to de Vries et al., 2007 the concentration of PTE's in animal organs can be given by:

$$[PTE]_{animal\_organ} = \left([PTE]_{feed} x \frac{I_{feed}}{I_{feed} + I_{soil}} + [PTE]_{soil} x \frac{I_{soil}}{I_{feed} + I_{soil}} \right) xBAF_{feed-animal} \qquad \textbf{4.10}$$

Where:

$[PTE]_{animal\_organ}$ = concentration of the PTE in an animal organ (kidney, liver or muscle), in mg kg^{-1} fresh weight (f.w.)

$BAF_{feed-animal}$= transfer coefficient or bioaccumulation factor from feed to animal organ for PTE's ((mg kg^{-1} in animal organ f.w.)/ (mg kg^{-1} in feed d.w.)).

Eq. 4.10 is based on the following assumptions: there is a linear relationship between the content of PTE's in feed and the respective element concentration in animal organs and is given by $BAF_{feed-animal}$; the transfer coefficient of PTE's from soil to animal organ is equal to the $BAF_{feed-animal}$; the concentration of a PTE in animal organs reflects the intake of the element through feed and soil and the intake by other sources (air and water) is negligible (de Vries et al., 2007).

A combination of Eq. 4.9 and Eq. 4.10 allows to calculate the acceptable daily intake (ADI) for animals. The ADI reflects the maximum daily intake of a given PTE so that the concentration of the element in the different animal organs (kidney, liver or muscle) does not surpass the limit organ concentrations given by food safety and animal health criteria and is given by:

$$ADI_{animals} = \frac{[PTE]_{\lim it\_animal\_organ} x(I_{feed} + I_{soil})}{BAF_{feed-animal}} \qquad \mathbf{4.11}$$

Where:

$ADI_{animals}$= acceptable daily intake of each PTE for animals, in mg d^{-1}

$[PTE]_{limit\_animal\_organ}$ = limit concentration of the PTE in an animal organ (kidney, liver or muscle), in mg kg^{-1} f.w, as given by food safety or animal health criteria.

4.7.3 Calculation of DI's of PTE's for humans due to soil ingestion and dietary intakes of vegetables and animal products

The routes for intake of PTE's by humans that were analysed within the scope of this include:

- Dietary intake of food crops (leaf vegetables);
- Direct intake through soil ingestion;
- Dietary intake of organs (kidney, liver and muscle/ meat) of grazing animals (cow and sheep).

The analysis of routes for intake of PTE's required an assessment of soil-plant-human, soil-human and soil-plant-animal-human pathways as described next.

a) Soil-plant-human relationships:

- Calculation of human DI of PTE's derived from the dietary ingestion of leaf vegetables using data on measured contents of PTE's in *Brassica oleracea* samples and average consumption of vegetables by the Portuguese population (according to most recent statistics, an adult is expected to consume on average 156.4 g of vegetables per day, we assumed that half of this value is of *Brassica* vegetables, INE, 2006).

b) Soil-human relationships:

- Calculation of human DI of PTE's derived from direct soil ingestion using measured soil concentrations at each sampling site and a value for daily intake of soil for an adult (we assumed a value of 50 mg soil d^{-1} d.w., according to Brand et al., 2007)

c) Soil-plant-animal-human relationships:

- Estimation of the concentration of PTE's in *Lolium perenne* at sites where element contents for feed crops were not measured using derived SPT functions – this plant was selected since this was the most common feed crop at sampling areas;
- Estimation of the concentration of PTE's in animal organs (kidney, liver and muscle) at each sampling site using contents in soil and feed according to Eq.4.10;
- Calculation of human DI of PTE's derived from the dietary ingestion of animal products using data on average consumption of offal and meat by the Portuguese population (according to most recent statistics, an average adult is expected to consume 37.8 g of cow meat per day; 6.8 g of sheep meat per day; 16.4 g of offal per day, INE, 2006; it was assumed that all offal consumed were liver and kidney of bovine origin).

The "worst case scenario" which is the consumption of all leaf vegetables and animal products from animals always at field and grazing always at the same sites, was assumed.

The DI of PTE's for humans was calculated according to the following equation:

$$DI_{humans} = [PTE]_{soil} xI_{soil} + [PTE]_{leaf\_vegatables} xI_{leaf\_vegetables} + [PTE]_{animal\_organ} xI_{animal\_organ} \quad \mathbf{4.12}$$

Where:

DI_{humans}= daily intake of a PTE for humans in mg d^{-1}

$[PTE]_{leaf\_vegetables}$ = concentration of a PTE in leaf vegetables (collard greens), in mg kg^{-1} f.w.

$I_{leaf\_vegetables}$ and I_{animal_organ} = daily intake of leaf vegetables (collard greens) and animal organs (kidney, liver and muscle/ meat), respectively, by humans in kg d^{-1} f.w.

Results obtained in the analyses of soil and plants samples as well as in the application of the model approaches described above will be discussed in Chapters 5-8.

Chapter 5

Total, reactive and available pools of PTE's in Portuguese soils

5.1 Introduction

Total concentrations of contaminants in soils may not be indicative of actually occurring adverse effects on biota, plants and aquatic systems since part of the potentially toxic elements (PTE's) stored in soils are present in what is called the "inert" fraction (Römkens et al., 2004; Rieuwerts et al., 2006; Peijenburg et al., 2007). In the short term, inorganic contaminants strongly bound within the crystalline matrix of the solid phase are not likely to contribute to the element fraction in the soil solution and therefore are not likely to become mobile and available to plants and organisms (Römkens et al., 2004; Rodrigues et al., 2010a). Hence, concepts dealing with reactivity and availability of PTE's in soils have been developed in recent years (Peijenburg et al., 2007).

The distribution of element concentrations in soils among three different pools (total, reactive and available) and most relevant soil processes associated to the concepts of reactivity and availability of contaminants are shown in Figure 5.1.

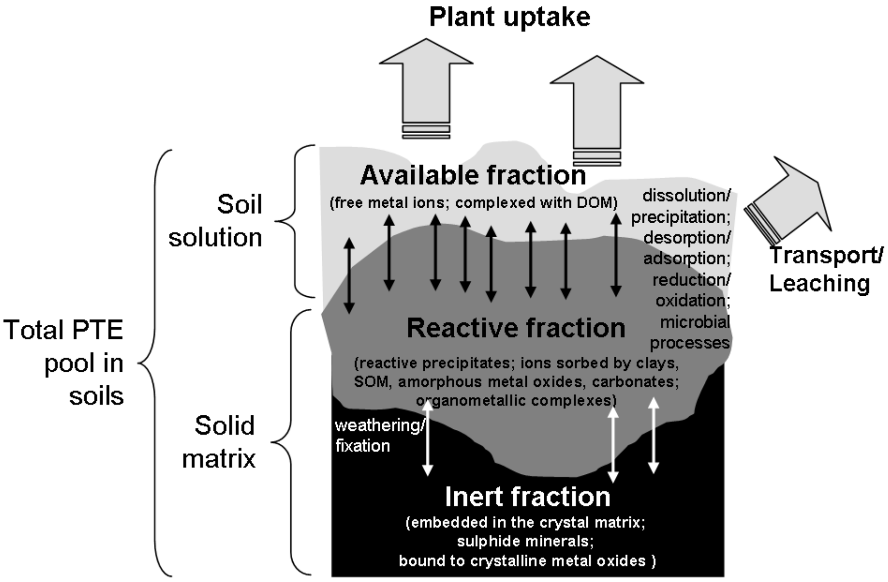


Figure 5.1: Distribution of total pools of potentially toxic elements in soils – the size of the different fractions and the most relevant soil processes vary according to the element of interest and environmental conditions (Rodrigues et al., 2010a)

The inert fraction is strongly bound within the crystal matrix of the solid phase and therefore unavailable for transport or plant uptake since its release is very slow and dependent on aggressive weathering processes (Römkens et al., 2009a). By contrary, the reactive fraction includes reactive precipitates of contaminants and metal ions adsorbed to the surfaces of clays, soil organic matter and amorphous metal oxides and that are readily exchangeable between solid and solution phases (Rieuwerts et al., 2006; Römkens et al., 2009a). The sorbed fraction of contaminants is in equilibrium with the directly available element pools which can be leached from soils by transport through the soil solution, be detrimental to soil (micro)organisms, and be incorporated into trophic chains (Sastre et al., 2007). The accuracy of risk assessments and the analysis of the probability of occurrence of adverse health effects are therefore constrained by our ability to distinguish the pool of the contaminant within the soil matrix that can potentially desorb and become mobile and/ or available to plants and organisms (Sauvé et al., 2000; Peijenburg et al., 2007; Römkens et al., 2009a).

Analytical techniques such as X-ray fluorescence (XRF) or soil extraction with *aqua regia* ($HCl+HNO_3$), hydrofluoric acid (HF) or a mixture of concentrated nitric and perchloric acids are generally applied to determine total element contents in soils. Soil chemical extraction can also be used to determine reactive pools of potentially toxic elements. Single extraction procedures with strong complexing agents (chelating agents such as EDTA) and dilute strong acids (such as HCl and HNO_3) have been applied in soil studies to determine the reactive metal contents (Peijenburg et al., 2007). Soil metal contents measured by extraction with 0.05/ 0.1 M EDTA and 0.43 M HNO_3 (Tipping et al., 2003; Römkens et al., 2004) and with 0.05 M EDTA, 0.43 M HNO_3 and 0.1 M HCl (Römkens et al. 2009a) showed that the different extractants provide comparable results and lower values than total soil concentrations. Synthetic chelating agents are able to form stable, water soluble complexes with polyvalent cations but in certain cases proved to be less specific for metals bound to carbonates and Fe and Al oxides (Peijenburg et al., 2007). In most recent years, the 0.43 M HNO_3 extraction procedure has been used to assess the geochemically reactive pools of potentially toxic elements (such as Cd, Zn, Cu, Ni, Cr and Pb) in soils (Gooddy et al., 1995; Marinussen, 1997; Temminghoff et al., 1997; Cancès et al., 2003; Tipping et al., 2003; Römkens et al., 2004; Pampura et al., 2007; Bonten et al., 2008; Koopmans et al., 2008; Römkens et al., 2009a).

Similarly, several chemical extraction procedures have been applied to quantify available pools of PTE's in soils. Weaker extractants such as neutral, non-buffered electrolyte solutions (e.g. $NaNO_3$, NH_4NO_3, $Ca(NO_3)_2$ or $CaCl_2$) have often been used for the assessment of available soil contents of potentially toxic elements (particularly for Cd, Zn, Cu, Ni and Pb) (Houba et al., 2000; Pueyo et al., 2004; Wang et al., 2004; Rieuwerts et al., 2006;

Meers et al., 2007a; Meers et al., 2007b; Menzies et al., 2007; Peijnenburg et al., 2007; Koopmans et al., 2008; Römkens et al., 2009b). The pool of potentially toxic elements extracted from soils with 0.01 M $CaCl_2$ was considered to give better indication of elements availability to plants than total contents or concentrations obtained with other extractants (Wang et al., 2004; Menzies et al., 2007; Meers et al., 2007a; Peijnenburg et al., 2007). This procedure was suggested to be used as a universal extractant for risk assessment practices (Houba et al., 2000). Moreover, the $CaCl_2$ extract was used to predict soil solution concentrations of potentially toxic elements such as Cd and Zn (Degryse et al., 2003; Peijnenburg et al., 2007).

5.2 Aim, scope and objectives

The results on total, reactive and available pools of PTE's in soils from different areas in Portugal will be discussed on this Chapter. Such a study will focus simultaneously on a rather large array of potentially toxic elements: As, Hg, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Li, Be, Se, B and Mo. The reactive pools of PTE's will be quantified using a chemical extraction procedure for soils (0.43 M HNO_3). The soil solution concentrations of the different elements (available pool) will be estimated on the basis of soil extraction with 0.01 M $CaCl_2$.

Available information on regional variation in naturally occurring background concentrations of PTE's in Portuguese soils and the variability of soil properties (pH, soil organic matter, clay content, and amorphous metal oxides) across Portugal will also be discussed.

The objectives of this Chapter are:

- Assessment of relevant soil properties at study areas (major elements contents; pH; Total and Org C %; sand, silt and Clay %; amorphous Fe and Al contents);
- Assessment of total pools of PTE's in soils from selected study areas and analysis of their geographical distribution;

- Comparison of observed total pools of PTE's in soils from study areas with background elemental contents;
- Quantification of reactive pools of PTE's in study areas;
- Quantification of available pools of PTE's in study areas.

5.3 Results and Discussion

5.3.1 Soil properties and soil chemical composition

A summary of properties of soils from Esposende, Estarreja, Lousal & Caveira and Aljustrel is shown in Table 5.1. Further details on description of the properties of soils included in the dataset is provided in Annex II: boxplots of key soil properties and soil chemical composition of samples clustered by the different sampling areas are shown in Figure 1 (Annex II); the distribution of values obtained for key soil properties (n=136: pH, OrgC %, Clay %, Al_{am-ox}, Fe_{am-ox}, P_{ox}) and soil chemical composition (including SiO_2 %, Al_2O_3 %, Fe_2O_3 %, MnO %, n=50) is shown in Figure 2 (Annex II). Applying the Kolmogorov-Smirnov statistical test it was observed that these values were generally not normally distributed. For most variables deviations from normality are associated with the presence of more than one population distributions within the data structure (Annex II, Figure 2.). Due to the occurrence of non-normal distributions median instead of mean values will be used to evaluate the central tendency of the entire dataset.

Table 5.1: Summary of key soil properties at the different sampling areas (mean ± standard deviation) and median value obtained for all samples together

Sampling area	Nº samples (n)	pH ($CaCl_2$)	Total C %	Org C %	Sand %	Silt %	Clay %	Al_{am-ox} mmol kg^{-1}	Fe_{am-ox} mmol kg^{-1}	P_{ox} mmol kg^{-1}	P_{total}* mg kg^{-1}	$P_{saturation}$** %	DOC mgC L^{-1}
Esposende	30	4.9±0.4	4.2±0.7	3.7±0.6	53.4±13.5	39.7±11.2	6.9±2.5	152±43	68±17	39±18	1345±469	17.7±6.8	6.0±1.5
Estarreja	39	5.0±0.7	2.8±0.5	2.2±0.4	37.7±24.0	53.6±20.5	8.7±3.6	62±26	50±33	23±10	1280±511	22.1±9.7	5.4±1.4
Lousal and Caveira	42	5.5±1.0	3.4±1.3	3.0±1.1	49.0±19.5	41.1±15.8	9.9±4.2	26±12	66±59	13±15	809±640	18.4±20.7	5.5±3.0
Aljustrel	25	5.7±1.1	2.4±0.9	2.1±0.8	3.3±4.5	75.4±3.0	21.3±4.7	34±17	70±55	7.1±4.9	572±243	9.3±7.2	4.5±1.1
all samples (median value)	*136*	*5.1*	*2.9*	*2.5*	*41.1*	*50.0*	*9.0*	*42*	*47*	*17*	*855*	*15.0*	*5.1*

*P_{total}=aqua regia extracted P concentrations; **$P_{saturation}$=(P_{ox}/(Al_{am-ox}+Fe_{am-ox}))x100 (van der Zee and van Riemsdijk, 1988; Koopmans et al., 2006)

As shown in Table 5.1, the pH values of the 136 samples varied from 3.1 to 7.0 (acidic to neutral), with a median of 5.1. Agricultural soil samples from Esposende, predominantly Cambisols from granitic areas were those showing the lowest mean pH (4.9) while Aljustrel samples had the highest mean pH values (5.7) (Table 5.1). This agrees with past surveys showing that agricultural soils in Portugal are generally predominantly acidic (Horta and Torrent, 2007a) and that the pH of Portuguese soils is generally lower in the northern areas of the country (pH ≤4.5) compared to the south (pH 5-8) (APA, 2007). Nevertheless, unusually low pH values (3.1-3.7) were observed in three samples from Aljustrel and in one sample from Caveira (pH 3.6). These areas are known to be affected by acid mine drainage (Cardoso Fonseca and Ferreira da Silva, 2000; Luis et al., 2009) which may explain the low pH. Acid mine drainage is formed when pyrite (FeS_2) and other metal sulphides are exposed to oxygen and water and subjected to oxidising conditions resulting in the production of sulphuric acid (low pH), sulphates and dissolved metal ions (Ziemkiewicz et al., 1997). Highly variable pH values were observed in soils collected around mining sites of the Iberian Pyrite Belt in Spain showing both soils affected by the acid mine drainage and soils protected from acid discharges (Fernández-Caliani et al., 2009). Such large pH variations were also observed in the Aljustrel area in our study (from 3.1 to 7.0).

Carbon contents of soils samples varied from low to medium. Total carbon % values varied between 1.2 and 6.0 % (median=2.9) while Org C % varied in the range of 1.1-5.3 % (median=2.5). A considerable fraction of the total carbon content is in the form of organic carbon, in the entire dataset. The highest total and Org C % were observed in samples from Esposende and Lousal and the lowest values were obtained for soils from Aljustrel and Estarreja (Table 5.1). The differences observed in Org C % across the different sampling areas and the higher values observed in samples from Esposende and Lousal are most likely related with variations in the intensity of agricultural practices and uneven uses of manure for soil fertilization. The organic carbon contents obtained in this study are slightly higher than those found in agricultural and mining soils around abandoned mine sites of the Iberian Pyrite Belt in South-West Spain (Fernández-Caliani et al., 2009). However, the majority of these soils have relatively lower organic matter contents than those commonly found in arable and grassland soils from temperate regions such as the Netherlands (Römkens et al., 2004).

Variable soil textures were obtained for the soil set: sand, loamy sand, sandy loam, loam, silt loam, and silty clay loam. Samples from Esposende showed a mean sand content of 53.4 % (Table 5.1) and were generally sandy loam. In Estarreja the silt loam texture was the most common. At Lousal and Caveira a combination of silt loam, sandy loam, sand and loam soils occurred. As shown in Table 5.1, highest Clay % were observed in soils from Aljustrel (mean=21.3 %) where almost all soils had silt loam texture. Small size soil particles generally exhibit higher reactivity (Ajmone-Marsan et al., 2008).

The "active" forms of Al and Fe (which occur as amorphous hydroxides and are bond to organic matter) extracted as oxalates from soil samples as well as the phosphorus measured in the ammonium oxalate-oxalic acid extract are also shown in Table 5.1. Amorphous Al and Fe oxides varied in the range of 7.9-240 mmol kg^{-1} and 11-183 mmol kg^{-1}, respectively. Median contents of $Al_{am\text{-}ox}$ and $Fe_{am\text{-}ox}$ in the soil set are 42 and 47 mmol kg^{-1}, respectively (Table 5.1). The $Al_{am\text{-}ox}$ are present in relatively higher concentrations in samples from Esposende (range: 26-240 mmol kg^{-1}; median=152 mmol kg^{-1}) than in the remaining sampling areas (Figure 2, Annex II). The highest $Fe_{am\text{-}ox}$ values (~180 mmol kg^{-1}) were observed in samples from the surroundings of Caveira and Aljustrel mining areas. The $Fe_{am\text{-}ox}$ contents of soils in the entire dataset and $Al_{am\text{-}ox}$ concentrations observed in Esposende and in Estarreja are relatively higher than those reported in a study of Portuguese agriculture acid soils (Horta and Torrent, 2007a,b). The ranges of Fe and particularly Al amorphous oxides contents found in our study were also larger than the concentration ranges observed in soils taken from all over the Netherlands, both in polluted and non-polluted areas (Bonten et al., 2008).

Total P concentrations varied between 160 and 2700 mg kg^{-1} (median=855 mg kg^{-1}) while P_{ox} varied between 0.27 and 78 mmol kg^{-1} (median=17 mmol kg^{-1}) (Table 5.1). Both total P and P_{ox} values obtained were also relatively higher than those obtained in a study of agricultural Portuguese acid soils (Horta and Torrent, 2007a,b). It has been observed that the uneven application of P fertilizers in Portuguese soils during the last decades resulted in wide and varied ranges of soil P contents (Horta and Torrent, 2007a). Highest values of total P and P_{ox} were observed in the areas of Esposende, Estarreja and Lousal (Table 5.1). Soils from Lousal and Caveira considerable differ for this parameter: total P and P_{ox} in Lousal were between 2700-2700 mg kg^{-1} (median=550 mg kg^{-1}) and 1.2-57.8 mmol kg^{-1} (median=8.5 mmol kg^{-1}), respectively; while in Caveira these values were between 290-800 mg kg^{-1} (median=550 mg kg^{-1}) and 0.27-16.0 mmol kg^{-1} (median=5.3 mmol kg^{-1}).

Since Al and Fe oxides are major phosphate-sorbing surfaces in soils, the degree of P saturation of a soil can be calculated with respect to its contents of amorphous Al and Fe oxides (van der Zee and van Riemsdijk, 1988; Koopmans et al., 2006). Values of P saturation for these soils are shown in Table 5.1 and were found to vary between 0.46 and 77.8% with a median value of 15.0%. These results are comparable to those obtained for other agricultural Portuguese acid soils (Horta and Torrent, 2007a,b). Highest values of P saturation were observed in Esposende, Estarreja and Lousal areas indicating that in these soils the competition for sorption sites between phosphate ions and anionic contaminants such as As may be higher.

The DOC concentrations (0.01 M $CaCl_2$ extracts) obtained for these samples are also shown in Table 5.1. These were found to be rather low (range: 2.0-17 mg C L^{-1}; median= 5.1 mg C L^{-1}) compared to the values commonly found in agricultural soils (Römkens et al., 2004; Bonten et al., 2008), reflecting the also relatively low organic carbon contents of the soils in this dataset. The DOC values for soils from Esposende are slightly higher than those from other areas (Figure 2, Annex II) also in accordance with the relatively higher organic carbon concentrations of these agricultural soils. The lowest DOC concentrations were observed in soils from Aljustrel (Figure 2, Annex II). Soil extracts from Lousal show slightly higher DOC values than soils from Caveira.

A summary of results of analysis of the chemical composition (expressed as oxides) of soils from the dataset performed in a total of 50 samples is shown in Table 5.2.

The median contents of major elements in soils (oxides form) in decreasing order was dominated by SiO_2 (67.9 %), followed by Al_2O_3 (10.8 %), Fe_2O_3-T (4.3 %), K_2O (1.9 %), TiO_2 (0.70 %), Na_2O (0.69 %), MgO (0.68 %), CaO (0.27 %), P_2O_5 (0.16 %) and MnO (0.055 %) (Table 5.2). These results indicate the siliceous character of the soil set. Particularly high levels of silica were found in soils from Estarreja and in certain samples from Lousal. The highest mean values of Al_2O_3 % were found in samples from Esposende (16.5%) while the highest values of Fe_2O_3-T % were obtained in samples from Lousal & Caveira and Aljustrel (7.9 % and 6.9 %, respectively) (Table 5.2). A significant ($p<0.01$) negative correlation was observed between the contents of SiO_2 and the contents of Al_2O_3 ($r= -0.91$) and of SiO_2 and Fe_2O_3-T ($r= -0.75$). The percentages of Al_2O_3 and Fe_2O_3-T are positively correlated ($r= 0.50$, $p<0.01$). Comparing these values with those obtained for agricultural soils around the Riotinto mining area in Spain (Iberian Pyrite Belt) (López et al., 2008) it was observed that: (a) soils from Estarreja and from a few sites in Lousal show considerably higher levels of silica than soils from around mining areas in both our set and in the Spanish dataset; (b) soils from Esposende show higher Al_2O_3 contents than samples from Estarreja and from mining areas in both datasets; (c) total Fe oxides contents observed in Lousal & Caveira and Aljustrel areas are comparable to those obtained around the Riotinto mining area and higher than those from Esposende and Estarreja; (d) finally, with the exception of soils from Aljustrel that show MnO percentages similar to those from the Spanish dataset, all our samples show relatively lower Mn oxides contents. In areas of higher contents of Al_2O_3 and Fe_2O_3-T (and lower SiO_2) an higher number of soil sorption sites in likely to occur since it has been reported that there is a positive relationship between soil sorption capacity and the ratio of (Al+Fe):Si of colloid surfaces (Shen, 1999).

Mineralogical analysis of soil from areas sampled may provide some further insight into the chemical composition of samples. According to past studies, the massive sulphides of Caveira and Lousal mining areas are composed predominantly by pyrite (FeS_2), together with minor chalcopyrite ($CuFeS_2$), galena (PbS), sphalerite (ZnS) (Ferreira da Silva et al., 2005). Pyrrhotite (FeS), marcasite (FeS_2), bournonite ($CuPbSbS_2$), tetrahedrite ($(Cu_2Ag_2FeZnHg)_3(SbAs)_2S_6$), arsenopyrite (FeAsS), cobaltite (CoAsS), magnetite (Fe_2O_4), were also observed in Caveira and Lousal as secondary minerals (Ferreira da Silva et al., 2005). Certain sulphates minerals including melanterite ($FeSO_4.7H_2O$), alunite ($KAl_3(SO_4)_2(OH)_6$ and anglesite ($PbSO_4$) and other minerals such as quartz, sericite and chlorite ($(Fe,Mg, Al)_6(Si,Al)_4O_{10}(OH)_8$ can also be found in these areas. Interstitial chalcopyrite ($CuFeS_2$), spharelite (Zn,Fe)S), galena (PbS), arsenopyrite and minor sulfossalts were observed in the massive pyrite ore of Aljustrel (Luis et al., 2009).

Table 5.2: Summary of chemical composition of soil samples at the different sampling areas expressed as oxides (mean ± standard deviation) and median value obtained for all samples together

Sampling area	**Nº samples (n)**	**SiO_2 %**	**Al_2O_3 %**	**Fe_2O_3-T %**	**MnO %**	**MgO %**	**CaO %**	**Na_2O %**	**K_2O %**	**TiO_2 %**	**P_2O_5 %**
Esposende	7	62.3±7.5	16.5±4.5	4.2±1.4	0.041±0.007	0.51±0.17	0.31±0.12	0.75±0.26	3.7±1.6	0.72±0.11	0.31±0.07
Estarreja	15	80.1±12.1	8.3±6.2	1.9±1.6	0.025±0.016	0.27±0.28	0.21±0.08	0.57±0.19	2.1±0.8	0.28±0.20	0.18±0.08
Lousal and Caveira	18	64.6±13.3	12.4±5.9	7.9±4.2	0.087±0.051	0.84±0.50	0.74±1.08	0.92±0.89	2.4±1.3	0.72±0.25	0.20±0.16
Aljustrel	10	69.8±5.3	11.2±2.0	6.9±2.4	0.17±0.11	1.1±0.3	0.76±0.53	1.1±0.4	1.4±0.4	1.3±0.7	0.10±0.05
all samples (median value)	*50*	*67.9*	*10.8*	*4.3*	*0.055*	*0.68*	*0.27*	*0.69*	*1.9*	*0.70*	*0.16*

A significant inverse correlation ($p<0.01$) was observed between the Org C % and the clay % ($r = -0.35$) suggesting organic carbon enrichment is lower in fine-textured soils such as those from Aljustrel. This is probably due to the fact that the most intensive agricultural practices were observed in the sampling areas of Esposende and in certain sites in the surroundings of Lousal and therefore it is possible that these soils are subject to higher fertilisation and manure application loads. The amorphous Al oxides contents were positively correlated with Org C and P contents at the $p<0.01$ level (r=0.51 and 0.62, respectively). As expected, a significant positive correlation ($p<0.01$) between soil organic carbon contents and DOC values was observed (r=0.53).

5.3.2 Total pools of PTE's

A summary of the total pools of PTE's is shown in Table 5.3. The values of "background" elemental concentrations for Portuguese soils are also shown in Table 5.3 for comparison purposes. The "background" values shown here are the 95th percentiles (P95) of "natural" topsoil concentrations reported for Portugal by Inacio *et al.* (2008).

Further details on the distribution of total pools of PTE's is shown in Annex II (Figure 3). The elemental content of the soil samples showed a considerable degree of variation, with many elements exhibiting large ranges extending far beyond the mean and showing a non-normal distribution (Kolmogorov–Smirnov test). These deviations from normality are often an indication of anthropogenic soil contamination. Similarly to soil properties, median values will be used to evaluate the central tendency of the entire dataset.

Table 5.3: Summary of total pools of PTE's in soil samples from this study and comparison with elemental concentrations of Portuguese "natural" soils as determined by Inacio *et al* (2008)

				percentiles							**"natural" soils***
	n	**mean**	**range**	**5%**	**10%**	**25%**	**median**	**75%**	**90%**	**95%**	**P95 values****
Hg (mg kg^{-1} dw)											0.080
total	136	7.1	0.013-98	0.030	0.038	0.077	0.34	1.5	26	54	
Cd (mg kg^{-1} dw)											n.a.
aqua regia	117	0.67	0.10-3.7	0.10	0.10	0.20	0.40	1.0	1.5	2.2	
Zn (mg kg^{-1} dw)											107
aqua regia	134	227	17-1194	40	52	80	137	310	532	606	
Cu (mg kg^{-1} dw)											46
aqua regia	134	205	7.4-7635	18	22	34	80	236	378	562	
Pb (mg kg^{-1} dw)											45
aqua regia	134	445	10-11546	17	20	35	58	224	856	2324	
Ni (mg kg^{-1} dw)											52
aqua regia	134	18	4.5-45	6.8	7.5	9.4	16	24	35	38	
Co (mg kg^{-1} dw)											27
aqua regia	134	11	0.50-49	3.1	3.4	4.4	8	18	23	28	
Mn (mg kg^{-1} dw)											1225
aqua regia	134	514	58-2439	80	118	168	304	730	1164	1406	
Fe (% dw)											4.8
aqua regia	132	2.7	0.32-10	1.1	1.3	1.6	2.1	3.3	4.8	5.7	
As (mg kg^{-1} dw)											48
aqua regia	134	218	6.3-2189	9	16	24	54	167	685	1336	
U (mg kg^{-1} dw)											n.a.
aqua regia	131	1.9	0.20-13	0.30	0.42	0.60	1.4	2.3	4.5	5.6	
Cr (mg kg^{-1} dw)											80
aqua regia	130	23	2.0-70	5.6	7.0	10	17	31	49	59	
Ba (mg kg^{-1} dw)											141
aqua regia	134	86	16-599	30	35	44	62	98	160	228	
Al (% dw)											3.3
aqua regia	134	1.4	0.42-3.4	0.72	0.82	1.0	1.2	1.6	2.1	2.7	
Sb (mg kg^{-1} dw)											n.a.
aqua regia	112	12	0.10-220	0.30	0.33	0.60	1.4	8.2	31	71	
Se (mg kg^{-1} dw)											n.a.
aqua regia	54	4.0	0.50-19	0.60	0.60	0.90	1.2	6.1	14	18	
Li (mg kg^{-1} dw)											n.a.
aqua regia	52	16	2.8-38	6.0	7.6	9.6	14	17	29	33	
Be (mg kg^{-1} dw)											n.a.
aqua regia	35	0.65	0.20-1.5	0.20	0.26	0.37	0.50	0.90	1.3	1.4	
Mo (mg kg^{-1} dw)											n.a.
aqua regia	23	0.92	0.50-1.6	0.50	0.54	0.70	0.90	1.0	1.4	1.6	
B (mg kg^{-1} dw)											n.a.
aqua regia	23	6.8	3.2-16	3.2	3.6	4.7	5.2	7.2	14	15	

**Values for "natural" soils were obtained from the "Soil Geochemical Atlas of Portugal" (Inacio et al., 2008). In this study, sampling sites were selected to represent "natural" soils (locations affected by pollution and arable soils were avoided). Results shown here refer to values obtained for 165 topsoil samples (upper mineral horizon, A; fraction <2mm).*

***Values shown refer to the 95th percentile of element contents extracted for1h with a mixture of HCl–HNO3–H2O (3-2-1) at 95 °C. Extracts were analysed by ICP-AES (and cold vapour for Hg).*

n.a.: not available, since most of the values were below the limit of quantification of the method used.

As shown in Table 5.3, total Hg concentrations obtained for the soil set (range: 0.013-98 mg kg^{-1}; median= 0.34) are significantly higher than those values determined by Inacio et al. (2008) (Hg extracted by *aqua regia*, P95: 0.080 mg kg^{-1}) showing severe Hg contamination in certain samples from our dataset and indicating that these soils are subject to relevant anthropogenic impacts. These results are in accordance with those from studies previously published reporting severe Hg contamination in Estarreja (Inacio et al., 1998; Cachada et al., 2009; Reis et al., 2009) and Lousal areas (Ferreira da Silva et al., 2005).

The results obtained for aqua regia extracted Ni, Co, Mn, Fe, Cr, and Al did not considerably differ from the values reported for "natural" Portuguese soils (Inacio et al., 2008) indicating that no significant anthropogenic contamination by these elements was observed for soils included in our set. Observed Ba concentrations only slightly exceeded the contents determined by Inacio et al (2008) (Table 5.3). By contrary, significantly higher values of As, Zn, Cu and Pb were obtained in this study in comparison with "natural" soils (Table 5.3). A past study (Ferreira da Silva et al., 2005) reported ranges of 164-2068, 31-1859, 103-3324 and 74-2831 mg kg^{-1} and median concentrations of 562, 339, 471 and 1070 mg kg^{-1} for As, Zn, Cu and Pb respectively for soils impacted by the mining activities, from the surroundings of the Lousal. Our results (Table 5.3) show lower median contents and broader concentrations ranges for these four elements indicating that the soil set includes both less and more contaminated samples than those analysed by Ferreira da Silva et al. (2005).

For Cd the concentrations observed for the soil set (Table 5.3) are similar to those found in contaminated soils from Lousal by Ferreira da Silva et al. (2005) who reported a range of Cd values of 0.2-3.6 mg kg^{-1}(n=57; median 0.4 mg kg^{-1}). A previous study developed in Aljustrel (Alvarenga et al., 2008) reported mean Cd values of 2.6 mg kg^{-1} (n=3) for those soils, also in accordance with the range of measured Cd concentrations in the present study. Regarding the remaining PTE's, only values for Mo and Sb are available for comparison from past studies developed in these study areas. Median concentrations obtained for these two elements (Table 5.3) are lower than those from Ferreira da Silva et al. (2005) although the concentration range of Sb is larger in the present study.

The Portuguese legislation (Decreto-Lei 276/2009) includes soil quality standards only for agricultural soils which are subject to sewage sludge application, and in this case only for the elements Cd, Cu, Ni, Pb, Zn, Hg and Cr (for $pH \leq 5.5$; $5.5 < pH \leq 7.0$; and $pH > 7.0$). Considering the lowest pH (5.5), it can be noticed that 75 % of Cd and Ni values obtained in our study are below the legislation limits (1 and 30 mg kg^{-1}, respectively) and 90 % of the Cr values are below the limit of 50 mg kg^{-1}. However almost 50 % of the soils show Zn and Hg concentrations higher than the respective limit values (150 and 1 mg kg^{-1}) and for Cu and Pb over 50 % of the results shown in Table 5.3 are considerably higher than the limit indicated by the legislation, which is 50 mg kg^{-1} for both elements. No limit values for the remaining PTE's analysed in this study were available from the Portuguese legislation.

Boxplots of the concentrations of 17 PTE's clustered by different sampling areas are shown in Annex II (Figure 4). Beryllium, Li, and B were not included since the number of results above detection limits per area was quite variable and did not allow an effective comparison of results.

Soils from Esposende showed the highest Al and U contents from the dataset (Annex II, Figure 4). The presence of Al in Esposende soils is likely to be geogenic since high background Al contents were also reported in Cambisols from granitic areas in the North-West of Portugal by Inácio et al. (2008). Similarly, the distribution of U contents in Portuguese soils is related with the geological background and with the presence of ore deposits (Inácio et al., 2008). Geogenic concentrations of Co, Cr, Ni in Cambisols from granitic areas from North-West Portugal are naturally low (Inácio et al.,

2008) which explains the low contents of these elements found in soils from Esposende. For the same reason, Cu contents in Esposende soils are also naturally low (Annex II, Figure 4), although the relatively high concentrations (100-500 mg kg^{-1}) found in four of these samples reflected the impact of agricultural activities in Esposende soils. It has been recognised that the use of Cu fungicides to control cultivated plant diseases is known to have led to long-term accumulation of Cu in the surface of some agricultural soils throughout the world (Brun et al., 1998).

Inácio et al. (2008) reported that the Western region of Portugal underlain by sedimentary detritic rocks, where Arenosols and Podzols develop (such as the Estarreja area), is naturally impoverished in most elements. However, soil samples from Estarreja used in our study showed the highest values of Hg, As, Cd and Se and relatively high concentrations of Zn, Cu, Pb and Ba (Annex II, Figure 4) indicating an anthropogenic origin for these elements in these soils. As stated before, soil Hg contamination in Estarreja in relation to the presence of a chlor-alkali plant located at the Chemical Complex has been reported by other studies (Inacio *et al.,* 1998; Costa and Jesus-Rydin, 2001; Cachada *et al.*, 2009; Reis *et al.*, 2009). The high levels of As, Cd and of other PTE's observed in Estarreja soils (Annex II, Figure 4) are also associated with the industrial activities at the Chemical Complex. Until 1975 liquid effluents highly contaminated with As, Hg, Zn, Pb and other elements were directly discharged into manmade streams around the Complex and solid pyrite wastes and Hg contaminated sludge have been stored within the Chemical Complex area (Costa and Jesus-Rydin, 2001).

Soils from Aljustrel showed the highest median concentrations of Ni, Co and Cr from this dataset. The highest values of Fe and Mn were also observed in soils from mining areas (Aljustrel, Lousal & Caveira) (Annex II, Figure 4). Naturally high concentrations of Ni, Co and Cr were found to be related to Leptosols and Vertisols developed from metasediments and mafic-ultramafic rocks such as those that can be found in the Lousal & Caveira and Aljustrel areas (Inácio et al., 2008). The presence of Fe and Mn is also related with the geological background and particularly with the higher clay contents of these soils.

The highest levels of Cu, Pb, Zn and Sb were observed in soils from Lousal & Caveira. Relatively high concentrations of As, Hg and to a less extend Cd were also found in both Lousal & Caveira and Aljustrel areas (Annex II, Figure 4). The erosion of mine tailing deposits and the formation of acid mine drainage in the mining areas can explain the high contents of these elements in these soils. Ferreira da Silva et al. (2005) reported that sulphides are still abundant at the surface around the Lousal mine and are likely to undergo further sulphur oxidation for a long time. Oxidation of the dominant sulphide minerals (pyrite, pyrrhotite, chalcopyrite, and arsenopyrite) gives rise to a great variety of secondary minerals on these two sites such as jarosite, melanterite, and scorodite among others. The solubility of these minerals is variable but the release of potentially toxic elements such as Cu, Pb, Zn, As into surrounding soils may occur (Ferreira da Silva et al., 2005). The waste tailings that can be found in Aljustrel mine are mainly composed by slag from Roman times, pyrite ore (blocks and brittle massive pyrite ore) and volcano-sedimentary complex host rocks (Luis et al., 2009). High levels of Fe, Cu, Pb, Zn, Ag, Sb, Hg, Se, Co, Au and Cd were observed in the massive pyrite ore (Luis et al., 2009). High concentrations of Au, Pb, Ag, Fe, Sb, Bi, Se, Cu, Zn and Mo were found in the roasted pyrite ore while large contents of Pb, Cu, Zn, Fe, As and Sb can be observed in the Roman slag (Luis et al., 2009). The erosion of these tailing deposits and the formation of acid mine drainage considerably affected the quality of sediments and waters from streams around the mining site by altering their pH, conductivity and potentially toxic elements concentrations (Luis et al., 2009). As observed by the low pH values of certain soil samples collected around mining areas in our study and the high contents of certain PTE's, the physical chemical characteristics of soils have also been affected.

5.3.3 Reactive pools of PTE's: soil extraction with 0.43 M HNO_3

The concentrations of PTE's extracted by 0.43 M HNO_3 and the extraction ratios in relation to respective total or *aqua regia* contents are shown in Table 5.4

Table 5.4: Concentrations of PTE's extracted by 0.43 M HNO_3 and ratios in relation to respective total or *aqua regia* (AR) contents

							percentiles						**ratio (%)**	
	n	**mean**	**range**	**5%**	**10%**	**25%**	**median**	**75%**	**90%**	**95%**		**mean**	**median**	**range**
Hg (µg kg^{-1} dw)														
0.43 M HNO_3	136	7.3	0.14-467	0.42	0.51	0.79	1.5	2.3	8.8	31	**HNO_3/Total**	0.96	0.40	0.012-7.1
Cd (mg kg^{-1} dw)														
0.43 M HNO_3	117	0.26	0.027-1.4	0.040	0.049	0.071	0.12	0.40	0.67	0.90	**HNO_3/AR**	40	38	3.0-100
Zn (mg kg^{-1} dw)														
0.43 M HNO_3	134	36	1.8-519	2.8	4.3	7.8	15	42	105	120	**HNO_3/AR**	13	12	1.4-46
Cu (mg kg^{-1} dw)														
0.43 M HNO_3	134	42	1.2-1492	3.7	4.1	6.6	21	40	87	109	**HNO_3/AR**	23	20	5.0-95
Pb (mg kg^{-1} dw)														
0.43 M HNO_3	134	62	1.5-581	3.9	5.3	8.9	16	34	182	444	**HNO_3/AR**	27	27	0.40-57
Ni (mg kg^{-1} dw)														
0.43 M HNO_3	134	1.3	0.22-8.4	0.30	0.35	0.53	0.85	1.6	3.2	4.5	**HNO_3/AR**	7.3	6.1	0.95-44
Co (mg kg^{-1} dw)														
0.43 M HNO_3	134	2.1	0.070-14	0.23	0.41	0.52	1.4	3.1	5.1	6.4	**HNO_3/AR**	17	15	3.8-63
Mn (mg kg^{-1} dw)														
0.43 M HNO_3	134	130	6.2-600	15	21	35	92	186	278	391	**HNO_3/AR**	26	23	2.5-69
Fe (% dw)														
0.43 M HNO_3	132	0.093	0.0070-0.33	0.017	0.029	0.051	0.080	0.11	0.17	0.22	**HNO_3/AR**	4.7	3.6	0.23-22
As (mg kg^{-1} dw)														
0.43 M HNO_3	134	23	0.037-384	0.24	0.32	0.80	2.8	9.2	38	228	**HNO_3/AR**	8.0	6.2	0.11-24
U (mg kg^{-1} dw)														
0.43 M HNO_3	131	0.54	0.044-4.1	0.08	0.10	0.19	0.27	0.68	2.5	9.4	**HNO_3/AR**	28	25	2.4-77
Cr (mg kg^{-1} dw)														
0.43 M HNO_3	130	1.0	0.072-8.9	0.14	0.19	0.39	0.62	1.4	2.1	3.0	**HNO_3/AR**	6.1	4.1	0.28-25
Ba (mg kg^{-1} dw)														
0.43 M HNO_3	134	32.0	2.3-208	4.8	6.2	10	20	46	79	91	**HNO_3/AR**	35	32	4.4-88
Al (% dw)														
0.43 M HNO_3	134	0.10	0.016-0.38	0.023	0.032	0.046	0.066	0.12	0.25	0.30	**HNO_3/AR**	6.9	5.8	1.5-22
Sb (mg kg^{-1} dw)														
0.43 M HNO_3	112	0.16	0.0060-1.4	0.011	0.016	0.026	0.048	0.090	0.67	0.87	**HNO_3/AR**	3.9	3.6	0.095-12
Se (mg kg^{-1} dw)														
0.43 M HNO_3	54	0.14	0.016-1.2	0.020	0.022	0.034	0.060	0.14	0.38	0.61	**HNO_3/AR**	5.6	3.8	0.33-21
Li (mg kg^{-1} dw)														
0.43 M HNO_3	52	0.17	0.010-1.0	0.018	0.043	0.060	0.095	0.25	0.40	0.46	**HNO_3/AR**	1.2	0.87	0.036-6.2
Be (mg kg^{-1} dw)														
0.43 M HNO_3	35	0.20	0.072-0.85	0.072	0.074	0.095	0.14	0.21	0.45	0.64	**HNO_3/AR**	33	33	10-67
Mo (mg kg^{-1} dw)														
0.43 M HNO_3	23	0.027	0.011-0.061	0.011	0.011	0.016	0.028	0.035	0.051	0.061	**HNO_3/AR**	2.4	2.3	0.21-5.9
B (mg kg^{-1} dw)														
0.43 M HNO_3	23	2.5	0.060-11	0.11	0.32	0.56	0.95	3.7	8.4	11	**HNO_3/AR**	22	13	1.8-82

Median reactive contents are low for most elements although high reactive concentrations were found for Cu, Pb, Zn and As (Table 5.4). For both Cu and Pb approximately 20% of the number of samples extracted by HNO_3 surpass the P95 *aqua regia* contents of Portuguese “natural” soils reported by Inacio *et al.* (2008) for these two elements. In addition, more than 5 % of the samples from the set show reactive Zn and As contents that are higher than the “pseudo-total” P95 contents found in the same “natural” soils. The median ratio of PTE’s contents extracted by HNO_3 was <38 % for all elements and decreased in the order Cd> Be> Ba> Pb> U> Mn> Cu> Co> B> Zn> As> Ni> Al> Cr> Se> Fe = Sb> Mo> Li> Hg. Nevertheless, the range of ratios obtained for the reactive element pools in the different samples was quite variable (from 0.012 up to 100 %) and rather high values were found for certain PTE’s such as Cd, Cu, Pb, U, Ba, B, Co, Mn and Be in some of the samples. Although the HNO_3: total ratios of Cd, Cu, Pb, Zn, Ni and Cr obtained in the present study were somewhat smaller than those observed for soils in the Netherlands (Römkens et al., 2004) as well as for paddy fields in Taiwan (Römkens et al., 2009), the ratios of extraction were found to decrease in the same order for the various elements in the different studies.

Similarly to what was observed by (Römkens et al., 2004; 2009a), Cd was the most reactive PTE in our soils (Table 5.4). A high Cd extractability ratio (up to 94%) by a diluted 0.5 M HNO_3 solution for acidic soils was also reported for Belgium soils by Meers *et al.* (2007a).

Copper and Pb are often reported as metals with strong affinity with soil constituents such as organic matter (McLean and Bledsoe, 1992; Römkens et al., 2009a). These two PTE’s proved to be considerably reactive in our soil set as well (Table 5.4). By contrary, Zn, Ni and Sb showed relatively low reactivity. Other authors have reported similar findings for Zn and Ni in association with metal fixation and long-term immobilization in soils by clay minerals (Buekers et al., 2008; Römkens et al., 2009a). Relatively low reactivity was also observed for Cr, As, Se and Mo (Table 5.4). According to McLean and Bledsoe (1992), contaminants with anionic forms such as As, Cr, Se and Mo can be strongly retained in soils through binding mechanisms with Fe, Al and Mn oxides.

Mercury was the element with the lowest 0.43 M HNO_3 extraction ratio in our study (Table 5.4). These low levels of reactivity can be explained by the presence of rather immobile forms of Hg in these soils but to some extent may also be related with the type of chemical extraction procedure used. To assess the biogeochemical reactivity of Hg is generally a more complex task than for other cationic metals particularly due to the several Hg-species that may occur in the geochemical cycle of this element. The so-called "reactive" species which are also the bioavailable and toxic forms are quite a complicated group which may include Hg^{2+}, $Hg(OH)_2$, $HgCl_2$, $HgBr_2$, Hg^{2+} complexes with organic acids and methylmercury among other species (Issaro et al., 2009). It has been observed that different Hg species have different environmental behaviour and that Hg compounds in the environment tend to convert to an ambient Hg speciation profile determined by the specific biogeochemical properties of the receiving matrix (Bloom et al., 2003). Moreover, according to the HSAB (Hard and Soft Acids and Bases) principle, the 'classic' trace metals (Cu, Co, Ni, Al, Cd, Zn) have as a binding partner hard ligands with O-containing functional groups. But for Hg (a soft metal), clay minerals (both their surface and interior lattice structure) and Fe/Mn-(hydr)oxides are generally not significant binding partners when in competition with the much stronger soft ligands such as organic matter and S-containing ligands (Wallschläger et al., 1998; Issaro et al., 2009). Moreover, in the case of Hg even the weakest complexes can be so strong that they hardly can be considered "reactive" in the sense of its "classical" definition as it is being applied in our study. Nevertheless, the analogy to other trace metals can be kept up by considering complexes with hard ligands, such as oxygen-containing surface groups on clay minerals, oxides, and amorphous oxides as weak and potentially reversible

(Wallschläger et al., 1998) and thus reactive. By applying the 0.43 M HNO_3 extraction and by comparison with the observations of Wallschläger et al. (1998) we are breaking down weaker Hg complexes (particularly with organic matter) and/ or partially dissolving Hg associated to Fe/Mn-(amorphous)oxides, by acidification. Further assessments of organo-chelated Hg (such as Hg-humics), other Hg-complexes, methylmercury or total elemental Hg must include the use of other extractants or concentrated nitric acid solutions (in the case of elemental Hg) (Wallschläger et al., 1998; Bloom et al., 2003; Issaro et al., 2009).

Boxplots of the reactive concentrations of 16 PTE's clustered by different sampling areas are shown in Annex II (Figure 5). Beryllium, Li, B and Se were not included since the number of results above detection limits per sampling area was quite variable and did not allow an effective comparison of results. The highest reactive contents of U, Al, Fe and Cr were observed in soils from Esposende (Annex II, Figure 5). The presence of these elements in soils was already considered to be primarily geogenic. Relatively higher total Fe and Cr contents have been observed in soils from around the mining sites, but in those areas these elements are generally present in more immobile forms. By contrary, the highest reactive contents of Ni, Co and Mn, also predominantly geogenic contaminants, were found around the mining areas, particularly in Aljustrel (Annex II, Figure 5). Soils from Estarreja showed the highest reactive pools of As, Sb and Mo and relatively high reactive contents of Pb (Annex II, Figure 5). The contamination of soils from our dataset by these elements was considered of anthropogenic origin and particularly high of total pools of As had also been observed in Estarreja. At the Caveira site, Cardoso Fonseca and Ferreira da Silva (2000) have concluded that crystalline Fe oxides and also the sulphides (in minesoils) were the main bearing-phases of As in the immediate vicinity of the mine. In addition, Ferreira da Silva et al. (2005) and Cardoso Fonseca and Ferreira da Silva (2000) showed that stable forms of As (such as arsenopyrite, cobaltite and tetrahedrite) in minesoils samples of both Lousal and Caveira and arsenopyrite was also observed in the Aljustrel area (Luis et al., 2009). The presence of stable forms of As in the mining areas may explain the relatively lower contents of these elements extracted by the 0.43 M HNO_3 in these soils. By contrary the highest reactive pools of Cu (Aljustrel and Lousal&Caveira) and Pb (Lousal&Caveira) were observed in soils collected around the mining sites (Annex II, Figure 5). For other anthropogenic contaminants such as Cd and Zn the highest reactive contents were obtained in spatially varied soils from the dataset, particularly from Estarreja, Aljustrel and to a less extent, Lousal&Caveira (Annex II, Figure 5).

5.3.4 Variability of total and reactive pools of PTE's in Portugal: Factor Analysis

Results of Factor Analysis for log transformed data including key soil properties and total pools of PTE's are shown in Table 5.5. The communalities extraction values and the percentage of the total variance explained were also included. Clay is the parameter less contributing to the common variance of the set of measured variables since the extracted factors explain only 60 % of the variance of clay contents and over 70 % for the remaining parameters (Table 5.5). The five extracted Factors explain around 84 % of the total variance in the data, with Factor 1 and 2 containing 30 and 24 % of the total variance, respectively (Table 5.5). As shown in Table 5.5, high correlation coefficients were found between Cu, Pb, Hg, As, Se, Sb and Mo and Factor 1. A medium loading of Zn (0.53) also occurs in this Factor (although this element is more heavily loaded in Factor 3). This confirms a common origin for these elements (Cu, Pb, Hg, As, Se, Sb, Mo and to a less extent Zn). Factor 1 represents anthropogenic soil contamination. Regarding Factor 2, the variables with higher loadings were Co, Ni, Mn, Fe, Cr and to a less extent, clay, and this is embodying the geological nature of soils. The Cd, Zn, Ba (and to a lesser extent pH) distributions in this set are strongly associated with one another within Factor 3. The variance of these parameters for these samples is somewhat different from other "anthropogenic" PTE's characterized by Factor 1. The variables U and Al are the most clearly and heavily loaded in Factor 4, while organic carbon is the only parameter with high loading in Factor 5.

Table 5.5: Rotated matrix for log transformed data including key soil properties and total pools of PTE's (n=117). Percentage of variance explained and communalities are also included.

Rotated Matrix(a)						
	Factor					**Communalities**
	1	**2**	**3**	**4**	**5**	**Extraction**
pH	-0.41	0.34	**0.60**	-0.31	0.15	0.77
Log(Org C)	0.0005	-0.16	-0.09	0.23	**0.85**	0.80
Log (Clay)	0.09	**0.61**	-0.06	0.19	-0.43	0.60
Log (Cd)	0.27	0.02	**0.84**	0.02	-0.16	0.81
Log (Zn)	0.53	0.2	**0.69**	-0.15	0.04	0.83
Log (Cu)	**0.80**	0.33	0.25	0.06	0.19	0.85
Log (Pb)	**0.93**	0.1	0.05	-0.22	0.04	0.93
Log (Hg)	**0.81**	-0.18	0.29	-0.18	-0.24	0.85
Log (Ni)	-0.12	**0.89**	0.24	-0.02	-0.16	0.89
Log (Co)	0.02	**0.91**	0.25	-0.16	0.06	0.91
Log (Mn)	0.11	**0.86**	0.03	-0.21	0.04	0.80
Log (Fe)	0.48	**0.76**	-0.12	0.21	0.27	0.93
Log (As)	**0.89**	-0.14	0.29	-0.01	-0.16	0.93
Log (U)	-0.02	-0.62	-0.03	**0.71**	0.07	0.90
Log (Cr)	-0.18	**0.75**	0.25	0.04	-0.35	0.79
Log (Ba)	0.32	0.26	**0.72**	0.14	-0.06	0.72
Log (Al)	-0.31	0.08	0.01	**0.88**	0.2	0.92
Log (Se)	**0.82**	-0.06	0.31	0.04	-0.08	0.78
Log (Sb)	**0.89**	0.24	0.10	-0.33	-0.03	0.97
Log (Mo)	**0.80**	-0.05	-0.22	0.09	0.27	0.77
Total variance explained (%)	30.4	23.7	13.3	9.2	7.1	
Cumulative variance explained (%)	30.4	54.1	67.4	76.6	83.7	

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

(a) Rotation converged in 9 iterations.

Note: Highest loadings (>0.6) are shown in bold style.

To investigate the possibility of discriminating between sampling areas on the basis of their soil properties and total PTE's content, the factor scores for the five factors extracted have been computed and are shown in Figure 5.2(a). According to Figure 5.2(a), Factor 2 which includes clay and the geogenic elements Co, Cr, Ni, Fe and Mn allows distinguishing samples of Estarreja and Esposende (on the left side of factor score graphs) from samples collected in Lousal & Caveira and Aljustrel (on the right). The presence of Al and U is also of geological origin, but their distribution appears to differ from the other "natural" elements from Factor 2 since it is strongly affected by samples from Esposende (and to a less extent, some samples from Aljustrel and Estarreja) which show the highest factor scores in Factor 4 (Figure 5.2(a)). This shows that samples from specific areas differ in terms of their geological nature, with soils from Aljustrel and Lousal & Caveira showing higher contents of naturally occurring Co, Cr, Ni, Fe and Mn and Esposende samples showing higher geogenic concentrations of Al and U. It is clear that there is a group of samples from Lousal & Caveira and also a few samples from Estarreja and Aljustrel showing higher scores for Factor 1. These are the most contaminated samples by the anthropogenically emitted PTE's embodied in the Component 1 (Figure 5.2(a)). The clearest difference between Factor 1 and Factor 3 is that there is higher number of samples from Estarreja with high scores for Factor 3. This is likely to occur because the distribution of Cd, Zn and Ba varies differently in relation to the other anthropogenic elements. The effects of contamination arising from industrial activities in Estarreja appear to be distributed between Factor 3 and Factor 1 whereas the impact of mining is more clearly present in Factor 1. The effect of the distribution of organic carbon contents in this set is not clearly associated with the other variables and allows only distinguishing samples from Esposende and Lousal & Caveira (with higher organic carbon loads) from those collected at Estarreja and Aljustrel.

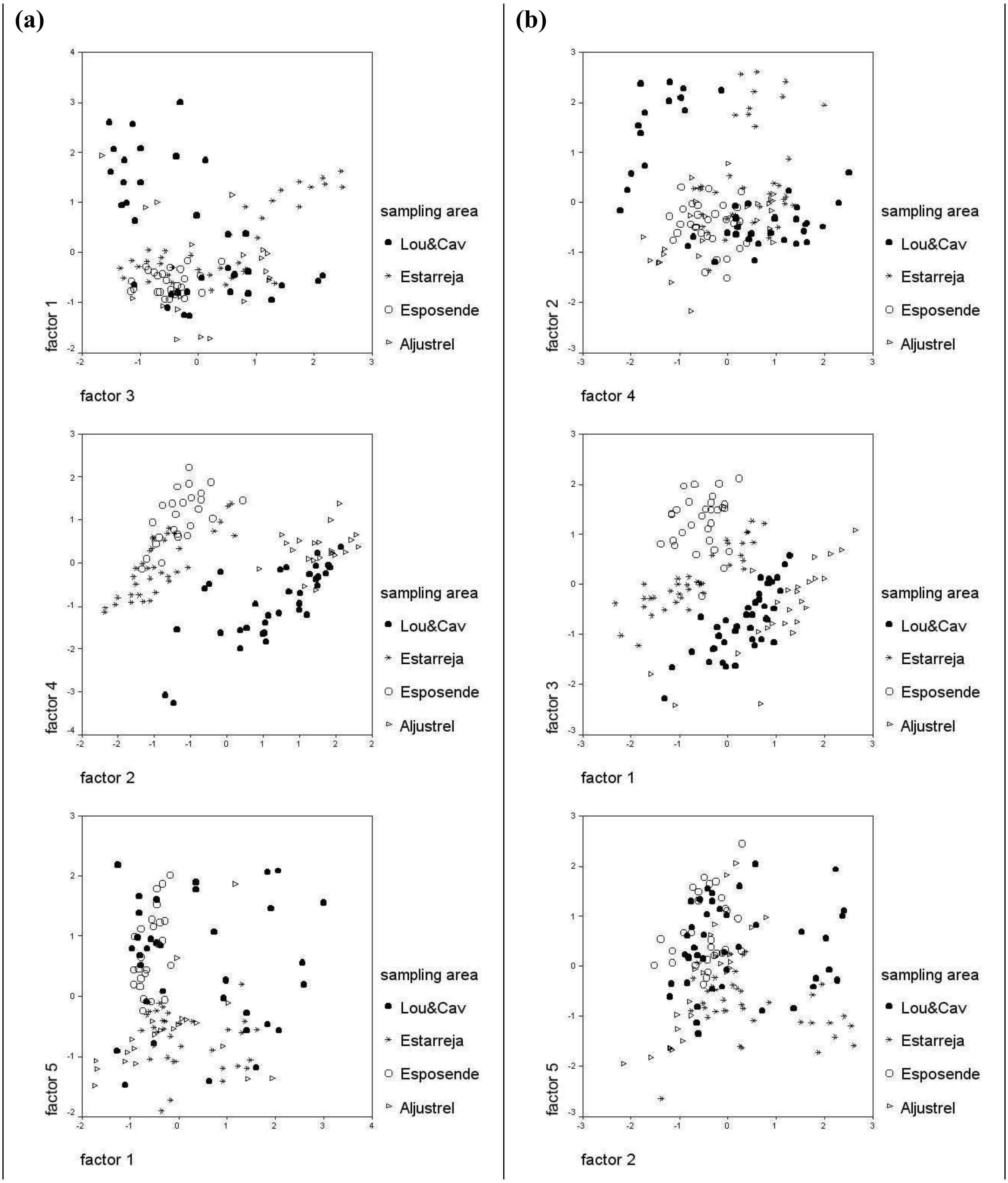


Figure 5.2: Factor scores for total (a) and reactive (b) pools of PTE's as obtained by factor analysis (rotated solution)

The variability of soil properties as well as the origin and distribution of PTE's in soils from the different sampling areas is summarized in Table 5.6. The variability of soil properties and soil chemical composition on these areas was also included to allow a more comprehensive interpretation of results. The (+) and (-) signs describe whether the values obtained for the different variables are higher or lower in that sampling area comparing to the other areas.

From Table 5.6 it is clear that most abundant geogenic elements in soils from the studied South-West areas of Portugal (Lousal & Caveira and Aljustrel) were Ni, Co, Cr, Mn and Fe while Al and U were the most abundant elements in soils from the North-West area of Portugal (Esposende) in relation to soil type and bedrock lithology. Soils from Esposende show relatively low levels of anthropogenic contaminants with the exception of a few sites where Cu levels indicate the use of Cu fungicides in agriculture. By contrary, Estarreja soils show relatively higher levels of anthropogenic contamination by Hg, As, Se, Cd, Zn, Ba and to some extent also Pb and Cu associated to the industrial activities. If the availability of such elements in Estarreja soils is high, considerable potential risks to human health and the environment may occur at these sites. It is therefore very important to further analyse the actual reactivity and availability of these contaminants in soils from Estarreja. It is interesting to notice that the origin of high levels of Cu, Pb, Hg, As, Se and to a less extent Sb and Mo in Estarreja soils appears to be associated with the anthropogenic contamination by the same elements around the mining areas. This must be related with the past use of pyrite (from mining activites) as an input for certain industrial processes in Estarreja (such as the production of sulphuric acid) and which originated highly contaminated liquid effluents and 150,000 ton of pyrite waste stored within the Chemical Complex of Estarreja (Costa and Jesus-Rydin, 2001). Soils from Lousal & Caveira are those showing highest levels of most geogenic elements as well as generally higher levels of anthropogenic soil contamination. The presence of high contents of potentially toxic elements in Aljustrel is mostly geogenic although high the levels of Cu, Pb, As, Sb, Mo, Cd and Zn in some of the samples are of anthropogenic origin.

Table 5.6: Soil properties, origin and distribution of total pools of PTE's in soils from the different sampling areas. The (+) and (-) signs describe whether the values obtained for the different variables are higher or lower in that sampling area comparing to the other areas.

			Esposende	**Estarreja**	**Lou&Cav**	**Aljustrel**
	Soil properties and soil chemical composition	**pH**	(-)	(-)	(-/+)	(+/-)
		OrgC	(+)	(-)	(+/-)	(-/+)
		Clay	(-)	(-)	(-/+)	(+)
		Al_ox	(+)	(+/-)	(-)	(-)
		Fe_ox	(-/+)	(-/+)	(+/-)	(+)
		P_ox	(+)	(+/-)	(+/-)	(-)
		SiO_2	(-)	(+)	(+/-)	(+/-)
		Al_2O_3	(+)	(-)	(-/+)	(-)
		Fe_2O_3-T	(-)	(-)	(+)	(+)
		MnO	(-)	(-)	(-/+)	(+/-)
Origin of total pools of PTE's in soils			**Esposende**	**Estarreja**	**Lou&Cav**	**Aljustrel**
Geogenic	soil type and bedrock lithology; U ore deposits	**Al**	(+)	(-/+)	(-)	(-/+)
		U	(+)	(-/+)	(-)	(-)
Geogenic	soil type and bedrock lithology	**Ni**	(-)	(-)	(+)	(+)
		Co	(-)	(-)	(+)	(+)
		Cr	(+/-)	(-)	(+)	(+)
		Mn	(-)	(-)	(+)	(+)
		Fe	(+/-)	(-)	(+)	(+)
Anthropogenic	erosion of mine tailings and acid mine drainage; erosion of pyrite waste and liquid effluents from industrial activities in Estarreja; agricultural practices (Cu)	**Cu**	(-/+)	(+/-)	(+)	(+)
		Pb	(-)	(+/-)	(+)	(+/-)
		Hg	(-)	(+)	(+)	(-/+)
		As	(-)	(+)	(+)	(+/-)
		Se	(-)	(+)	(+)	(-/+)
		Sb	(-)	(-/+)	(+)	(+/-)
		Mo	(-)	(-/+)	(+/-)	(+/-)
Anthropogenic	Liquid effluents from industrial activities in Estarreja and erosion of pyrite waste	**Cd**	(-)	(+)	(+/-)	(+/-)
		Zn	(-)	(+)	(+)	(+/-)
		Ba	(-)	(+)	(-)	(+/-)

Factor analysis was also conducted to better understand how the reactive pools of the different PTE's were distributed among the sampling areas. The elements Be, Li, B, Se and Mo were not included in the analysis since the nº of samples with values of reactive contents below the detection limit was considerably higher than that of other elements. The rotated matrix for log transformed PTE's reactive contents is shown in Table 5.7. Four Factors explained 81 % of the variance contained in the samples. The reactive concentrations of Cu, Pb, Hg, As and Sb have high loadings in Factor 1 (Table 5.7), similarly to what have happened with total/ *aqua regia* element contents. Ni, Co, Mn and Ba show higher correlation coefficients with Factor 2. It appears that the variance of the distribution of reactive contents of Fe and Cr are more related with those of Al and U in Factor 3, than with the distribution of Ni, Co and Mn as was observed for *aqua regia* extraction. Again, Cd and Zn have related variance distributions in the soil set and exhibit higher loadings in Factor 4 (Table 5.7).

Figure 5.2(b) shows the factor scores for reactive PTE's contents. There is a group of soils from Estarreja and Lousal & Caveira with higher reactive contents of the anthropogenic elements Pb, Cu, Hg, As and Sb while soils from Esposende show generally higher reactive contents of Fe, Al, U and Cr (Figure 5.2(b)). Highest concentrations of reactive Mn, Co, Ba and Ni were observed in soils from Aljustrel and in some samples from Lousal & Caveira (Figure 5.2(b)). For Cd and Zn the highest reactive concentrations were again obtained for a group of soils including samples from Estarreja, Lousal & Caveira and Aljustrel (Figure 5.2(b)).

Table 5.7: Results of Factor Analysis (rotated solution) for log transformed data on reactive pools of PTE's (*n*=134). Percentage of variance explained and communalities are also included.

Factor loadings$^{(a)}$

	Factor					**Communalities**
	1	**2**	**3**	**4**	**5**	**Extraction**
pH	0.53	-0.38	-0.07	0.41	-0.31	0.69
Log(Org C)	-0.06	-0.02	0.40	0.13	**0.75**	0.74
Log (Clay)	**0.63**	0.07	-0.18	-0.38	-0.22	0.63
Log(Cd_HNO3)	0.34	0.33	0.19	**0.71**	0.06	0.76
Log(Zn_HNO3)	0.26	0.37	-0.03	**0.80**	0.17	0.87
Log(Cu_HNO3)	0.36	**0.62**	-0.07	0.21	0.50	0.81
Log(Pb_HNO3)	0.11	**0.89**	-0.07	0.01	0.18	0.84
Log(Hg_HNO3)	-0.14	**0.87**	0.13	0.04	-0.05	0.80
Log(Ni_HNO3)	**0.79**	-0.24	0.20	0.31	-0.14	0.83
Log(Co_HNO3)	**0.82**	-0.13	-0.20	0.25	0.18	0.83
Log(Mn_HNO3)	**0.84**	-0.05	-0.21	0.00	0.22	0.80
Log(Fe_HNO3)	-0.18	0.18	**0.82**	0.13	0.05	0.75
Log(As_HNO3)	-0.31	**0.72**	0.34	0.36	-0.20	0.90
Log(U_HNO3)	-0.09	0.10	**0.77**	-0.18	0.31	0.74
Log(Cr_HNO3)	0.15	-0.02	**0.84**	0.33	-0.24	0.89
Log(Ba_HNO3)	**0.82**	0.17	0.05	0.26	-0.09	0.78
Log(Al_HNO3)	-0.11	-0.06	**0.92**	-0.05	0.20	0.90
Log(Sb_HNO3)	-0.11	**0.91**	0.02	0.22	-0.06	0.90
Total variance explained (%)	21.5	21.3	18.4	11.5	7.6	
Cumulative variance explained (%)	21.5	42.8	61.2	72.7	80.3	

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

(a) Rotation converged in 10 iterations.

Note: Highest loadings (>0.6) are shown in bold style.

A summary overview of reactive pools of PTE's in the soils of the dataset within the different sampling areas in comparison with the total PTE's pools is shown in Table 5.8. A relevant observation from Table 5.8 is that the distribution of the reactive pools of PTE's is not entirely coincident with the distribution of the total pools of PTE's. For instance in the Esposende area, although low contamination levels regarding most PTE's are present, considerable reactive for pools for some of the elements were observed. It is therefore crucial to better understand the underlying factors (such as soil properties) explaining these differences.

Table 5.8: Origin and distribution of reactive and total pools of potentially toxic elements in soils from the different sampling areas

Origin of total pools of PTE's in soils			Total pools of PTE's			
			Esposende	**Estarreja**	**Lou&Cav**	**Aljustrel**
Geogenic	soil type and bedrock lithology; U ore deposits	**Al**	(+)	(-/+)	(-)	(-/+)
		U	(+)	(-/+)	(-)	(-)
Geogenic	soil type and bedrock lithology	**Ni**	(-)	(-)	(+)	(+)
		Co	(-)	(-)	(+)	(+)
		Cr	(+/-)	(-)	(+)	(+)
		Mn	(-)	(-)	(+)	(+)
		Fe	(+/-)	(-)	(+)	(+)
Anthropogenic	erosion of mine tailings and acid mine drainage; erosion of pyrite waste and industrial activities in Estarreja	**Cu**	(-/+)	(+/-)	(+)	(+)
		Pb	(-)	(+/-)	(+)	(+/-)
		Hg	(-)	(+)	(+)	(-/+)
		As	(-)	(+)	(+)	(+/-)
		Se	(-)	(+)	(+)	(-/+)
		Sb	(-)	(-/+)	(+)	(+/-)
		Mo	(-)	(-/+)	(+/-)	(+/-)
Anthropogenic	industrial activities in Estarreja and erosion of pyrite waste	**Cd**	(-)	(+)	(+/-)	(+/-)
		Zn	(-)	(+)	(+)	(+/-)
		Ba	(-)	(+)	(-)	(+/-)

Origin of reactive pools of PTE's in soils			Reactive pools of PTE's			
			Esposende	**Estarreja**	**Lou&Cav**	**Aljustrel**
Anthropogenic	Anthropogenic contaminants with higher reactive pools in both industrial and mining areas (particularly Lousal&Caveira)	**Cu**	(-/+)	(-/+)	(+)	(+)
		Pb	(-/+)	(+)	(+)	(-/+)
		Hg	(-/+)	(+)	(+)	(-)
		As	(+/-)	(+)	(+/-)	(-)
		Sb	(-/+)	(+)	(+)	(-)
Geogenic	Geogenic elements with higher reactive pools in both industrial and mining areas	**Ni**	(-/+)	(-/+)	(+)	(+)
		Co	(-/+)	(-/+)	(+)	(+)
		Mn	(-/+)	(-/+)	(+)	(+)
		Ba	(-/+)	(-/+)	(+)	(+)
Geogenic	Geogenic elements with higher reactive pools in the non-polluted area of Esposende	**Al**	(+)	(+/-)	(-)	(-)
		U	(+)	(+/-)	(-)	(-)
		Cr	(+)	(+/-)	(-)	(+/-)
		Fe	(+)	(+/-)	(-/+)	(-/+)
Anthropogenic	Anthropogenic contaminants with higher reactive pools in both industrial and mining areas (particularly Aljustrel)	**Cd**	(-)	(+)	(+/-)	(+)
		Zn	(-)	(+)	(+)	(+)

5.3.5 Available pools of PTE's: soil extraction with 0.01 M $CaCl_2$

The concentrations of PTE's extracted by 0.01 M $CaCl_2$ and the extraction ratios in relation to the respective total and reactive pools are shown in Table 5.9. The median concentrations of PTE's in $CaCl_2$ extracts were generally < 1 mg kg^{-1}, with the exception of Zn (3.1 mg kg^{-1}), Mn (10 mg kg^{-1}) and Ba (2.9 mg kg^{-1}) (Table 5.9). Nevertheless, considerably high contents of available PTE's concentrations, particularly Cu, Pb, As, Mn, Zn, Ba, Ni, Co and Cr were obtained in certain samples. As shown in Table 5.9, the 95th percentile of Cu, Ba, As, Zn and Pb concentrations varied between 15 and 37 mg kg^{-1} and it was of 70 mg kg^{-1} in the case of Mn. The maximum values of available concentrations were exceptionally high for Zn, Cu, Pb, Mn, As and Ba (46, 173, 208, 118, 103 and 55 mg kg^{-1}, respectively) and were between 1.9 and 2.5 mg kg^{-1} for Cr, Co, B and Ni (Table 5.9). The maximum $CaCl_2$ concentration of Sb was also above 1 mg kg^{-1} (Table 5.9).

The results for Cd available contents obtained in our study (0.001-0.44 mg kg^{-1}) were comparable to those obtained in soils from the UK (Rieuwerts et al., 2006). The concentrations of Pb and Zn in $CaCl_2$ extracts in samples from our dataset were generally higher than those observed by Rieuwerts et al. (2006). Nevertheless, the soil samples from the UK also presented considerably lower contamination levels than soils from our study (total Pb<177 mg kg^{-1}; total Zn <115 mg kg^{-1}) (Rieuwerts et al., 2006). When comparing our Cd, Zn, Cu and Pb 0.01 M $CaCl_2$ extraction results with those obtained for contaminated soils affected by pyrite particles and acid wastewater from a mining area in South Spain (Pueyo et al., 2004) it was observed that our values of Cd and Zn were relatively lower than those in Span (above 0.9 and 200 mg kg^{-1}, respectively, in Spanish soils). However, available contents of Pb and Cu in our dataset were considerably higher than those reported by Pueyo et al. (2004) which were <6 mg kg^{-1} for both elements in $CaCl_2$ extracts.

Table 5.9: Concentrations of PTEs extracted by 0.01 M $CaCl_2$ and ratios in relation to respective total/ AR and reactive (0.43 M HNO_3) contents

					percentiles								ratio (%)		
Element (unit)	**Extractant**	***n***	**mean**	**range**	**5%**	**10%**	**25%**	**median**	**75%**	**90%**	**95%**		**mean**	**median**	**range**
Hg (µg kg^{-1} dw)	0.01 M $CaCl_2$	136	6.5	0.10-234	0.13	0.21	0.34	0.69	2.3	17	35	**$CaCl_2$/Total**	0.61	0.20	0.009-9.8
												$CaCl_2$/HNO_3	43	39	6.0-95
Cd (mg kg^{-1} dw)	0.01 M $CaCl_2$	117	0.051	0.001-0.44	0.0027	0.0034	0.010	0.031	0.059	0.15	0.19	**$CaCl_2$/AR**	8.3	6.2	0.21-51
												$CaCl_2$/HNO_3	24	16	0.98-99
Zn (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	6.7	0.032-46	0.16	0.33	0.93	3.1	6.8	19	27	**$CaCl_2$/AR**	2.9	1.9	0.12-37
												$CaCl_2$/HNO_3	25	18	0.62-100
Cu (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	3.7	0.072-173	0.12	0.18	0.34	0.88	2.4	8.2	15	**$CaCl_2$/AR**	1.8	1.2	0.046-12
												$CaCl_2$/HNO_3	9.4	5.7	0.69-71
Pb (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	2.0	0.016-208	0.070	0.10	0.20	0.78	2.1	14	37	**$CaCl_2$/AR**	1.7	1.0	0.067-14
												$CaCl_2$/HNO_3	8.2	4.8	0.31-79
Ni (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	0.31	0.029-2.5	0.056	0.088	0.12	0.25	0.40	0.66	0.90	**$CaCl_2$/AR**	2.0	1.6	0.21-8.0
												$CaCl_2$/HNO_3	32	24	1.6-98
Co (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	0.23	0.0055-2.4	0.020	0.025	0.057	0.14	0.29	0.59	0.77	**$CaCl_2$/AR**	2.3	1.6	0.13-10
												$CaCl_2$/HNO_3	16	11	0.74-82
Mn (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	19	0.55-118	0.980	2.0	4.2	10	28	46	70	**$CaCl_2$/AR**	4.0	3.2	0.10-16
												$CaCl_2$/HNO_3	19	13	0.49-92
Fe (% dw)	0.01 M $CaCl_2$	132	0.014	0.0010-0.66	0.0010	0.0020	0.0042	0.010	0.018	0.034	0.050	**$CaCl_2$/AR**	0.67	0.43	0.001-3.7
												$CaCl_2$/HNO_3	19	12	0.039-97
As (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	3.6	0.016-103	0.031	0.038	0.078	0.24	1.1	5.6	20	**$CaCl_2$/AR**	0.94	0.48	0.057-7.0
												$CaCl_2$/HNO_3	16	12	0.50-82
U (mg kg^{-1} dw)	0.01 M $CaCl_2$	131	0.032	0.0010-0.33	0.0017	0.0022	0.0052	0.015	0.033	0.072	0.13	**$CaCl_2$/AR**	1.8	1.0	0.058-10
												$CaCl_2$/HNO_3	8.6	4.6	0.10-89
Cr (mg kg^{-1} dw)	0.01 M $CaCl_2$	130	0.19	0.0042-1.9	0.011	0.018	0.042	0.10	0.22	0.49	0.66	**$CaCl_2$/AR**	1.1	0.67	0.013-13
												$CaCl_2$/HNO_3	23	14	0.34-85
Ba (mg kg^{-1} dw)	0.01 M $CaCl_2$	134	5.0	0.61-55	1.0	1.1	1.6	2.9	5.4	9.8	16	**$CaCl_2$/AR**	5.6	4.7	1.1-19
												$CaCl_2$/HNO_3	20	15	2.0-73
Al (% dw)	0.01 M $CaCl_2$	134	0.010	0.0001-0.72	0.00075	0.0012	0.0030	0.0061	0.012	0.024	0.032	**$CaCl_2$/AR**	0.86	0.51	0.005-7.0
												$CaCl_2$/HNO_3	14	8.2	0.069-75

Sb (mg kg^{-1} dw)	0.01 M $CaCl_2$	112	0.049	0.004-1.1	0.0011	0.0018	0.0044	0.0088	0.024	0.12	0.25	**$CaCl_2$/AR**	0.66	0.56	0.042-2.3
												$CaCl_2$/HNO_3	22	17	3.6-85
Se (mg kg^{-1} dw)	0.01 M $CaCl_2$	54	0.051	0.010-0.27	0.022	0.024	0.028	0.034	0.049	0.088	0.19	**$CaCl_2$/AR**	3.1	2.8	0.18-9.5
												$CaCl_2$/HNO_3	43	42	14-95
Li (mg kg^{-1} dw)	0.01 M $CaCl_2$	52	0.17	0.020-0.63	0.035	0.046	0.068	0.14	0.22	0.36	0.49	**$CaCl_2$/AR**	1.3	1.0	0.077-5.0
												$CaCl_2$/HNO_3	40	30	4.4-97
Be (mg kg^{-1} dw)	0.01 M $CaCl_2$	35	0.019	0.040-0.10	0.0063	0.0072	0.010	0.014	0.023	0.029	0.076	**$CaCl_2$/AR**	3.8	2.6	0.74-19
												$CaCl_2$/HNO_3	11	8.0	0.33-43
Mo (mg kg^{-1} dw)	0.01 M $CaCl_2$	23	0.0067	0.0011-0.016	0.0012	0.0016	0.003	0.0058	0.008	0.015	0.016	**$CaCl_2$/AR**	0.69	0.65	0.049-2.3
												$CaCl_2$/HNO_3	25	22	4.2-64
B (mg kg^{-1} dw)	0.01 M $CaCl_2$	23	0.71	0.11-2.1	0.12	0.15	0.19	0.56	1.0	1.4	2.0	**$CaCl_2$/AR**	11	9.7	2.0-31
												$CaCl_2$/HNO_3	44	39	8.3-98

The median percentages of PTE's present in the soil that are extractable by $CaCl_2$ in relation to total pools of PTE's are also shown in Table 5.9. These percentages were generally low and varied between 0.2 % (Hg) and 9.7 % (B). The median ratio of $CaCl_2$ extractable element pools in relation to total contents for the various elements decreased in the order: B> Cd> Ba> Mn> Se> Be> Zn> Ni=Co> Cu> Pb=U=Li> Cr> Mo> Sb> Al> As> Fe> Hg. Although median ratios were all < 10 %, differences between soils were clear and in certain samples the PTE's $CaCl_2$: *aqua regia* ratio was as high as 51 % (for Cd) and 37 % (for Zn). These results indicate that PTE's such as B, Cd, Ba, Se, Be and Zn can be considerably mobile and available over the short-term to plants and soil microorganisms. By contrary, under the current conditions, elements such as Hg, Fe, As, Al and Sb are more strongly bound to the soil matrix. These findings agree with reports from other authors indicating higher $CaCl_2$ extration ratios and soil solution concentrations of contaminants such as Cd, Mn, B, Se and Be (Goody et al., 1995; Sauvé et al., 2000) and lower mobility and availability for As (Burgos et al., 2008), Fe and Hg (Goody et al., 1995; Sauvé et al., 2000). In fact, Cd is well known as a highly labile element particularly at low pH (McBride et al., 2006; Degryse et al., 2007; Römkens et al., 2009a). Zinc and Ni are also often considered more mobile elements when in comparison with Cu and Pb which tend to be more strongly sorbed by soils constituents such as organic matter and Fe hydroxides (McLean and Bledsoe, 1992; Temminghoff et al., 1997; Weng et al., 2001; Yin et al., 2002; Tipping et al., 2003; Römkens et al., 2009a).

In the case of Hg, which in our soils showed the lowest $CaCl_2$ extraction ratio in relation to total contents, a study by Jing et al. (2008) has similarly reported low extractability by $CaCl_2$. Jing et al. (2008) also found a significant correlation between $CaCl_2$ soil-extractable Hg and Hg contents in edible plant tissues, indicating that this extractant can indeed be used to assess soil-available Hg. Nevertheless, when comparing different extractants ($CaCl_2$, DPTA, NH_4OAc and HCl), 0.01 M HCl provided the highest extractability ratios in relation to Hg total contents and the best indication of Hg phytoavailability (Jing et al., 2008).

When comparing the $CaCl_2$-extractable contents and the respective reactive concentrations (extracted by 0.43 M HNO_3), the median ratio between the two element pools varied between around 40 % for elements such as Se, B and Hg and 4-6 % for Cu, Pb and U (Table 5.9). The available element pools in relation to reactive contents decreased in the order: Se> B> Hg> Li> Mo> Ni> Zn> Sb> Cd> Ba> Cr> Mn>As> Fe> Co> Al> Be> Cu> Pb> U. These results are in accordance with those found by Römkens et al. (2009a) who reported considerably higher ratios of $CaCl_2$-extractable pools in relation to soil reactive contents for Cd, Zn and Ni compared to Cr, Cu and Pb. With the exception of Be, Mo, Ba, Al, Cu and Pb all elements showed maximum $CaCl_2$:HNO_3 extraction ratios between 80 and 100 % (Table 5.9), indicating that it is possible that almost even concentrations of most contaminants occur in the soil solution and in the soil reactive fraction, in certain samples from our dataset.

Boxplots of the $CaCl_2$-extractable pools of 18 PTE's clustered by different sampling areas are shown in Figure 5.3. Molybdenum and B were not included since the number of results above detection limits per sampling area was quite variable and did not allow an effective comparison of results. Soil samples from Estarreja showed the highest $CaCl_2$-extractable concentrations of Cd, Zn, Hg, As, U, Al, Be and relatively high concentrations of Pb, Cu, Cr and Se compared to the other sampling areas (Figure 5.3). Soils from the mining areas (Aljustrel and Lousal & Caveira) showed the highest available pools of Cu, Pb, Ni, Co, Mn, Ba, Sb and Li and also relatively high $CaCl_2$-extractable contents of Cr, Cd and Zn (Figure 5.3). Relatively low concentrations of Hg and As were extracted by $CaCl_2$ in soil samples from Aljustrel. Finally, as shown in Figure 5.3, soils from Esposende showed the lowest available contents for all contaminants except

U, Al and Se and $CaCl_2$-extractable Hg and As pools similar to those from Aljustrel.

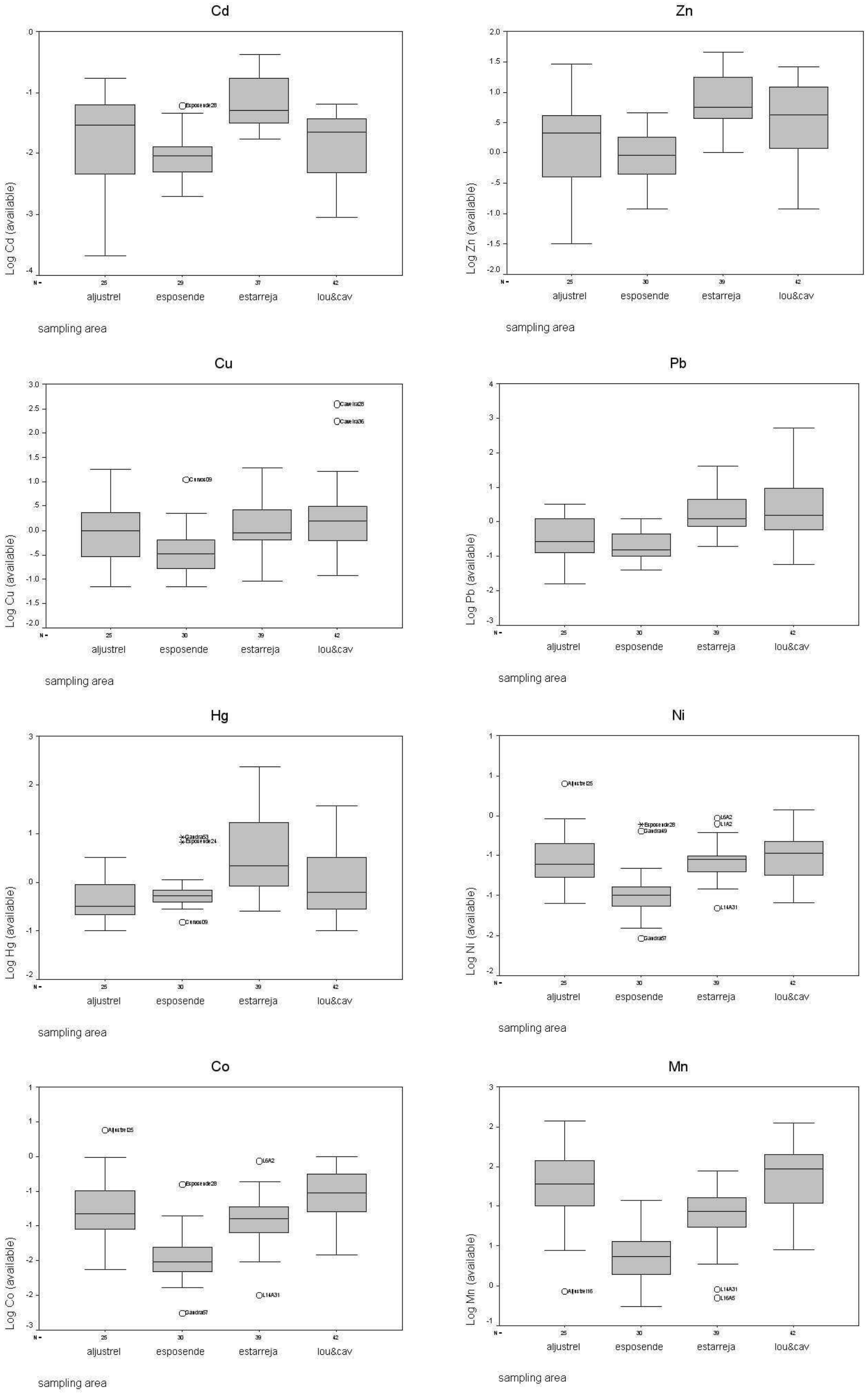


Figure 5.3: Boxplots of $CaCl_2$-extractable concentrations of PTEs in the different sampling areas (Log_{10} data of μg kg^{-1} for Hg and mg kg^{-1} for other elements is shown)

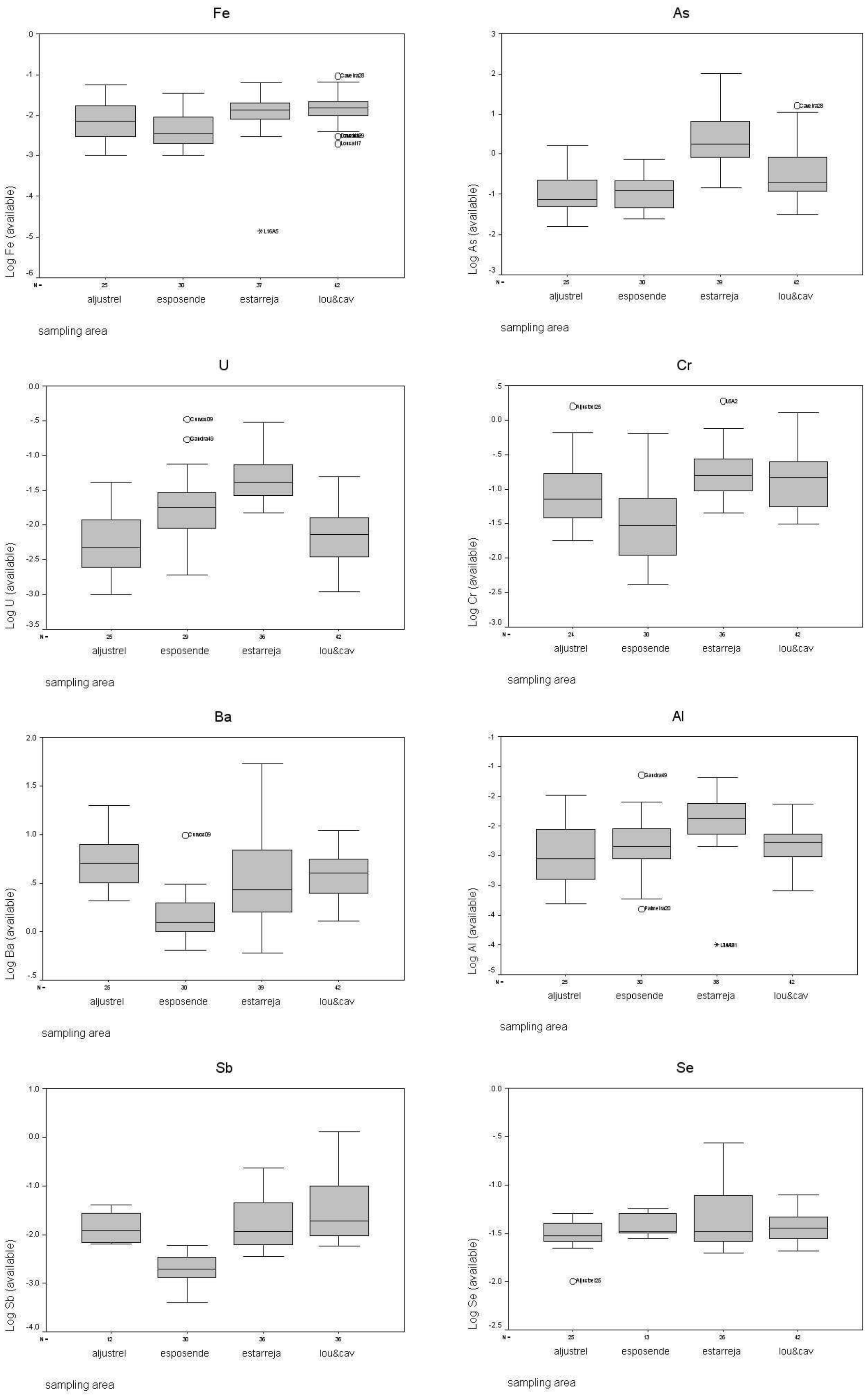


Figure 5.3 *(cont.)*

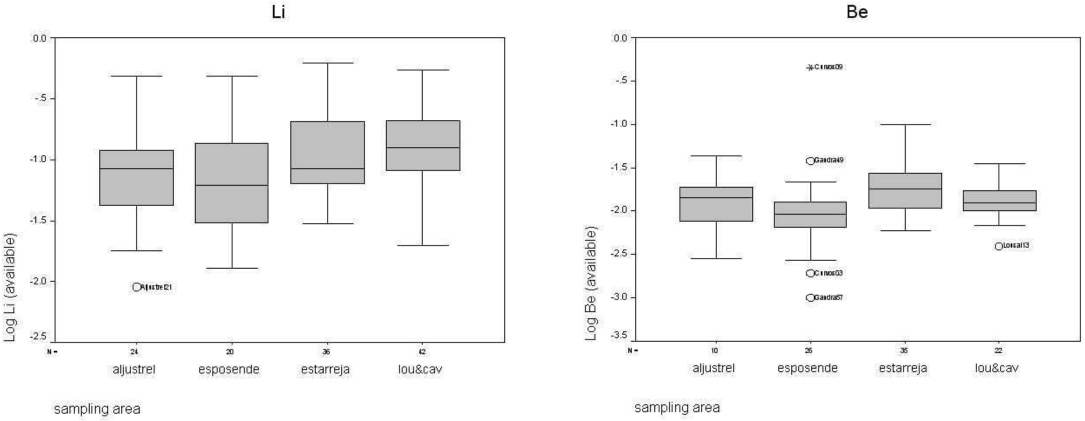


Figure 5.3 *(cont.)*

5.4 Summary and Conclusions

This study was undertaken to assess total, reactive and directly available pools of PTE's in Portuguese soils and to evaluate the variability of soils' reactivity and elements' availability. One of the main objectives was to provide the basis to understand the partition of elements within the different soil phases which will be further analysed in the next Chapter. Through a better understanding of these factors most relevant considerations on potential risks to the environment and human health arising from soil contamination can be drawn providing key information for the definition of future monitoring schemes and risk assessment strategies in Portugal.

The dataset produced included information on soils from across Portugal. The pH values varied from very acidic (pH~3, samples from mining areas subject to the influence of acid mine drainage) to neutral, with soil pH generally increasing from North to South. Soil textures varied from sandy loam (mostly in the northern sampling areas) to silty clay loam, with higher clay contents being observed in the country southern areas. Organic C contents were generally low, with the exception of a few samples from more intensive cultivated areas and DOC contents in $CaCl_2$-extracts were for most samples extremely low. The reasons for such low DOC values should be further investigated in future studies. Wide ranges of metal total oxides and amorphous oxides contents were observed, with amorphous Al oxides being particularly abundant in the North of the country and Fe amorphous oxides showing higher contents in soils from Centre and South areas. The area of Estarreja (North/Central-West Portugal) was identified as that with potentially lowest soils' reactivity (and consequently highest availability of contaminants) due to low pH values, low Org C and low clay contents and only intermediate levels of amorphous metal oxides. These observations are in accordance with expected results for the occurring soil type, Podzols.

Severe anthropogenic contamination of soils by Cu, Pb, Hg, As, Zn and to a less extent Se, Sb and Ba was observed in certain samples of the dataset. This contamination was associated to both industrial and mining activities. For elements such as Cd, Zn and Ba, industrial activities appear to be the main contamination source. Soil Cu contamination by agricultural practices was also observed in some samples from the most intensively cultivated areas. Three areas were found to be particularly impacted by anthropogenic soil contamination: Estarreja (industrial) and Lousal & Caveira (mining). High contents of certain geogenic contaminants were also observed in these soils. Nickel, Co, Cr, Mn and Fe were particularly abundant in soils from South-West areas of Portugal around mining sites (Lousal & Caveira and Aljustrel) while Al and U were the most abundant elements in soils from the North-West area of Portugal (Esposende) and were associated to soil type and bedrock lithology.

Very large reactive pools of particularly Zn, Cu, Pb, As and Ba were observed in certain areas with some samples showing HNO_3-extractable concentrations of these five elements up to 519, 1492, 581, 384 and 208 mg kg^{-1}, respectively. Highest HNO_3 extractability ratios were obtained for Cd which agrees with other studies from the Netherlands and Belgium (Römkens et al., 2004; Meers et al., 2007a), followed by Be, Ba, Pb, U, Mn, Cu and Co in decreasing order. Similarly to findings by other authors Zn and Ni showed relatively low HNO_3: *aqua regia* extraction ratios which have been associated with metal fixation and long-term immobilization by clay minerals (Buekers et al., 2008; Römkens et al., 2009a). Relatively low reactivity was also observed for Cr, As, Se and Mo which can be strongly retained in soils through binding mechanisms with Fe, Al and Mn oxides (McLean and Bledsoe, 1992). The lowest HNO_3-extractability ratio and smaller soil reactive pools were observed for Hg. The low levels of reactivity were related to the presence of highly immobile forms of Hg in these soils, particularly in samples from around mining areas but also to the type of chemical extraction procedure used. Soil extraction with 0.43 M HNO_3 may not be the most appropriate methodology to assess the several Hg-species that can be considered "reactive" in soils since this is more likely to only break down weaker Hg complexes (particularly with organic matter) and/ or partly dissolve Hg associated to Fe/Mn-(amorphous)oxides by acidification. Mercury strongly associated to organic matter and S-containing ligands and species that can still be considered "reactive" (Issaro et al., 2009) are not likely to be effectively extracted by this method. Further assessments of the reactivity of organo-chelated Hg, methylmercury, total Hg or other Hg species in soils must include the use of other extractants or more concentrated nitric acid solutions (for elemental Hg) (Wallschläger et al., 1998; Bloom et al., 2003; Issaro et al., 2009).

The variability of the distribution of reactive pools of PTE's in soils from the dataset was not entirely coincident with that of total element pools. The highest reactive pools of Cu, Sb, Hg, As and Sb were observed in soils from around the mining areas of Lousal & Caveira and in some samples from the industrial area of Estarreja. The distribution of Cd and Zn reactive contents differed from that of the other anthropogenic contaminants, with higher pools being observed in samples from around the industrial complex. The southern Aljustrel area showed the highest reactive pools of the geogenic contaminants Ni, Co, Mn and Ba. Soils from the northern area which showed relatively low contamination levels by most PTE's showed relatively high reactive contents for some elements, particularly for Fe, Cr, Al and U.

The available pools of PTE's were also assessed in this study by means of the assessment of $CaCl_2$-extractable concentrations. Large $CaCl_2$-extractable pools were observed for contaminants such as Zn, Cu, Pb, As, Mn, Ba and to a less extent, Ni, Co, Cr and Sb. The largest available pools of Cd, Zn, Hg, As, U, Al, Be and relatively high $CaCl_2$-extractable concentrations of Pb, Cu, Cr and Se were observed in soils from the industrially contaminated area (Estarreja). Soils from the mining areas (Aljustrel and Lousal & Caveira) showed the highest available pools of Cu, Pb, Ni, Co, Mn, Ba, Sb and Li and also relatively high $CaCl_2$-extractable contents of Cr, Cd and Zn. The smallest available pools of most PTE's were observed in soils from the non-contaminated northern area of the country (Esposende).

Chapter 6

Partition relationships: the influence of soil properties on the reactivity and direct availability of PTE's at contaminated sites

6.1 Introduction

Processes controlling the fate of PTE's in soils, such as adsorption, are metal-specific and dependent on soil properties including pH, organic matter, clay and amorphous Al and Fe oxides since the equilibrium of element concentrations between soil and soil solution is strongly affected by competition for surface exchange sites by other cations (especially H^+) and by the presence of binding surfaces (Sauvé et al., 2000; Weng et al., 2001; Rieuwerts et al., 2006). Moreover, the chemical forms and oxidation states in which PTE's occur in soils determine their relative reactivity and availability (Peijnenburg et al., 2007).

For cationic metals such as Cu, Zn, Cd, Pb and Hg, concentrations in soil solution generally depend negatively upon pH since increases in pH values decrease proton competition, and thus favour metal binding (McLean and Bledsoe, 1992; Yin et al., 2002; Tipping et al., 2003; Römkens et al., 2004). In addition, changes in pH also cause alterations in soil components (e.g. promoting dissolution of soil organic matter) thus affecting metal distribution among soil phases (Yin et al., 2002). It has been observed that under certain conditions the addition of pH-increasing fertilizers to soils enhanced Hg methylation and increased the loosely bound and ion-exchangeable Hg in the top organic soil thereby increasing the availability of this element (Matilainen et al., 2001).

Soil components including soil organic matter, metal oxides and clay minerals (especially 2:1 clay minerals) have shown large sorption capacities for certain metals, affecting their solid: solution partition (Yin et al., 2002). The increase of these soil constituents generally contributes to higher pools of sorbed metals (McLean and Bledsoe, 1992; Yin et al., 1997; Gustafsson et al., 2003). By contrary, the microbial mineralization of soil organic materials can be an important factor for the release and mobilisation of metals bound to soil organic matter (Munthe et al., 2001; Matilainen et al., 2001; Yin et al., 2002). Other soil constituents like carbonates and sulphides may also play a role in the immobilization/ mobility of PTE's in soils. It has been reported that the presence of sulphides in soils (H_2S or HS^-) contributes for the precipitation of HgS (cinnabar), a rather immobile form of Hg in soils due to its low solubility (Ravichandran et al., 1998).

For cationic metals, total dissolved metal concentrations include a sum of the free metal pool (Me^{n+}), plus inorganic ion pairs, plus organic complexes (Sauvé et al., 2000). Any factor affecting one of the components will impact total dissolved element concentrations. Elements' speciation are also affected by the composition of the soil solution (and its ionic strength) since the presence of competing divalent cations (such as Ca^{2+}) in high concentrations may contribute to increase desorption of divalent metals (e.g. Cd and Zn) and solubilize them through cation exchange reactions (Sauvé et al., 2000; Römkens et al., 2004). Dissolved organic carbon has an important influence, since it may contribute to the formation of stable metal-organic complexes in the soil solution that are particularly relevant for increasing the solubility of metals, particularly Cu and Pb (Sauvé et al., 2000; Weng et al., 2002; Römkens et al., 2004). Ravichandran et al. (1998) have reported that dissolved organic matter (particularly the humic fractions) enhance the release of Hg from cinnabar both under oxic and anoxic conditions and that, humic substances could enhance Hg mobilization in soils and affect its bioavailability even in areas favourable for HgS formation.

The availability of anionic contaminants in soils like As, Se and Cr is highly dependent on their oxidation states. Certain oxide surfaces such as Fe, Mn and Al oxides, carbonate surfaces and insoluble organic matter can generate positive charges at low pH values and contribute for the adsorption of element anions, although the adsorption capacity for anions is generally small relatively to cation adsorption capacity of soils (McLean and Bledsoe, 1992). The availability of Cr in soils is significantly related to soil oxidation capacity and it has been observed that extractable Mn is a main factor controlling net Cr oxidation in soils (Chon et al., 2008). Arsenic in soils is subject to both biological and chemical transformations and forms several organic and inorganic compounds and solid precipitates with Fe, Al, Ca, Mg, and Ni (Mahimairaja et al., 2005). Available soil concentrations of As are predominantly determined by the redox potential, pH, the contents of organic matter, Fe, Mn, P, Ca-carbonate, and biological activity (soil microbes) (Mahimairaja et al., 2005; Brammer and Ravenscroft, 2009). In aerated soils As is present mainly as As(V) and, as such tends to be adsorbed by Fe amorphous oxides in the solid phase and to become unavailable to plants (Brammer and Ravenscroft, 2009). But when anaerobic soil conditions occur, as such during flooding events, As is mainly present as As(III) and is dissolved in the soil solution (Xu et al, 2008). The adsorption and availability of As in soils can also be affected more strongly by the presence of $H_2PO_2^-$ ion than any other anions (Mahimairaja et al., 2005). The soluble forms of Se are selenite (Se(IV)) and selenate (Se(VI)), which are more mobile in soils than elemental Se (Rosen and Liu, 2009). Selenites and selenates in soil tend to be adsorbed on clay particles, Fe and Mn minerals, and organic matter (Environment Agency, 2009a). In general, pH, oxidation potential and biological processes are the main factors controlling Se speciation and availability. Selenates occur under oxidising conditions and are very weakly adsorbed at alkaline pH values, therefore becoming available. Lower availability of Se is observed in acidic soils and under mildly reducing conditions, since this element occurs primarily as selenite which is readily sorbed onto metal oxides, organic matter and/ or precipitated as Fe complexes (Environment Agency, 2009a). At very reducing conditions, such as poorly drained acidic soils, another form of Se (selenide, Se^{2-}) may occur. This is generally very immobile since it tends to be removed from the soil solution by the formation of strong organo-metallic complexes with soil organic matter (Environment Agency, 2009a).

The assessment of transfer of contaminants between total and available pools in soils can be performed through the analysis of solid:solution partition relationships and it is often expressed by the distribution coefficient between the solid and the solution phase, K_d (Sauvé et al., 2000; Degryse et al., 2003; Tipping et al., 2003; Sastre et al., 2007). Models of different levels of complexity have been used to predict metal partition and speciation and to describe relationships between soil and soil solution composition (Bonten et al., 2008). Both complex process-based models and empirical multiple regression analysis describing relationships between concentrations of either total dissolved metal or free metal ions and soil properties such as pH, organic carbon content and total metal burden have provided relevant descriptions of the solid:solution partition of elements in contaminated soils (Jopony and Young, 1994; McBride et al., 1997; Sauvé et al., 1997; Sauvé et al., 2000; Yin et al., 2002; Bonten et al., 2008). Empirical models that relate the solid:solution partition relationships of elements to soil properties have also been recently used to describe the adsorption:desorption equilibria between reactive and available pools of potentially toxic elements in soils (Degryse et al., 2003; Tipping et al., 2003; Römkens et al., 2004; Rieuwerts et al., 2006; Meers et al., 2007a; Koopmans et al., 2008; Römkens et al., 2009a). The use of either complex mechanistic based models (including speciation and advanced surface complexation modelling for ion adsorption to soil particles) or empirical models depends on the requirements and objectives of the study being developed. The analysis of empirical relationships between solid:solution partition and soil properties can be quite useful for risk assessment studies, mass transport calculations and contaminated soil remediation (Bonten et al., 2008). With different degrees of complexity, these two approaches have the advantage of describing the processes determining elements lability (mobility and bioavailability) from contaminated soils and of providing a significant understanding of the soil properties and environmental conditions that control uptake of contaminants by plants allowing more adequate assessments of actual risk levels.

6.2 Aim, scope and objectives

In this Chapter the most relevant soil properties controlling the reactivity of PTE's in soils will be analysed. Furthermore, the solid: solution partition relationships for PTE's in Portuguese soils will be assessed and the influence of soil and soil solution properties on these relationships will be discussed.

A conceptual framework for the assessment of total, reactive and available pools of PTE's in Portuguese soils is shown in Figure 6.1. A similar approach has been recently successfully applied to soils from the UK and the Netherlands (Tipping et al., 2003; Römkens et al., 2004). This study focused on a Southern European area (Portugal), since empirical functions for metal partition to date were mainly developed in temperate regions

(Römkens et al., 2004) and it is important to extend these studies to different climatic and geological areas towards an EU-wide application of such concepts.

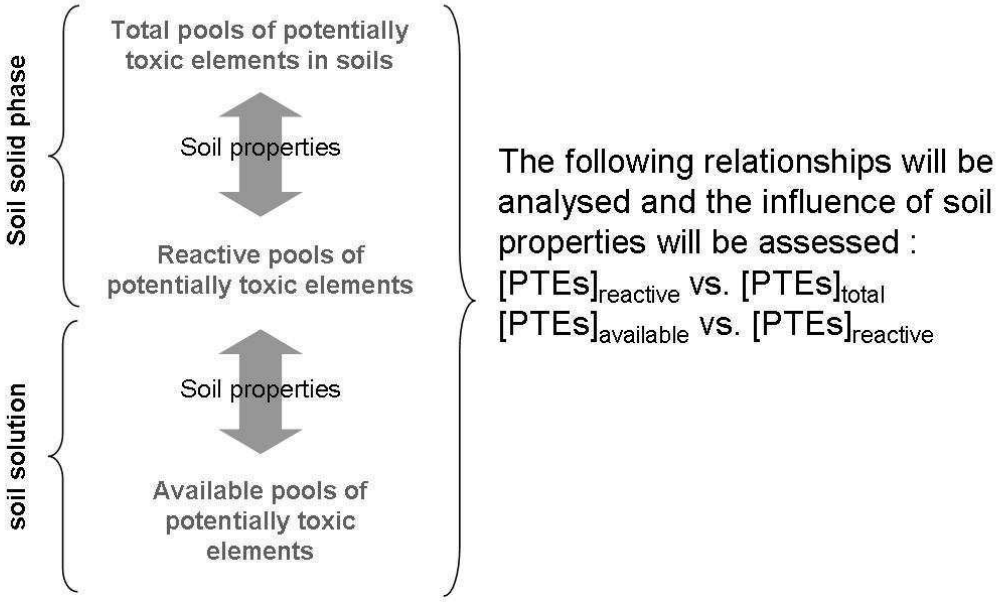


Figure 6.1: Conceptual framework for the assessment of total, reactive and available pools of PTE's in Portuguese soils

In summary, the main objectives of this Chapter are:

- Analysis of the influence of key soil properties in the development of reactive and available pools of PTE's in soils and assessment of potential alterations in reactivity and availability of contaminants under changing environmental conditions;
- Calibration of a soil ⇔ soil solution model for a Southern European area;
- Analysis of potential for alterations in mobility and uptake of contaminants by plants in association with changes in environmental conditions.

6.3 Reactive pools of PTE's: analysis of the influence of key soil properties

In this section I will test the hypothesis that the reactive element pool can be estimated from a function that combines the total element pool and key soil properties. The most relevant soil properties determining the reactivity of a contaminant in soil will depend on the element in question.

Multiple linear regression analysis was performed using experimental data according to eq. 4.4 (Chapter 4). The following relationships were tested:

- Reactive element content= *f* (total element content) (empirical model 1)
- Reactive element content= *f* (total element content, pH, Org C%, Clay%) (empirical model 2)
- Reactive element content= *f* (total element content, pH, Org C%, Clay%, total Fe, total Al, total Mn) (empirical model 3)
- Reactive element content= *f* (total element content, pH, Org C%, Clay%, total Fe, total Al, total Mn, $Fe_{am\text{-}ox}$, $Al_{am\text{-}ox}$) (empirical model 4)

Table 6.1 shows the results of the multiple linear regression analysis for the reactive pools of 19 elements (B was not included since the number of samples analysed for this element was rather low). Furthermore, graphs plotting HNO_3-extractable concentrations predicted by the linear multiple regression models against measured data are shown in Annex III (Figure 1). The performance of the model in each sampling area is also shown.

Table 6.1: Linear regression coefficients and r^2 values of respective equations for reactive concentrations of PTE's

	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	r^2
	interc.	(log[PTE$_{total}$])	(pH)	(log % OrgC)	(log % Clay)	(log[Fe$_{total}$])	(log[Al$_{total}$])	(log[Mn$_{total}$])	(log[Fe$_{am\ ox}$])	(log[Al$_{am\ ox}$])	
Cu	**-0.58**	**0.95**	x	x	x	x	x	x	x	x	**0.86**
	-0.74	**0.94**	n.s.	**0.45**	n.s.	x	x	x	x	x	**0.87**
	-0.80	**1.0**	n.s.	**0.35***	n.s.	**-0.22***	**0.36***	n.s	x	x	**0.89**
	-0.49	**0.95**	n.s.	**0.26***	n.s.	**-0.26***	**0.26***	n.s	**0.20**	n.s	**0.90**
Pb	**-0.19***	**0.77**	x	x	x	x	x		x	x	**0.78**
	n.s.	**0.78**	n.s.	**0.35***	**-0.23***	x	x	x	x	x	**0.81**
	-1.2	**1.0**	n.s.	**0.47***	n.s.	**-1.0**	**0.88**	**0.17***	x	x	**0.89**
	-1.2	**2.0**	n.s.	**0.47***	n.s.	**-1.0**	**0.88**	**0.17***	n.s	n.s	**0.89**
Zn	**-1.1**	**1.1**	x	x	x	x	x	x	x	x	**0.68**
	-1.9	**1.1**	**0.11**	**0.55**	n.s.	x	x	x	x	x	**0.74**
	-1.9	**1.1**	**0.098**	**0.63**	n.s.	**-0.35***	n.s.	n.s	x	x	**0.76**
	-1.6	**1.1**	**0.13**	**0.60***	n.s.	**-0.74**	n.s.	n.s	**0.61**	n.s	**0.81**
Cd	**-0.47**	**0.90**	x	x	x	x	x	x	x	x	**0.78**
	-1.1	**0.91**	**0.041***	**0.59**	**0.17***	x	x	x	x	x	**0.83**
	-0.93	**0.92**	**0.050***	**0.38***	n.s.	n.s.	**0.46**	n.s	x	x	**0.85**
	-1.1	**0.99**	**0.051***	**0.55**	n.s.	**-0.32***	**0.78**	n.s	**0.33***	**-0.30***	**0.87**
Ni	**-1.2**	**0.96**	x	x	x	x	x	x	x	x	**0.47**
	-1.9	**0.84**	**0.14**	**0.42***	n.s.	x	x	x	x	x	**0.60**
	-2.0	**1.3**	**0.082***	**0.69**	n.s.	**-0.57**	n.s.	n.s	x	x	**0.67**
	-2.1	**1.3**	**0.088**	**0.63**	n.s.	**-0.54**	**-0.80**	**0.22***	n.s	**0.47**	**0.73**
Co	**-0.99**	**1.2**	x	x	x	x	x	x	x	x	**0.79**
	-1.7	**1.1**	**0.11**	**0.51**	n.s.	x	x	x	x	x	**0.86**
	-1.7	**1.1**	**0.1**	**0.6**	n.s.	n.s.	**-0.24***	n.s	x	x	**0.86**
	-1.6	**1.2**	**0.11**	**0.51**	n.s.	n.s.	**-0.51***	n.s	n.s	**0.21***	**0.87**
Mn	**-0.57**	**0.98**	x	x	x	x	x	x	x	x	**0.72**
	-1.5	**0.98**	**0.11**	**0.80**	n.s.	x	x	x	x	x	**0.83**
	-1.5	**0.98**	**0.11**	**0.80**	n.s.	n.s.	n.s.	x	x	x	**0.83**
	-1.5	**0.98**	**0.11**	**0.80**	n.s.	n.s.	n.s.	x	n.s	n.s	**0.83**
As	**-1.2**	**0.93**	x	x	x	x	x	x	x	x	**0.52**
	n.s.	**0.95**	n.s.	n.s.	**-1.1**	x	x	x	x	x	**0.63**
	-1.6	**1.3**	**0.11***	**0.44***	**-0.45**	**-1.9**	**1.3**	n.s	x	x	**0.88**
	-1.4	**1.2**	**0.12**	**0.41***	**-0.49**	**-2.4**	**1.5**	n.s	**0.56**	n.s	**0.90**
U	**-0.60**	**0.92**	x	x	x	x	x	x	x	x	**0.70**
	-1.1	**0.95**	n.s.	**0.30***	n.s.	x	x	x	x	x	**0.72**
	-1.1	**0.80**	n.s.	n.s.	n.s.	n.s.	**0.61**	n.s	x	x	**0.76**
	-1.1	**0.80**	n.s.	n.s.	n.s.	n.s.	**0.61**	n.s	x	x	**0.76**
Ba	**-0.92**	**1.2**	x	x	x	x	x	x	x	x	**0.67**
	-1.7	**1.0**	**0.16**	**0.34***	**0.22***	x	x	x	x	x	**0.79**
	-2.2	**0.99**	**0.11**	**0.60**	**0.20***	**-0.38**	n.s.	**0.32**	x	x	**0.83**
	-2.2	**0.99**	**0.11**	**0.60**	**0.20***	**-0.38**	n.s.	**0.32**	n.s	n.s	**0.83**
Hg	**-0.86**	**0.41**	x	x	x	x	x	x	x	x	**0.61**
	-0.46*	**0.42**	n.s.	n.s.	**-0.43**	x	x	x	x	x	**0.65**
	n.s.	**0.46**	n.s.	n.s.	**-0.24***	n.s.	**0.56***	**-0.31**	x	x	**0.72**
	n.s.	**0.45**	n.s.	n.s.	**-0.28***	n.s.	n.s.	n.s.	n.s	**0.43***	**0.73**
Al	**-1.3**	**1.3**	x	x	x	x	x	x	x	x	**0.47**
	-1.2	**1.2**	n.s.	**0.55**	**-0.25***	x	x	x	x	x	**0.60**
	-0.89	**1.2**	n.s.	**0.60**	n.s.	**-0.37**	x	**-0.19**	x	x	**0.76**
	-0.88	**0.24***	**0.056**	**0.23***	**n.s.**	**n.s.**	x	**n.s**	**n.s**	**-0.72**	**0.84**
Cr	**-0.58**	**0.32***	x	x	x	x	x	x	x	x	**0.06***

	-1.1	**0.57**	n.s.	**0.75***	**-0.52**	x	x	x	x	x	**0.26**
	-0.49*	**0.84**	**0.098***	**0.60***	**-0.23***	**-0.53**	**0.79**	**-0.46**	x	x	**0.64**
	n.s.	**0.75**	**0.18**	n.s.	**-0.35**	**-0.52**	n.s.	**-0.25***	**0.36**	**0.58**	**0.76**
Fe	**-1.1**	n.s.	x	x	x	x	x	x	x	x	n.s.
	-0.95	n.s.	n.s.	**0.69**	n.s.	x	x	x	x	x	**0.21**
	-0.54*	n.s.	n.s.	**0.32***	n.s.	x	**0.59**	**-0.31**	x	x	**0.38**
	n.s.	**-0.42***	**0.065***	n.s.	**-0.16***	x	**0.67***	**-0.3**	**0.77**	n.s	**0.63**
Se	**-1.3**	**0.56**	x	x	x	x	x	x	x	x	**0.33**
	-2.0	**0.62**	**0.12***	n.s.	n.s.	x	x	x	x	x	**0.38**
	-3.1	**0.93**	n.s.	n.s.	n.s.	**-1.2***	**1.2***	**0.79**	x	x	**0.57**
	-3.1	**0.93**	n.s.	n.s.	n.s.	**-1.2***	**1.2***	**0.79**	n.s	n.s	**0.57**
Sb	**-1.5**	**0.60**	x	x	x	x	x	x	x	x	**0.52**
	-0.73	**0.66**	n.s.	n.s.	**-0.80**	x	x	x	x	x	**0.62**
	-0.76	**1.1**	n.s.	n.s.	**-0.49**	**-1.6**	**1.6**	n.s	x	x	**0.82**
	-0.36	**0.95**	n.s.	n.s.	**-0.46**	**-1.7**	**1.3**	n.s	**0.58**	n.s	**0.85**
Mo	**-1.7**	**0.78**	x	x	x	x	x	x	x	x	**0.22**
	-1.7	**0.78**	n.s.	n.s.	n.s.	x	x	x	x	x	**0.22**
	-1.4	**1.1**	n.s.	n.s.	n.s.	**-0.82**	n.s.	n.s	x	x	**0.43**
	-0.92	**0.93**	n.s.	n.s.	n.s.	**-1.2***	n.s.	n.s	**0.7***	n.s	**0.57**
Li	**-1.5**	n.s.	x	x	x	x	x	x	x	x	n.s.
	-2.2	**0.53***	**0.13***	n.s.	n.s.	x	x	x	x	x	**0.16***
	-2.2	**0.53***	**0.13***	n.s.	n.s.	x	x	x	x	x	**0.16***
	-2.5	n.s.	**0.27**	n.s.	n.s.	**-0.65***	n.s.	n.s	**0.89**	n.s	**0.55**
Be	**-0.62**	**0.68**	x	x	x	x	x	x	x	x	**0.39**
	-0.62	**0.68**	n.s.	n.s.	n.s.	x	x	x	x	x	**0.39**
	-0.31	**0.61**	n.s.	n.s.	n.s.	**-0.52**	**0.9**	n.s	x	x	**0.81**
	-0.31	**0.61**	n.s.	n.s.	n.s.	**-0.52**	**0.9**	n.s	x	x	**0.81**

Notes:

- All coefficients are significant at the $p<0.001$ level, with the exception of those indicated by an asterisk (*) which are significant at the $p<0.05$ level
- n.s.= not significant at the $p<0.05$ level

As shown in Table 6.1, r^2 values ≥0.80 were obtained for Cu, Pb, Zn, Cd, Co, Mn, As, Ba, Al and Sb and $0.70 \leq r^2 < 0.80$ were observed for Ni, U, Hg, Cr and Be. The lowest model performances ($r^2 < 0.70$) were obtained for Fe, Se, Mo and Li (Table 6.1). The *aqua regia* content of the elements alone (model 1) provided very good predictions of the reactive contents of Cu, Pb, Cd, Co, Mn and U ($r^2 > 0.70$). By contrary the aqua regia contents of Cr, Fe and Li provide little or no significant contribution for the regression models of the respective elements. The inclusion of pH was significant for some of the elements providing small improvements of performance of model 2 for Zn, Cd, Ni, Co, Mn, Ba, Se and Li while the contribution of organic carbon was significant for Cu, Pb, Zn, Cd, Ni, Co, Mn, U, Ba, Al, Cr and Fe (Table 6.1). The contribution of clay slightly improved the performance of model 2 for Pb, Cd, Ba, Al and Cr and was more important for elements such as As, Hg and Sb. The inclusion of these three parameters (pH, organic C and clay) on the regression model provided relevant contributions for elements such as Zn, Cd, Ni, Co, Mn, As, Ba, Al, Cr, Fe, Sb and Li. Adding the "pseudo-total" soil contents of Fe, Al and Mn to the regression analysis (model 3) was significant for Pb, Ni, As, Hg, Al, Cr, Fe and Se (Table 6.1). Finally, the inclusion of amorphous Al and Fe oxides in model 4 provided relevant contributions only for Ni, Al, Cr, Fe, Mo and Li.

From Table 6.1, reactive Cu pools appear to have a highly significant linear relationship with the total metal pool (b=0.95; r^2=0.86, $p<0.01$) although soil Org C and amorphous Fe oxides appear to also contribute to predict the range in reactivity for Cu at a given metal total metal content. Copper extractable with HNO_3 has also been found to be positively correlated with soil organic matter by other authors (Tipping et al., 2003; Römkens et al., 2004). When including soil properties into the regression model a r^2=0.90 ($p<0.01$) is obtained. It is clear from Figure 1 (Annex III) that for Cu, samples from the different sampling areas are equally distributed along the regression prediction line.

For Pb a significant linear relationship between reactive and total metal pools was also observed (*b*=0.77; r^2=0.78, $p<0.01$) although Fe, Al and Mn "pseudo-total" contents as well as Org C appear to play a relevant role (Table 6.1). The contribution of amorphous metal oxides appear not be significant when considering "pseudo-total" Fe, Al and Mn in the equation. It appears that the sorption of Pb into metal amorphous oxides is in this case surpassed by the role of crystalline oxides (particularly Fe) and of the retention of Pb within the crystalline solid matrix rather than in the expression of its sorption onto reactive surfaces. The performance of the regression prediction model is quite high for the total population (r^2=0.89, $p<0.01$) and it is exceptionally good in the more contaminated areas of Lousal & Caveira and Estarreja (Figure 1, Annex III).

For both Zn and Cd, significant linear relationships were also observed between reactive and total metal pools (Table 6.1). Using aqua regia as a predictor of the reactive metal pool provided a slightly higher r^2 value for Cd (r^2=0.78, $p<0.01$) than for Zn (r^2=0.68, $p<0.01$). Including Org C and amorphous Fe oxides (particularly in the case of Zn) which contribute positively to the retention of these contaminants on these soils allow to increase model performance. At the same time, pH has also a positive contribution (Table 6.1) indicating that when pH increases a relatively higher amount of these metals is retained in the reactive fraction. Other studies had also reported on the role of pH (McBride et al., 2006; Bonten et al., 2008) and organic matter (Tipping et al., 2003; Römkens et al., 2004) on the sorption of Cd and Zn in soils. When including soil properties, the model performance for both elements for the total population is high (r^2>0.80, $p<0.01$). For Zn, a slightly lower r^2 (r^2=0.81, $p<0.01$) was obtained in comparison with Cd (r^2=0.87, $p<0.01$) (Figure 1, Annex III). When considering the different sampling areas, the model performance for both Cd and Zn is slightly weaker in the Esposende area (Figure 1, Annex III) where predominantly low total and reactive concentrations of the two metals were observed. It also appears from Figure 1, Annex III that Zn reactive contents measured in a group of samples from Lousal & Caveira are slightly higher than predicted values. Other authors have reported that nitric acid may dissolve certain amounts of Zn containing precipitates present in soils and therefore overestimate metal reactive contents, particularly in highly contaminated samples (Bonten et al., 2008).

For Ni, when considering only the total metal pool for estimating reactive contents the model performance is relatively low (b=0.96; r^2=0.47). Immobilization of Ni in soils due to fixation and surface precipitation onto oxyhydroxides was reported by other authors (Buekers et al., 2008; Römkens et al., 2009a) and may have contributed to reduce model performance for this element when considering only the total and reactive pools. Nevertheless, model performance for Ni can be improved by adding Org C and particularly pH into the model (Table 6.1). In fact pH and Org C are most relevant factors contributing for a better prediction of the range in reactivity for Ni, Co, Mn and Ba at a given metal total metal content (Table 6.1). Amorphous Al oxides (for Ni and Co) as well as Fe and Mn contents (for Ni and Ba) also contribute to slightly improve model performance (Table 6.1). A relatively wide range of reactive contents of Ni, Mn, Co and Ba were observed in the samples from the various sampling areas and considerably high model performances for the total population of these elements was attained ($r^2>0.73$, $p<0.01$) (Figure 1, Annex III). When considering the different sampling areas, lowest fitting values were observed for Esposende in the case of Ba and in Lousal & Caveira for Mn due to the occurrence of predominantly low reactive Ba contents in Esposende and by contrary predominantly high reactive Mn contents in Lousal & Caveira (Figure 1, Annex III).

Reactive U contents can be quite well predicted from the total metal concentrations (b=0.92; r^2=0.70) although the introduction of "pseudo-total" Al concentrations slightly contributes to improve model performance (Table 6.1). The levels of reactive U show a significantly high correlation with reactive contents of Al (r=0.73, $p<0.01$) indicating that the reactive pools of these two elements may have a similar distribution within the dataset. Other soil components do not significantly contribute to explain the variability of U reactive contents on these samples. A relatively wide range of U contents was observed in the entire dataset and a very good prediction of the U range of reactivity for the total population was attained ($r^2=0.76$, $p<0.01$). The model performance tends to decrease when considering the sampling areas separately, particularly Esposende and Aljustrel where U levels are predominantly high and low, respectively (Figure 1, Anne II).

The reactive (0.43 M HNO_3 extracted) levels of Hg could only be fairly predicted from total Hg soil contents ($b=0.41$; $r^2=0.61$, $p<0.01$) (Table 6.1). Amorphous Al oxides appear to slightly contribute to increase the metal reactive fraction. Clay contents show the opposite effect possibly because in soils with higher clay contents, Hg is present in more immobile forms. Nevertheless, it seems clear from Figure 1 (Annex III) that the linear regression model could only be used to predict Hg reactive pools due to the very wide range of Hg contents in these soils and particularly due to the very high contamination levels observed in Estarreja and Lousal&Caveira (up to 100 mg kg^{-1} of total Hg).

Reactive concentrations of Sb in soils can only be fairly predicted from total element contents ($b=0.60$; $r^2=0.52$) (Table 6.1). Nevertheless, adding soil properties to the regression analysis considerably improves model performance (Table 6.1). From Table 6.1, most relevant soil properties contributing to improve reactive Sb contents predictions are soil "pseudo-total" contents of Fe and Al, amorphous Fe oxides, and to a less extent, clay. These observations are in accordance with studies from other authors who reported that the presence of amorphous Fe (along with Mn) oxides is a most relevant factor enhancing the oxidation of Sb(III) to Sb(V) and that in aerobic soils, Sb(V) is the dominant redox species and it is mainly associated with Fe (hydr)oxides (Oorts et al., 2008). Including soil properties into the analysis allowed a high model performance both for total population ($r^2=0.85$, $p<0.01$) and for the different sampling areas, particularly in Lousal & Caveira ($r^2=0.92$, $p<0.01$) and Estarreja ($r^2=0.86$, $p<0.01$) areas, where the wider ranges of reactive Sb were observed (Figure 1, Annex III).

For Cr (an anionic contaminant), using aqua regia as a sole predictor of the reactive metal content is clearly insufficient since very low *b* and r^2 values were found ($b=0.32$; $r^2=0.06$, both significant at $p<0.05$) (Table 6.1). By including soil properties into the regression model, not only the performance of the model increases significantly but also a considerably higher *b* coefficient is obtained, expressing a stronger relationship between reactive and total Cr contents (Table 6.1). The contents of amorphous Al oxides, followed by pH, "pseudo-total" Fe, amorphous Fe oxides, "pseudo-total" Mn and soil clay contents contribute to increase the model performance (Table 6.1). The ability of Al and Fe oxides to adsorb soil Cr has also been reported by other authors (Prokisch et al., 1997; Hopp et al., 2008). Moreover, other studies reported that soil pH and Mn oxides affect soils oxidation capacity and thus affecting Cr speciation (Chon et al., 2008). Changes in element speciation alter the balance of Cr distribution between the reactive and available soil pools. At low pH, Mn oxides increase the mobility of Cr by oxidizing Cr(III) to Cr(VI) in soils and thus decreasing sorbed element pools (Kim et al., 2002; Chon et al., 2008). Adding soil properties into the model allowed to obtain a relatively high performance for Cr both for total population ($r^2=0.76$, $p<0.01$) and for the different sampling areas ($0.55<r^2<0.88$, $p<0.01$) (Figure 1, Annex III).

In the case of As a relatively high *b* coefficient ($b=0.93$) and a significant r^2 value ($r^2=0.52$, $p<0.01$) was obtained when considering aqua regia as a predictor of the reactive element content (Table 6.1). Adding soil properties into the model considerably increased its performance (Table 6.1). Both "pseudo-total" Fe and Al play a very important role, with Fe showing a negative coefficient - in areas with higher soil Fe contents, such as the mining areas, As is apparently present in more immobile forms in soils. Nóvoa-Muñoz et al. (2007) have reported that in acid agricultural soil from Spain, As was also strongly bound by crystalline Fe. The presence of Fe amorphous oxides proved to be relevant factors to increase As reactive pools in our dataset and contributed to increase model performance (Table 6.1). Other authors have also reported that under oxidising conditions, arsenate [As(V)] is the stable redox state in soils and that As(V) adsorption is significantly positively correlated with amorphous Fe oxides (Yang et al., 2002; Goldberg et al., 2005; Krysiak and Karczewska, 2007). Clay, pH and Org C also played a small role in increasing our model performance (Table 6.1). The relatively wide range of reactive contents of As observed in the samples from the various sampling areas allowed to obtain considerably high model performances both for the total population and separate sampling areas (Figure 1, Annex III). Estarreja is the area showing the best model fit (r^2=0.92) (Figure 1, Annex III).

The reactive concentrations of Se were below the detection limit for many samples which difficult the derivation of relationships among variables. Nevertheless, a significant relationship (r^2=0.33, $p<0.01$) was obtained when considering aqua regia as a sole predictor for Se reactive contents, although a relatively low b coefficient was obtained (b=0.56) (Table 6.1). Adding "pseudo-total" concentrations of Fe, Al and Mn allowed to improve Se reactive contents prediction. The inclusion of soil properties in the regression model allowed to both improve model performance (r^2=0.57, $p<0.01$) and to obtain a b coefficient closer to 1 (Table 6.1). Redox potential is known to be a most relevant factor controlling Se speciation and mobility in soils. In general, Se(VI) which adsorbs weakly on soil surfaces (or not at all) is the more stable redox state under oxidising conditions (Goldberg et al., 2007; Environment Agency, 2009a). However the transformation of Se(VI) to Se(IV) is slow and both redox states often co-exist (Goldberg et al., 2007). Selenite [Se(IV)] adsorbs strongly on soil surfaces particularly at low pH, and has been found to adsorb specifically on Fe, Al and Mn oxides and on amorphous Al and Fe oxides (Hiemstra and Van Riemsdijk, 1999; Goldberg et al., 2007). The regression model derived from our dataset also provides an indication that Fe, Al and Mn may have a relevant role on the sorption of Se by our soils. As shown in Figure 1 (Annex III), the derived regression model is only effective in the case of Estarreja sampling area where a wide range of reactive Se concentrations was observed. At the other sites, Se concentrations were predominantly very low and did not provide a good model fit. No results for Esposende are shown since all reactive Se contents were below the detection limit.

Reactive contents of Li in our dataset could not be significantly predicted from the total Li contents (Table 6.1). The main factors controlling Li reactivity were amorphous Fe oxides, followed by pH and "pseudo-total" Fe which allowed to derive a regression model with a $r^2=0.55$ (Table 7). Nevertheless only 54 samples (and all from Estarreja and Lousal & Caveira) were included in this analysis (Figure 1, Annex III) and therefore the factors controlling Li reactive pools should be subject of further consideration.

Total contents of Be provided to some extent a relevant prediction of the reactive element pool, although the model performance was considerably improved by "pseudo-total" contents of Fe and Al (Table 6.1). A relatively high r^2 value was obtained for the Be regression model, particularly in the Estarreja sampling area (Figure 1, Annex III). Nevertheless, similarly to Li only 54 samples, and all from Estarreja and Lousal & Caveira, were included in this analysis and therefore the factors controlling Be reactivity should also be subject of further consideration.

For Mo a significant improvement in the regression model is attained when "pseudo-total" Fe and amorphous Fe oxides are taken into account (Table 6.1). With 76 samples showing reactive Mo contents above the detection limit, the derived model proved to be more effective in the

Aljustrel area where a wider range of Mo reactive contents was observed (Figure 1, Annex III).

For Fe no significant relationship could be found between aqua regia and 0.43 M HNO_3 extracted contents (Table 6.1). Although relatively high r^2 values are obtained for both Fe and Al, particularly when including Al, Mn and amorphous Fe oxides (for Fe) or amorphous Al oxides, pH and organic C (for Al) the interpretation of these results cannot be performed as for the other contaminants in terms of their total and reactive contents since the geochemistry of these two elements in soils is quite specific.

6.4 Available pools of PTE's: analysis of the influence of key soil properties

In this section I will test the hypothesis that the available element pool can be estimated from a function that combines the reactive element pool and key soil properties. The most relevant soil properties determining the availability of a contaminant in soil will depend on the element in question.

Multiple linear regression analysis was performed using experimental data according to eq. 4.5 (Chapter 4). The following relationships were tested:

- Available element content= *f* (reactive element content, pH, Org C%, Clay %) (empirical model 1)
- Available element content= *f* (reactive element content, pH, Org C%, Clay %, total Fe, total Al, total Mn) (empirical model 2)

Table 6.2 shows the results of the multiple regression analysis for 19 elements analysed (B was not included because many of both reactive and available concentrations were below the detection limit).

Table 6.2: Linear regression coefficients, r^2 values and standard error of the estimates (S.E.) for available pools of PTE's

	Linear regression coefficients (model 1)							***Linear regression coefficients (model 2)***									
	a	***b***	***c***	***d***	***e***	r^2	**S.E.**	***a***	***b***	***c***	***d***	***e***	***f***	***g***	***h***	r^2	**S.E.**
PTE's	***(interc.)***	**(log[PTE$_{reactive}$])**	**(pH)**	**(log % OrgC)**	**(log % Clay)**			***(interc.)***	**(log[PTE$_{reactive}$])**	**(pH)**	**(log % OrgC)**	**(log Al)**	**(log % Clay)**	**(log Fe)**	**(log Mn)**		
Cu	n.s.	0.87	-0.15	-0.98		0.61	0.39	n.s.	0.90	-0.16	-0.73*	-0.88	-	-	-	0.66	0.37
Pb	n.s.	1.0	-0.15*	-1.2		0.66	0.47	n.s.	0.94	-0.18	-0.81*	-1.2	-	-	-	0.71	0.44
Zn	1.7	1.0	-0.4	-1.1		0.69	0.37	1.8	0.98	-0.41	-0.69*	-1.0	-	-	-	0.75	0.34
Cd	1.8	1.0	-0.43	-0.95		0.80	0.28	2.0	1.1	-0.44	-0.78	-0.63	-	-	-	0.82	0.26
Ni	0.56*	0.56	-0.18	-0.84		0.40	0.27	0.8	0.62	-0.2	-0.7	-0.56	-	-	-	0.46	0.26
Co	n.s.	0.73	-0.23	-1.0		0.46	0.37	0.77*	0.77	-0.25	-0.77	-0.91	-	-	-	0.54	0.34
Mn	1.1	0.86	-0.3	-1.3		0.58	0.36										
As	n.s.	0.89	-0.14*	-0.94	0.61	0.77	0.40	n.s.	0.87	-0.16	-0.55*	-0.98	0.68	-	-	0.81	0.37
U	n.s.	0.45	-0.2	-0.81*		0.29	0.48										
Ba	0.83	0.84	-0.22	-0.85		0.68	0.22	0.92	0.89	-0.24	-0.74	-0.40*	-	-	-	0.70	0.21
Hg	n.s.	1.1	-0.096*	-0.86		0.69	0.41										
Al	n.s.																
Cr	n.s.	0.36		-1.3		0.20	0.48	-1.4	0.82	-0.14*	-	-1.8	-	-	0.58	0.36	0.43
Fe	n.s.																
Se	n.s.	0.39	-0.072*			0.50	0.19										
Sb	-1.0	1.0		-0.75	0.65	0.82	0.3	-1.1	0.91	-	-0.58*	-0.98	0.42	0.6	-	0.89	0.23
Mo	-2.0	0.48*	0.24*	-1.40*		0.74	0.18										
Li	n.s.																
Be	-0.81*	0.52		-1.1		0.24	0.32										

- All coefficients are significant at the $p<0.001$ level, with the exception of those indicated by an asterisk (*) which are significant at the $p<0.05$ level
- n.s.= not significant at the $p<0.05$ level

The availability of PTE's as measured by 0.01 M $CaCl_2$ could be well predicted by the reactive metal content and pH, Org C and clay as independent variables (Table 6.2). As shown in Table 6.2, between 20% (Cr) and 82% (Sb) of the variance of available pools could be explained using the empirical model. The highest r^2 values were obtained for Sb, Cd, As, Mo, Zn, Hg and Ba while the lowest r^2 were observed for Cr, Be and U (Table 6.2). Available concentrations of Fe, Al and Li could not be significantly predicted ($p>0.05$) from this set of independent variables (Table 6.2).

Soil pH was significantly ($p<0.01$) negatively correlated with the Cd, Zn, Cu, Pb, Hg, As, U and Al $CaCl_2$ extractable pool, with highest r values found for Cd and U (r~0.4). This indicates an increasing availability with decreasing pH as shown for cationic species in the pH range 3-7 (Sauvé et al., 2000; Tipping et al., 2003; Römkens et al., 2004; Wang et al., 2004; Rieuwerts et al., 2006; Degryse et al., 2007; Horckmans et al., 2007; Römkens et al., 2009a) due to competition between protons and metal cations. pH allowed to improve predictions of the extractability for models of all elements except Cr and Be. The relation between the availability of anionic species and pH is less straightforward with a decrease in availability of Mo and an increase in availability of As and Se with decreasing pH.

Further improvements to predict metals availability were achieved by including Org C soil contents into the regression model, which improved predictions all elements except Se. Negative coefficients were obtained for all elements (Table 6.2) which is also found for cationic species in other studies fraction (Sauvé et al., 2000; Yin et al., 2002; Gustafsson et al., 2003; Tipping et al., 2003; Römkens et al., 2004; Rieuwerts et al., 2006) due to binding of these species by the solid phase Org C. The similar effect on anionic species is not well understood as in general, these bind primarily to Al and Fe oxides in soil.

Generally, the addition of clay into the empirical models only resulted in minor improvements of their performance (Table 6.2). This agrees well with results of mechanistic models which predict a marginal contribution of clays to the binding of metal cations (Weng et al, 2001; Bonten et al., 2008) Only for As and Sb a significant effect of clay on the available pool was observed, which in both cases proved to be positive. The positive effect of clay, on the availability of As and Sb is not well understood.

The effect of the inclusion of total Fe, Al and Mn pools on the performance of the regression models was also analysed (Table 6.2). For most elements the reactive pool of PTE's proved to be the major factor explaining the observed variation in available pools followed by pH for Cd, Zn, Ni, Co and Ba and by total Al contents for Cu, Pb, Cr, As and Sb. Significant negative coefficients between -1.8 and -0.43 were obtained for total Al contents in the models of Cu, Pb, Zn, Cd, Ni, Co, Mn, As, U, Ba, Cr and Sb (Table 6.2). The inclusion of total Al contents in the regression models provided higher improvements in the performance of the models (by increasing r^2 values of a wider range of elements) than clay contents. The variation of the pools of total Al in these soils expresses the sorptive capacity of aluminosilicates and Al oxides at the surfaces and edges of clay minerals better than the actual variability of clay contents.

The explained variance of the models for Cr is rather low but was improved by the inclusion of Al contents into the model (Table 6.2). It is important to distinguish between Cr(III) and Cr(VI) because the retention mechanisms are clearly different. Cr(III) is adsorbed by soil organic matter and is mainly present in soils in the form of insoluble oxides. Cr(VI) is better soluble and mainly adsorbed by Al and Fe oxides (Prokisch et al., 1997). Mn oxides stimulate the oxidation of Cr(III) to Cr(VI) (Chon et al., 2008) which possibly explains the positive coefficient of Mn oxides (Table 6.2).

Aluminium contents also contribute to explain the availability of Ni, Zn and Co. A possible explanation for the impact of Al in the models of these elements is the formation of mixed metal-Al hydroxide surface precipitates. A study by Peltier et al. (2010) showed that for soils, the formation of Ni-Al hydroxide surface precipitates resulted in a reduction in the fraction of desorbed and available Ni. Similar processes may also occur for Zn and Co (Peltier et al., 2010).

Although DOC has earlier been reported as and important variable in empirical models for Cu and Pb (Temminghoff et al., 1997; Weng et al., 2002; Tipping et al., 2003; Römkens et al., 2004; Rieuwerts et al., 2006) in Portuguese soils, the inclusion of DOC proved not to be significant for any of the elements. This relates primarily to the fact that DOC concentrations in the $CaCl_2$ extracts from these soils were low and showed little variation. A better understanding of the role of DOC in the soil: solution partition of PTE's in Portuguese soils is necessary.

Contrary to what occurred when modelling the reactivity of contaminants for these soils, the inclusion of amorphous oxides of Fe and Al in the multiple regressions for the assessment of elements availability did not have a significant effect on the regression coefficients or r^2 values. This indicates that the contribution of the pool of elements adsorbed to amorphous metal oxides to the dissolved pool of contaminants in these soils is either negligible or better expressed by other variables such as the total Al contents. Either way, and since variables such as amorphous metal oxides are not so commonly included in soil quality monitoring programs, a model using pH, Org C and clay (in the cases of As and Sb) is considered to be appropriate for modelling the availability of PTE's in Portuguese soils. When available, total Al contents may also provide useful information.

Graphs plotting $CaCl_2$-extractable concentrations predicted by the linear multiple regression models against measured data are shown in Annex III

(Figure 2). The performance of the model in each sampling area is also shown. The best fitting was obtained for Cd, Zn, Sb, Ba, As, Cu and Pb showing higher r^2 values and almost all samples within small 95% confidence intervals based on individual data (Figure 2, Annex III). The models for these elements provided significant ($p<0.001$) predictions of $CaCl_2$-extractable contents for all sampling areas although with generally lower r^2 values for Esposende, the least contaminated area (Figure 2, Annex III). Nickel and Cr models performed worst in both Esposende and Aljustrel areas. Selenium and Be regression models were only able to effectively predict elements extractability in Estarreja and Aljustrel, respectively. Finally, Hg $CaCl_2$-extractable contents showed the most effective predictions in the contaminated areas (Estarreja and Lousal & Caveira, up to 100 mg kg^{-1} of total Hg).

6.4 Available pools of PTE's: Comparison with other empirical models from literature

To assess whether the derived models have generic validity I compared our results with those available from literature for most commonly studied elements Cd, Zn, Cu and Pb (Sauvé et al., 2000; Tipping et al., 2003; Römkens et al., 2004; Rieuwerts et al., 2006; Unamuno, 2009). Coefficients around -0.4 for pH in Cd extractability models (similar to what was obtained for our samples) have also been reported for other soils from the Netherlands (Römkens et al., 2004) and from the UK (Rieuwerts et al., 2006). This suggests that the response of Cd in the $CaCl_2$ extracts to changes in pH is similar in samples from varied environmental settings either in North or Southern European areas.

When applying empirical models from literature based on a large array of soil conditions (such as those of Sauvé et al. (2000), similar model performances were obtained. Sauvé et al. (2000) developed empirical models for solid-solution partition for Cd, Cu, Ni, Pb and Zn using total metal contents, pH and soil organic matter and based on very large literature datasets compiled from over 70 different studies. Applying the model developed by Sauvé et al. (2000) to our dataset, the r^2 values obtained showed performances similar to our equations (r^2 of 0.80, 0.66, 0.44, 0.70 and 0.74 for Cd, Cu, Ni, Pb and Zn respectively). As shown in Figure 6.2, there was also a very good agreement between predictions of dissolved element concentrations by the regression model developed in this study and values predicted by Sauvé et al. (2000), particularly for Cu, Pb and Zn. The results for Ni were found instead to be poor. The concentrations of PTE's predicted by both models were of the same order of magnitude.

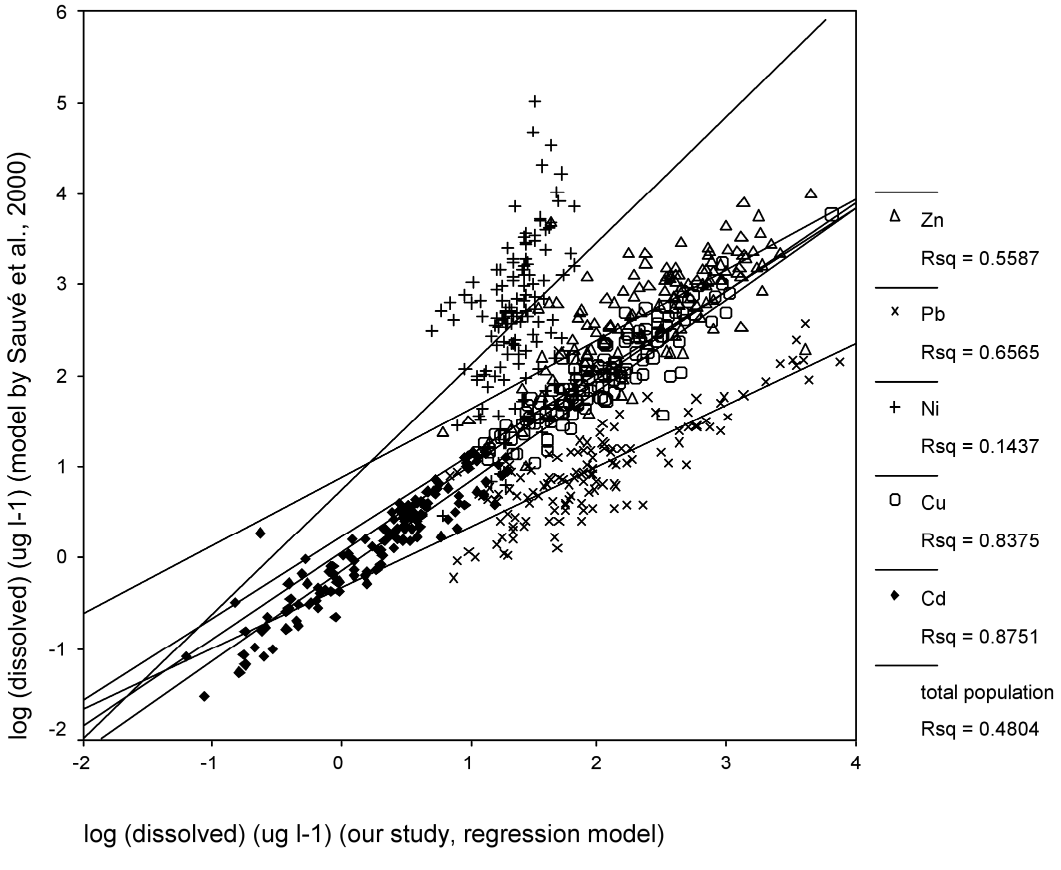


Figure 6.2: Scatterplot of dissolved concentrations (μg L^{-1}) predicted by the model by Sauvé et al., (2000) *versus* dissolved concentrations predicted by the regression model developed during the present study.

Tipping et al. (2003) performed a study to identify the factors responsible for solid–solution partition in upland soils of England and Wales. However, regression equations derived by Tipping et al. (2003) predicted our data poorly since these relationships were derived for a very particular group of soils with high organic matter contents (Org C > 5%) which are clearly not representative for the soils of this study (Org C < 5%).

For the remaining PTE's included in the present study no comparable regression models were found in literature.

6.6 Summary and Conclusions

It was clear from this study that in the event of an input of anthropogenic contaminants into the environment (as occurred in Estarreja and Lousal & Caveira areas), soils will have more or less ability to retain these contaminants in its solid phase depending on the presence of reactive surfaces such as soil Org C, clay, Fe, Al and Mn oxides. The role of each soil property varies according to the element in question. Through multiple regression empirical models we were able to provide an explanation for the most part of the variance of the reactive pools of almost all contaminants studied. For elements such as Cu and Pb, the reactive element pools could be almost entirely predicted from the aqua regia element pool although organic C (for Cu) and organic C in association with Fe, Al and Mn *aqua regia* contents (for Pb) also played a significant role. For elements such as Ni, Co, Mn and Ba, the inclusion of both pH and Org C, and to a less extent metal oxides, played a most relevant role in improving the predictions of HNO_3-extractability. This was particularly relevant for Ni and Ba reactive pools which could only be fairly predicted from "pseudo-total" element contents. The inclusion of pH, Org C and amorphous Fe oxides was also significant for Cd and Zn and Al oxides were particularly important for Cd. The inclusion of aqua regia Al contents in the model of U, provided considerably best estimations of U reactivity in these soils. The inclusion of soil properties in the empirical model was also highly significant for anionic contaminants such as As, Cr and Se. In fact, Cr reactive contents could not be significantly predicted from only the aqua regia element contents, but with the inclusion of soil characteristics 76 % of the variance of the distribution of reactive pools could be explained. With the exception of Hg (for which significant contamination was only observed in samples from Estarreja and Caveira) and Se (for which significant contamination was only observed in a few samples from Estarreja), the empirical models allowed to obtain significant predictions of HNO_3-extractable pools both at national level (considering the entire dataset) and at regional/ local level (considering the 4 sampling areas individually). In the case of Hg, HNO_3-extractable concentrations could be better predicted from total Hg concentrations by quadratic regression instead of linear regression since it is only above certain soil contamination levels (>1 mg kg^{-1}) that the diluted HNO_3 solution is able to extract Hg contents that effectively correlate with total element concentrations.

The findings of this work make a strong point for the assessment of elements' reactivity in soils and particularly for the inclusion of key soil properties into soil quality monitoring programs towards the assessment of risks associated to soil contamination. It was clear from our results that in the event of an alteration in soil constituents particularly Org C contents, amorphous Fe and Al oxides, the reactivity of soils can be significantly impacted and the pools of contaminants such as Pb, Cu, Zn, Pb, Ni, Ba, Mn, Co, Sb, As, Cr, Se and Mo retained by soils' reactive surfaces may decrease, and, by implication, increase the mobility and availability of these elements. It was also clear that there are highly contaminated soils in Portugal (such as samples from Estarreja) where both total and reactive pools of certain PTE's are quite high but in which the contents of soils' reactive surfaces are relatively low compared to other sites and therefore sensitive receptors may be at risk.

Soil: solution partition regression models including (pseudo-)total/ reactive element contents and soil properties provided the best estimations for $CaCl_2$-extractability (r^2 of 0.7-0.9) for Sb, As, Zn and Cd and the worst predictions (r^2 of 0.3-0.5) for Cr, Ni and U. Using the HNO_3-extractable pool as an independent variable to predict $CaCl_2$ extractability instead of the (pseudo-)total element contents allowed to obtain slightly better predictions for Cd, Ni, Cr, Co, Ba, Sb, Se and Mo.

The inclusion of pH into the multiple regression models allowed to improve predictions of the availability of Zn, Cd, Ba, Ni, Co, Mn, Cu, Pb and U but further improvements of elements extractability were achieved by including organic C (for Cu, Pb, Zn, Cd, Ni, Co, Mn, As, U, Ba, Hg, Sb and Be) and Fe and/ or Al *aqua regia* contents (for Pb, Cu, Zn, Cd, Ni, Co, Mn, As, U, Ba, Cr, Se, Sb and Mo). The inclusion of Fe and Al pseudo-contents outweighed the contribution of amorphous Fe and Al oxides and of clay contents in explaining the variability of $CaCl_2$- extractable contents. Similarly, DOC did not provide significant contributions for explaining the variance in $CaCl_2$ elements extractability due to the very low DOC contents of the samples from this dataset and its strong correlation with Org C contents. It is expected that particularly in the most contaminated soils from this dataset, elements such as Cu and Pb are present in the soil solution mostly as free ions instead of metal-organic complexes.

Finally, empirical regression models provided significant estimations for Cd, Zn, Sb, Ba, As, Cu and Pb both at national level (all samples from the dataset) and for the different sampling areas individually (although with generally lower r^2 values for Esposende, the least contaminated area). Thus, it appears to be feasible to use such an approach to derive useful estimations of available concentrations of these elements on both a national and a regional basis.

Chapter 7

Concentrations of PTE's in Portuguese crops and derivation of soil to plant transfer functions

7.1 Introduction

Consumption of crops that have accumulated potentially toxic elements (PTE's) from soil is one of the major routes of exposure of animals and humans to inorganic contaminants (Millis et al., 2004; Moyano et al., 2009; Römkens et al., 2009b). In certain industrialized areas, the contents of the contaminants in food crops considerably exceeded food quality standards due to elevated concentrations of PTE's in soils (Römkens et al., 2009b). Besides the introduction of contaminants into the food chain, other aspects of risk to the environment through element uptake by plants are the loss of vegetation cover due to phytotoxicity and the cycling of metals to surface soil horizons and to other environmental compartments by tolerant plants (Kabata-Pendias, 2004). Therefore, the calculation of regulatory soil quality standards that are protective of the environment, animal and human health requires models to estimate chemical concentrations in plants based on soil contents.

Despite considerable progress in recent years the availability and uptake of contaminants from contaminated soils as well as the associated risks remain an important area of research (Almås et al., 2006). The transfer of PTE's within the soil-plant chain is know to occur through processes of the biochemical cycle of chemical elements, as is illustrated in Figure 7.1.

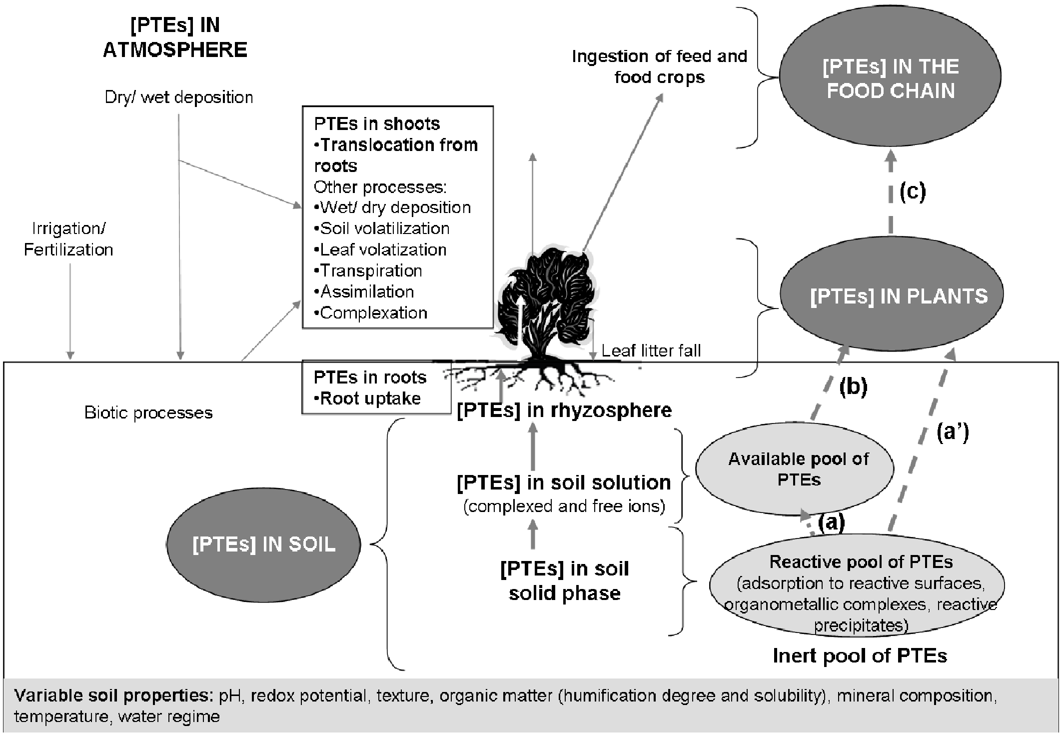


Figure 7.1: General overview of processes related to the uptake of contaminants from contaminated soils and impacts on terrestrial food chains

Figure 7.1 describes the cycle of elements regarding general soil-plant and plant-atmosphere interactions, the processes occurring within plants and the transfer of contaminants into the food chain. Uptake and internal transport mechanisms into and through plants are highly metal specific and also depend on element speciation in the soil and soil-water compartment. As of now it is believed that plants readily take up elements that are in the soil solution in either free ionic or complexed forms (Kabata-Pendias, 2004;

Kalis et al., 2007). The fraction of elements in solution is generally referred to as the available pool, the later being in equilibrium with the reactive or sorbed element content in the soil (Römkens et al., 2009a; Rodrigues et al., 2010b). The degree to which PTE's are available for uptake by plants therefore depends on their abundance, chemical form and nature of the binding to soil reactive surfaces and is mainly controlled by sorption, complexation and redox processes (Sauvé et al., 2000; Rieuwerts et al., 2006; Rodrigues et al., 2010b). Rhizosphere-plant interactions may influence metal availability since certain root exudates can affect both pH and redox conditions in the rhyzosphere thereby lowering or increasing the availability of PTE's (Kabata-Pendias, 2004; Patra et al., 2004; Feng et al., 2005; Sterckeman et al., 2005; Kalis et al., 2007). Plants growing on contaminated sites may also develop mechanisms to actively restrict translocation of certain toxic elements such as metal sequestration by specially produced organic compounds, organic ligand exudation or root cell wall immobilization (Patra et al., 2004; Válega et al., 2009).

As described in Chapter 6, soil chemical processes affecting contaminants' availability strongly depend on the variability of soil properties including pH, organic matter content and clay content as well as Al and Fe-oxides for anionic metalloids including As (Kabata-Pendias, 2004; Clemente et al., 2005; Moreno et al., 2005; Kalis et al., 2007; Anawar et al., 2008; Bakkaus et al., 2008; Rodrigues et al., 2010b). The implicit relationships between the occurrence of PTE's in soil and the impact of soil properties on PTE availability and uptake can be used as a starting point to improve risk assessment methodologies (Wang et al., 2004; Römkens et al., 2009b). The accuracy of predictions of the risk to both environment and human health can be increased including those soil parameters that control the behaviour of inorganic contaminants in soils.

Mechanistic models describing soil-plant transfer of PTE's require soil and plant parameters that are difficult to determine given the complexity of the varied processes involved in soil-plant uptake and translocation of contaminants to plant aerial tissues (Krauss et al., 2002). Empirical models can be used as an approximation of soil-plant transfer mechanisms and to correlate the metal availability to plants with common soil properties (Efroymson et al., 2001; Krauss et al., 2002; Römkens et al., 2009b). Such empirical models have been applied recently to assess metal uptake regarding wildlife plants and specific food crops (wheat, rice, Chinese cabbage, spinach, celery and cole) growing in soils from USA, Slovakia, China and Taiwan (Efroymson et al., 2001; Krauss et al., 2002; Wang et al., 2004; Römkens et al., 2009b). Elements commonly studied were Cd, Zn, Cu, Pb, Cr and Ni but information on other relevant PTE's like Hg, As, Se, U and many others is still lacking.

7.2 Aim, scope and objectives

The aim of this Chapter was to derive relationships between concentrations of eighteen metals and metalloids (Hg, As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Se, B and Mo) in a variety of contaminated and non-contaminated soils in Portugal and their contents in field-grown feed and food crops. The hypothesis that soil-plant transfer of PTE's can be predicted either from the available contents of these elements in soil solutions or from their reactive concentrations in combination with soil properties was tested. The use of such soil-plant relationships in risk assessment and land use management practices will be evaluated as well.

Special attention was given to Hg due to severe Hg soil contamination problems reported in Portugal (Reis et al., 2009; Rodrigues et al., 2010a; Rodrigues et al., 2010b) and since high Hg contents have been observed in terrestrial and salt marsh plants particularly in the area of the "Aveiro Lagoon" due to contamination associated with past effluent emissions from the Estarreja Industrial Complex (Portugal) (Válega et al., 2008; Reis et al., 2009).

7.3 Concentrations of PTE's in plant tissues

The concentrations of PTE's in plant tissues from this dataset are shown in Table 7.1. For Hg both root and shoot samples were analysed while for the remaining elements only shoot contents were measured.

Table 7.1: Concentrations of PTE's observed in plant samples. For Hg both root and shoot samples were analysed while for the remaining elements only shoot contents were measured.

Ryegrass (*Lolium perenne*) (n=73)						**Italian ryegrass (*Lolium multiflorum*) (n=9)**						**Collard greens (*Brassica oleracea)* (n=23)**					
(concentrations d.w.) (mg kg^{-1})						**(concentrations d.w.) (mg kg^{-1})**						**(concentrations d.w.) (mg kg^{-1})**					
		mean	**median**	**min**	**max**			**mean**	**median**	**min**	**max**			**mean**	**median**	**min**	**max**
Hg	shoots	**0.28**	0.038	0.0098	**5.4**	Hg	shoots	**0.29**	**0.30**	0.0089	**0.98**	Hg	shoots	0.047	0.027	0.010	0.18
	roots	1.1	0.055	0.0087	42		roots	1.7	0.99	0.018	5.8		roots	0.079	0.047	0.0050	0.44
Cd	shoots	0.52	0.31	0.010	**5.0**	Cd	shoots	0.15	0.16	0.010	0.27	Cd	shoots	0.11	0.10	0.040	0.38
Zn	shoots	105	100	14	**395**	Zn	shoots	54	38	12	134	Zn	shoots	49	42	19	138
Cu	shoots	15	13	3.2	**56**	Cu	shoots	10	9.8	3.5	**20**	Cu	shoots	3.9	3.6	2.1	8.9
Pb	shoots	25	1.4	0.080	**554**	Pb	shoots	24	19	0.57	**68**	Pb	shoots	0.67	0.68	0.050	1.9
As	shoots	**5.3**	2.2	0.10	**56**	As	shoots	**5.7**	**6.2**	0.10	**13**	As	shoots	0.44	0.30	0.20	1.1
Ni	shoots	5.1	3.2	0.50	48	Ni	shoots	4.7	5.2	2.7	7.3	Ni	shoots	1.1	0.90	0.30	3.8
Cr	shoots	9.4	5.6	0.80	110	Cr	shoots	6.1	6.2	3.3	8.9	Cr	shoots	1.8	1.6	0.80	4.4
Co	shoots	0.61	0.37	0.050	3.4	Co	shoots	0.32	0.23	0.120	1.0	Co	shoots	0.20	0.17	0.040	0.65
Ba	shoots	16	12	1.4	80	Ba	shoots	19	18	8.2	35	Ba	shoots	26	19	9.2	70
U	shoots	0.049	0.040	0.010	0.20	U	shoots	0.02	0.02	<0.01	0.02	U	shoots	0.014	0.010	<0.01	0.020
Sb	shoots	1.0	0.18	<0.02	15	Sb	shoots	0.94	0.77	0.020	2.6	Sb	shoots	0.049	0.050	0.020	0.12
B	shoots	3.9	2.0	1.0	20	B	shoots	1.4	1.0	<1	3.0	B	shoots	27	24	12	58
Se	shoots	0.8	0.4	0.1	28	Se	shoots	0.3	0.3	0.1	0.4	Se	shoots	0.5	0.4	0.1	1.3
Mo	shoots	1.1	0.86	0.10	5.0	Mo	shoots	0.30	0.26	0.080	0.58	Mo	shoots	2.4	1.3	0.11	7.3
Mn	shoots	156	91	11	935	Mn	shoots	154	156	83	214	Mn	shoots	59	52	10	176
		(%)						**(%)**						**(%)**			
Fe	shoots	0.075	0.041	0.010	0.31	Fe	shoots	0.054	0.046	0.022	0.11	Fe	shoots	0.031	0.029	0.015	0.059
Al	shoots	0.04	0.03	0.01	0.13	Al	shoots	0.02	0.01	<0.01	0.03	Al	shoots	0.03	0.01	<0.01	0.14

Note: Values exceeding quality criteria shown in Table 7.2 are underlined and marked in bold

Table 7.1 (cont.)

Orchard grass (*Dactilis glomerata*) (n=12)						**Rye (*Secale cereale*) (n=11)**					
(concentrations d.w.) (mg kg^{-1})						**(concentrations d.w.) (mg kg^{-1})**					
		mean	**median**	**min**	**max**			**mean**	**median**	**min**	**max**
Hg	shoots	0.031	0.012	0.0060	0.10	Hg	shoots	0.014	0.011	0.0071	0.027
	roots	0.044	0.015	0.010	0.16		roots	0.043	0.042	0.013	0.084
Cd	shoots	0.040	0.035	0.010	0.11	Cd	shoots	0.10	0.11	<0.01	0.20
Zn	shoots	44	47	11	63	Zn	shoots	58	31	18	144
Cu	shoots	8.4	8.0	2.6	15	Cu	shoots	8.3	6.6	3.9	15
Pb	shoots	1.4	0.44	0.26	5.6	Pb	shoots	**0.74**	0.58	0.29	**1.8**
As	shoots	0.87	0.65	0.40	1.8	As	shoots	0.54	0.40	0.10	1.6
Ni	shoots	3.8	3.4	1.70	7.7	Ni	shoots	3.2	3.1	1.4	5.5
Cr	shoots	8.9	7.4	4.6	17	Cr	shoots	6.7	6.2	3.2	11
Co	shoots	0.41	0.34	0.090	0.97	Co	shoots	0.31	0.27	0.13	0.55
Ba	shoots	10	8.4	4.1	25	Ba	shoots	10	9.8	6.2	16
U	shoots	0.038	0.035	0.010	0.11	U	shoots	0.012	0.010	<0.01	0.020
Sb	shoots	0.18	0.21	0.02	0.29	Sb	shoots	<0.02	<0.02	<0.02	<0.02
B	shoots	3	3	2	5	B	shoots	2	2	1	3
Se	shoots	0.3	0.3	0.2	0.5	Se	shoots	0.2	0.2	0.1	0.4
Mo	shoots	1.4	1.2	0.62	3.0	Mo	shoots	0.25	0.19	0.12	0.57
Mn	shoots	73	68	47	107	Mn	shoots	40	30	19	83
		(%)						**(%)**			
Fe	shoots	0.052	0.032	0.016	0.16	Fe	shoots	0.043	0.040	0.023	0.079
Al	shoots	0.04	0.04	0.01	0.09	Al	shoots	0.03	0.03	0.01	0.07

Although Hg concentrations in roots appear to slightly exceed shoot contents in all types of plants (Table 7.1), the analysis of variance of results did not show a statistically significant difference (p=0.18) between root and shoot Hg contents, indicating that roots are not always a barrier to soil-shoot transfer of Hg, as suggested by past studies (Patra et al., 2004; Peralta-Videa et al., 2009). As shown in Figure 7.2, a positive log-linear relationship between shoot and root Hg concentrations was also observed. A study with salt marsh plants conducted in Portugal also reported a linear relationship between roots and stem Hg concentrations (Válega et al., 2008). These results are also in line with those from Heeraman et al. (2001) who observed high Hg concentrations in above ground plant tissues at a mining area.

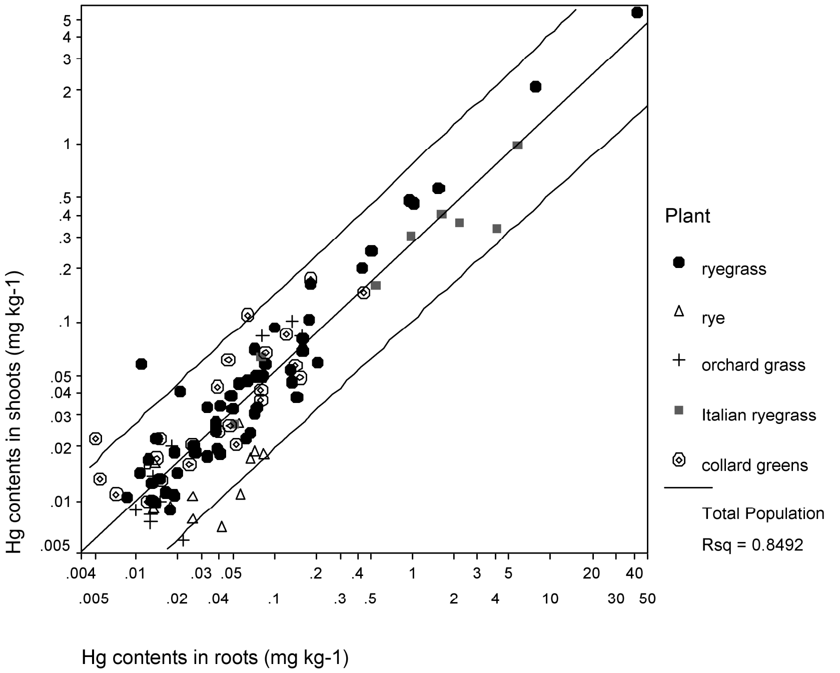


Figure 7.2: Scatterplot of Hg contents in roots vs. shoot concentrations

To further evaluate plant uptake of Hg from our soils, the relationship between root Hg contents and total pools of Hg in soils was analysed (Figure 7.3). A positive log-linear relationship between total pools of Hg in soils and roots concentrations occurs particularly for ryegrass and Italian ryegrass showing that for plants growing on contaminated soils the uptake of Hg can effectively be important (Figure 7.3). These results are particularly relevant since perennial ryegrass cultures have recently been used for biomonitoring of airborne mercury (De Temmerman et al., 2007).

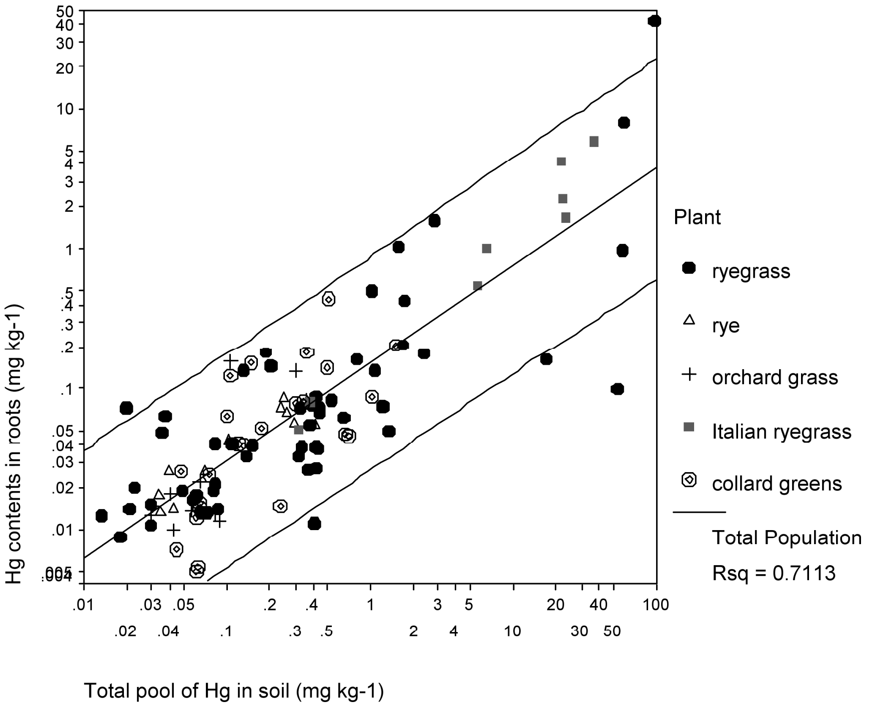


Figure 7.3: Scatterplot of Hg contents in roots vs. soil total concentrations

According to Table 7.1, concentrations of PTE's in feedstuffs (particularly ryegrass and Italian ryegrass) generally exceeded those in collard greens and rye. The high contents of PTE's observed in ryegrass may lead to the impairment of animal health as well as accumulation of contaminants in animal products (organs and muscle) indirectly leading to risks to human health.

To evaluate potential risks to environment, animals and humans, results given in Table 7.1 were compared with data for quality criteria for food and feedstuffs and limits in view of phytotoxicity available from literature (Table 7.2). Levels of Hg, Cd, Zn, Cu, Pb and As in ryegrass exceeded limits for green fodder. For Cd the green fodder limit was exceeded in 7 samples of ryegrass collected at the industrial area. For Zn, the majority of contaminated ryegrass shoots (8 samples) were also from Estarreja, while for Pb, the contamination occurred in 7 samples from the Caveira mining area. For Hg, contaminated ryegrass samples were collected both from the industrial area (7 samples) and from the mining areas (1 in Lousal and 6 in Caveira). The same was observed for As, for which the green fodder limit was surpassed in 17 samples from Estarreja, 4 samples from Lousal, 7 samples from Caveira and 6 samples from Aljustrel. Copper limits in green fodder were exceeded in 29 ryegrass samples, distributed among all sampling areas, including 1 sample from Esposende. Since this site is not located in the vicinity of urban, industrial or mining activities, the Cu level in this sample is expected to be related with agricultural practices, particularly fungicide application.

Along with ryegrass, green fodder limits of Hg (6 samples), Cu (1 samples), Pb (2 samples) and As (6 samples) were also exceeded in Italian ryegrass from the Caveira mining area. Concentrations of Pb in 5 rye samples from the Aljustrel mining area samples were above the food safety limit for cereals.

Finally, phytotoxicity limits for Hg were exceeded in 1 ryegrass sample from the industrial area and in 1 sample from the Caveira mining area. In 3 samples from Caveira, both Pb and As concentrations of ryegrass shoots were above phytotoxicity limits.

Since plant growth as well as animal and human health may be at risk at the most contaminated sites it is crucial to better understand factors controlling uptake processes, particularly the transfer of contaminants into feed crops.

Table 7.2: Quality criteria for feed and food crops in view of food safety, animal health protection and toxicity to plants

Quality criteria (mg kg-1 d.w.)				
Criteria	**Food safety**		**Animal health**	**Phytotoxicity**
PTE	**Cereals***	**Leaf vegetables****	**Feed materials (green fodder)*****	**Grass**
Hg	0.09****(c)	0.2****(c)	0.11 (b)	1-3 (c;j)
Cd	0.30 (a)	1.3 (a)	1.1 (b)	5-30 (c;j)
Pb	0.61 (a)	2 (a)	34 (b)	30-300 (c;j)
As			2.3 (b)	5-20(j)
Mo			10 (d)	10-50 (j)
Ni				10-100(j)
Cr				5-30(j)
Co			10 (e)	15-50 (f;j)
Cu			15 (g,h)	20-100 (j)
Zn			150 (h)	100-400(j)
Sb		100 (i)		150(j)
Ba				500(j)
Se				5-30(j)
Mo				10-50(j)
B				50-200(j)

* excluding bran, germ, wheat and rice for Cd. Cereals quality criteria is originally given as 0.20, 0.10 and 0.03 f.w. (EC, 2006e; de Vries et al., 2007). For d.w. calculations a 67% moisture content (rye samples, this study) was considered.

** leaf vegetables quality criteria is originally given as 0.30, 0.20 and 0.03 for Pb, Cd and Hg f.w. (EC, 2006e; de Vries et al., 2007) For d.w. Calculations a moisture content of 85% was considered (Brassica vegetables, this study)

*** green fodder criteria is originally given as 30, 1, 2 and 0.1 for Pb, Cd, As and Hg on the basis of 12% moisture content (EC, 2002b)

**** currently not applicable

(a) Maximum levels in foodstuffs (EC, 2006e)

(b) Undesirable substances in animal feed (EC, 2002b)

(c) de Vries et al., 2007

(d) O'Connor et al., 2001

(e) Gal et al., 2008

(f) Scheckel et al., 2009

(g) Advisory Committee on Animal Feedingstuffs (ACAF, 2000)

(h) Additives in Feedingstuffs (EC, 2006f)

(i) Tschan et al., 2009 (assuming a TDI of 100 mg/ day for humans and consumption of 1kg of leaf vegetables per day)

(j) Kabata-Pendias, 2001, general crop-unspecific overview

7.4 Soil-to-plant transfer functions and analysis of the role of soil properties on uptake of PTE's by crops

Multiple regression models with soil concentration and soil properties as variables have been used for estimating the uptake of inorganic contaminants by plants (Efroymson et al., 2001). Regression models were derived using experimental data from this study (PTE's concentration in plants; total pools of PTE's; and soil properties) and Eq.4.6 (Chapter 4), linearized by log-transformation. Results obtained for the various soil-to-plant (STP) transfer models are shown in Table 7.3. Data for orchard grass was not included in Table 7.3 because regression analysis did not allow to obtain significant model fits for the majority of the elements.

Regression analysis produced significant model fits for 11 elements studied in ryegrass samples, although the r^2 values of Ni, Ba and U are relatively low. Soil pH had a significant contribution for Cd, Zn, Cu, Co and Sb models while Org C contributed to improve the performance of Cd, Zn, Cu and Co model. The Pb model was improved by the inclusion of $Al_{am\text{-}ox}$.

For Italian ryegrass samples, regression analysis produced significant model fits for 8 elements. The inclusion of soil properties did not have a significant effect in performance of the models for Italian ryegrass.

In the cases of rye and collard greens significant model fits were obtained for 7 and 4 PTE's, respectively and no effect of the inclusion of soil properties was observed as well.

The hypotheses that significant STP transfer models can be derived from available pools of PTE's (Eq. 4.7, Chapter 4) or from a combination of reactive pools and soil properties (Eq. 4.8, Chapter 4) were tested. The coefficients of the STP transfer functions for the various elements included in our study are shown in Table 7.4. The r^2 values for Cd, Zn, Hg, Pb and Cu in ryegrass samples varied between 0.4 and 0.6 for both models (Table 7.4), showing that between 40 and 60 % of the variability of ryegrass contents of these contaminants could be explained using both the $CaCl_2$ extraction and the SPT model. Likewise, these two models were successful in explaining most part of the variability of Cd, Zn, Hg, Pb and Cu as well as As, Sb and Ba concentrations in Italian ryegrass (r^2 values between 0.5 and 0.9) (Table 7.4).

For Co, U and Ni only a few relationships of poor quality could be derived (Table 7.4). The generally low Co (<3.4 mg kg^{-1}), U (<0.2 mg kg^{-1}) and Ni (48 mg kg^{-1}) plant contents as well as respectively low contamination and availability of these elements in soils (available pools were <2.5 mg kg^{-1} for Ni, <2.4 mg kg^{-1} for Co and <0.33 mg kg^{-1} for U) caused difficulties in the derivation of significant relationships. Nickel phytotoxicity has been observed only for highly contaminated soils (total Ni >200 mg kg^{-1}) (Siebielec et al., 2007). Other processes such as competition of Ca during element translocation may have affected the performance of the empirical models for Ni (Kalis et al., 2007). For the remaining studied elements, particularly Cr, B, Se and Mo no significant empirical relationships could be obtained from neither their available nor their reactive pools and soil properties. Despite certain variability in concentrations of these elements in plant samples the ranges of their respective available and reactive concentrations in soil were relatively narrow which did not allow to effectively evaluate the impact on plant uptake. Given the complexity of the geochemistry of these elements and the possible occurrence of different oxidation states, particularly for Cr, Se and Mo, other variables (e.g. redox potential) not included in this analysis may also be needed to explain the availability of this element to plants and the variability of shoot contents. Moreover, other authors reported that due to their toxicity few plants accumulate Cr (Peralta-Videa et al., 2009).

Table 7.3: Computed multiple linear regression coefficients

			Linear regression coefficients							
			log(a)	***b***	***c***	***d***	***e***	***f***	***r^2***	**S.E.**
	PTEs		***(interc.)***	**(log[PTE$_{total}$])**	**(pH)**	**(log % OrgC)**	**(log Al$_{ox}$)**	**(log Fe$_{ox}$)**		
ryegrass (n=73)	**Hg**	**roots**		0.61					**0.62**	0.44
		shoots	0.83	0.52			-0.29		**0.64**	0.38
	Cd	**shoots**	0.76	0.82	-0.15	-0.79			**0.43**	0.39
	Zn	**shoots**	1.5	0.53	-0.11	-0.51			**0.60**	0.19
	Cu	**shoots**	1.1	0.34	-0.097	-0.41			**0.50**	0.19
	Pb	**shoots**		0.95			-0.56		**0.72**	0.41
	As	**shoots**	-0.99	0.70					**0.53**	0.38
	Ni	**shoots**		0.49					**0.10***	0.36
	Co	**shoots**		0.94	-0.16	-0.66			**0.51**	0.32
	Ba	**shoots**	0.50	0.32					**0.08***	0.31
	U	**shoots**	-1.5	0.39					**0.16***	0.33
	Sb	**shoots**		0.69	-0.22				**0.60**	0.45
Italian ryegrass (n=9)	**Hg**	**roots**		0.87					**0.98**	0.15
		shoots		0.63					**0.95**	0.16
	Cd	**shoots**		1.1	-0.54				**0.73**	0.18
	Zn	**shoots**		0.88					**0.75**	0.17
	Cu	**shoots**	-0.34	0.55					**0.83**	0.09
	Pb	**shoots**	-1.3	0.83					**0.91**	0.19
	As	**shoots**	-1.7	0.98					**0.86**	0.25
	Ni	**shoots**	n.s.							
	Co	**shoots**	-2.3	1.3					**0.48***	0.22
	Ba	**shoots**	n.s.							
	U	**shoots**	n.s.							
	Sb	**shoots**	-0.96	0.58					**0.83**	0.13
Collard greens (n=23)	**Hg**	**roots**	1.8	0.59				-0.95	**0.69**	0.30
		shoots	1.6	0.37				-0.56	**0.54***	0.26
	Cd	**shoots**	n.s.							
	Zn	**shoots**	n.s.							
	Cu	**shoots**	n.s.							
	Pb	**shoots**	-1.9	0.86					**0.26***	0.43
	As	**shoots**	n.s.							
	Ni	**shoots**	n.s.							
	Co	**shoots**		0.74	-0.23		-0.31		**0.55***	0.20
	Ba	**shoots**	n.s.							
	U	**shoots**	n.s.							
	Sb	**shoots**	n.s.							
Rye (n=11)	**Hg**	**roots**		0.64					**0.85**	0.12
		shoots		0.28					**0.40**	0.15
	Cd	**shoots**	n.s.							
	Zn	**shoots**		0.65					**0.77**	0.17
	Cu	**shoots**		0.39					**0.76**	0.11
	Pb	**shoots**	-0.84	0.40					**0.45***	0.18
	As	**shoots**	-1.2	0.58					**0.73**	0.15
	Ni	**shoots**	n.s.							
	Co	**shoots**	n.s.							
	Ba	**shoots**		0.82					**0.40***	0.11
	U	**shoots**	n.s.							
	Sb	**shoots**	n.s.							

All r^2 coefficients are significant at the $p<0.001$ level, with the exception of those indicated by an asterisk (*) which are significant at the $p<0.05$ level; n.s.= not significant at the $p<0.05$ level

Table 7.4: Computed multiple linear regression coefficients for Freundlich-type models and respective model performance parameters (r^2 and standard error of estimates)

			Linear regression coefficients (Eq. 4.7)				***Linear regression coefficients (Eq. 4.8)***							
			log(a)	***b***	***r^2***	**S.E.**	***log(a)***	***b***	***c***	***d***	***e***	***f***	***r^2***	**S.E.**
	PTE's		***(interc.)***	**(log[$PTE_{available}$])**			***(interc.)***	**(log[$PTE_{reactive}$])**	**(pH)**	**(log % OrgC)**	**(log Al_{ox})**	**(log Fe_{ox})**		
ryegrass (n=73)	**Hg**	**roots**	1.9	0.64	**0.32**	0.59	2.8	1.0			-0.68		**0.44**	0.55
		shoots	1.7	0.56	**0.40**	0.48	2.5	0.91			-0.59		**0.52**	0.43
	Cd	**shoots**	0.44	0.70	**0.53**	0.36	1.5	0.82	-0.20	-1.1			**0.52**	0.38
	Zn	**shoots**	1.7	0.36	**0.68**	0.17	2.4	0.41	-0.15	-0.52			**0.59**	0.19
	Cu	**shoots**	1.1	0.28	**0.38**	0.20	1.4	0.35	-0.10	-0.54			**0.50**	0.19
	Pb	**shoots**	0.31	0.66	**0.39**	0.60	2.2	0.80	-0.24		-1.0		**0.61**	0.49
	As	**shoots**	0.46	0.36	**0.24**	0.49	2.1	0.28	-0.22		-0.48		**0.22***	0.50
	Ni	**shoots**	0.70	0.28	**0.06***	0.37	n.s.							
	Co	**shoots**		0.51	**0.33**	0.37	1.0	0.72	-0.22	-0.96			**0.48**	0.33
	Ba	**shoots**	0.90	0.37	**0.17**	0.29	0.72	0.29					**0.14**	0.30
	U	**shoots**	n.s.					0.74	-0.13		-0.50		**0.37**	0.29
	Sb	**shoots**		0.69	**0.24***	0.61	n.s.							
Italian ryegrass (n=9)	**Hg**	**roots**	2.2	1.1	**0.87**	0.34	2.0	2.0					**0.90**	0.30
		shoots	1.8	0.76	**0.74**	0.35	1.6	1.4					**0.88**	0.24
	Cd	**shoots**	0.85	1.1	**0.92**	0.12	4.0	1.4	-0.74				**0.89**	0.16
	Zn	**shoots**	1.4	0.39	**0.53**	0.23	0.89	0.71					**0.90**	0.11
	Cu	**shoots**	0.85	0.28	**0.49**	0.16		0.53					**0.68**	0.13
	Pb	**shoots**	0.74	0.59	**0.71**	0.35		0.78					**0.78**	0.30
	As	**shoots**	0.64	0.71	**0.74**	0.34		0.64					**0.63**	0.41
	Ni	**shoots**	n.s.				n.s.							
	Co	**shoots**	n.s.				-0.79	0.63					**0.54**	0.21
	Ba	**shoots**	0.76	0.58	**0.66**	0.11		0.63					**0.47**	0.14
	U	**shoots**	n.s.				n.s.							
	Sb	**shoots**	0.32	0.37	**0.71**	0.17	0.17	0.36					**0.81**	0.14

- All r^2 coefficients are significant at the p<0.001 level, with the exception of those indicated by an asterisk (*) which are significant at the p<0.05 level
- n.s.= not significant at the p<0.05 level

Table 7.4 (cont.)

			Linear regression coefficients (Eq. 4.7)				*Linear regression coefficients (Eq. 4.8)*							
			log(a)	***b***	r^2	**S.E.**	***log(a)***	***b***	***c***	***d***	***e***	***f***	r^2	**S.E.**
	PTEs		***(interc.)***	**(log[PTE$_{available}$])**			***(interc.)***	**(log[PTE$_{reactive}$])**	**(pH)**	**(log % OrgC)**	**(log Al$_{ox}$)**	**(log Fe$_{ox}$)**		
Collard greens (n=23)	**Hg**	**roots**												
		shoots												
	Cd	**shoots**	n.s.					0.41		-0.57			**0.35**	0.21
	Zn	**shoots**	1.6	0.20	**0.17**	0.21	n.s.							
	Cu	**shoots**	n.s.				n.s.							
	Pb	**shoots**						0.80		-1.7			**0.40***	0.40
	As	**shoots**	n.s.				n.s.							
	Ni	**shoots**		0.32	**0.20***	0.21		0.28					**0.17***	0.22
	Co	**shoots**	-0.34	0.4	**0.37***	0.22		0.70	-0.19	-0.77			**0.62**	0.18
	Ba	**shoots**	1.1	0.56	**0.26***	0.23	n.s.							
	U	**shoots**	n.s.				n.s.							
	Sb	**shoots**		0.4	**0.68***	0.12	n.s.							
Rye (n=11)	**Hg**	**roots**	n.s.				n.s.							
		shoots	n.s.				n.s.							
	Cd	**shoots**	0.45	0.97	**0.94**	0.11	n.s.							
	Zn	**shoots**	1.6	0.74	**0.87**	0.13	1.1	0.39					**0.71**	0.19
	Cu	**shoots**	0.95	0.42	**0.65***	0.13	0.50	0.28					**0.72***	0.11
	Pb	**shoots**	n.s.				-0.68	0.49					**0.44***	0.18
	As	**shoots**	n.s.				n.s.							
	Ni	**shoots**	n.s.				n.s.							
	Co	**shoots**	n.s.				n.s.							
	Ba	**shoots**	0.73	0.38	**0.58***	0.08	n.s.							
	U	**shoots**	n.s.				n.s.							
	Sb	**shoots**	n.s.				n.s.							

- All r^2 coefficients are significant at the $p<0.001$ level, with the exception of those indicated by an asterisk (*) which are significant at the $p<0.05$ level

n.s.= not significant at the $p<0.05$ level

According to Table 7.4, log(a) values varied between 0.31 and 1.7 for ryegrass and between 0.32 and 1.8 for Italian ryegrass when using the available pool of PTE's as a predictor for element concentrations in plant shoots. These log(a) coefficients decreased in the order Zn=Hg> Cu> Ba> Ni> As> Cd> Pb> Co and Hg> Zn> Cu=Cd> Ba> Pb> As> Sb for rygrass and Italian ryegrass respectively. These results are in line with those obtained by Krauss et al. (2002) who observed intercept values decreasing in the same order for Zn, Cu, Cd and Pb. The coefficient log(a) obtained for the transfer functions reflects the capability of a plant to uptake PTE's from soils and it may be used to identify plants accumulating inorganic contaminants at certain contaminated areas for example for monitoring and/ or remediation purposes (Krauss et al., 2002). In this study we observed log(a) values above 1 for Zn (1.7), Hg (1.7) and Cu (1.1) in ryegrass shoots and for Hg (1.8) and Zn (1.4) in Italian ryegrass (Table 4) which indicates an ability of these crops to accumulate these elements. The log(a) values for Hg were slightly higher in roots (1.9/2.2) that in shoots (1.7/1.8) for both plants (Table 4) indicating a significant contribution of Hg roots uptake and translocation within plants to the shoot Hg contents in comparison with other processes such as soil volatilization and atmospheric deposition as well as root immobilization of this metal, similarly to what was observed by Válega et al. (2008).

According to Table 7.4, coefficients b varied between 0.70 and 0.28 for ryegrass shoots using Eq. 4.8 and decreased in the following order: Cd> Sb> Pb> Hg> Co> Ba> Zn=As> Cu=Ni. For Italian ryegrass, b coefficients decreased in the order Cd> Hg> As> Pb> Ba> Zn> Sb> Cu, varying between 1.1 and 0.28 (Table 7.4). The b coefficient, or slope, reflects the ability of the plant to actively influence element accumulation, with a small coefficient for these Freundlich-type models resulting in a strong curvature and indicating a higher active uptake at low soil available concentrations as well as a reduced uptake at higher contamination levels (Krauss et al., 2002). This is particularly the case of Zn, As, Ni and Cu for ryegrass plants and of Cu for

Italian ryegrass samples included in this study which showed b coefficients between 0.28 and 0.36 (Table 7.4).

Low slope (b) values (0.13) associated with high intercepts (log(a)) (2.81) for linearized Freundlich-type models for Cu in wheat plants were obtained by Krauss et al. (2002) indicating that the plants included in both studies have high ability to accumulate Cu although showing lower uptake at higher soil available concentrations. Thus plants growing in highly contaminated soil may have similar or even lower Cu concentrations in aboveground parts than plants grown in soils with low Cu concentrations (Krauss et al., 2002). In contrast, Hg showed relatively high values for both a (1.7/1.8) and b (0.56/ 0.76) for shoots of feed crops included in our study indicating that these plants have capability to accumulate Hg from soil available contents and do not show a significant ability to reduce uptake at higher available levels. For Hg, the b coefficient of roots is higher than that of shoots and is even higher than 1 (b=1.1) in the case of Italian ryegrass samples (Eq. 4.8, Table 7.4) indicating that roots have lower ability to actively control metal accumulation compared to the above ground plant parts and that root uptake can even increase with contamination levels. This also contributes to explain why higher concentrations of Hg were observed in roots when compared to shoot contents.

For Cd, which showed b coefficients around 1 for Eq. 4.8 (Table 7.4), the transfer functions approached a linear relationship between soil available pools and shoot concentrations (particularly for Italian ryegrass) indicating that Cd contents in shoots can only be controlled by the plants to a lesser extent.

Results from the multiple linear regression analysis (Table 7.4) proved that Org C, pH and Al_{ox} were important variables that influenced the transfer of PTE's from soil to Italian ryegrass. Except for Ni and Cr, highly significant ($p < 0.001$) SPT models can be derived which explain between 47 % (for Ba) and 90 % (for Hg and Zn) of the observed variation on levels in plants. Also the solution model based on $CaCl_2$ was able to explain between 53 % (Zn) and 87 % (Hg) of the observed variation in plant levels. These results confirm that the uptake by Hg, Zn, Cd, Cu, Pb, As, Ba, and Sb is related to the chemical availability in soil.

From Table 7.4 we observed that in the case of ryegrass the log(a) values decreased in the order Hg> Zn> Pb> As> Cd> Cu> Co> Ba and that in these soils three properties (Org C, Al_{ox} and pH) were the most important variables affecting plant levels. Regression coefficients shown in Table 7.4 indicate that at lower levels of Org C and Alox or lower pH, the uptake of certain PTE's increases. This is justifiable since processes such as adsorption of contaminants to the soil solid-phase are metal-specific and depend on soil properties like pH, organic matter and amorphous Al and Fe oxides. (Sauvé et al., 2000; Weng et al., 2001; Yin et al., 2002; Gustafsson et al., 2003; Rieuwerts et al., 2006). In this study, Org C was particularly relevant in the cases of Cd, Co, Cu and Zn whereas Alox was most important for Pb, Hg, As and U and increasing pH affected negatively the uptake of Cd, Pb, As, Co, Zn, U and Cu by ryegrass (Table 4). For Italian ryegrass, only pH in the case of Cd revealed a significant control over plant uptake (Table 7.4). The role of pH in controlling Cd uptake from contaminated soils has also been previously observed for several crops (Efroymson et al., 2001; Römkens et al., 2009b).

The fact that soil properties had no significant impact in the SPT model for Italian ryegrass (except for Cd) and that generally higher r^2 values (with the exception of As, Cd and Ba) were obtained for Eq.4.8 (r^2 0.5-0.7) compared to Eq.4.7 (r^2 0.5-0.9) (Table 7.4) can be explained by an ability of these plants to increase the availability of certain PTE's, i.e. to increase the pool of ions on the solid reactive phase that can supply the soil solution without sole depending on the soil conditions to be favourable to cation's desorption. These results are consistent with those from Sterckeman et al. (2005) who observed that Italian ryegrass was able to mobilize non-labile metals from soils. According to these authors this could relate to root excretion of Fe-selective chelators in response to Fe deficiency and which can be involved in the mobilization of Fe as well as other metals and/ or relate to the decrease in pH in the rhizosphere, due to the release of protons and organic acids by roots (Sterckeman et al., 2005).

7.5 Summary and Conclusions

Although food crops included in our study showed levels of contaminants that were generally below food safety limits, the contents of Hg, Pb, As, Cu, Cd and Zn in feed products were higher than limits in feedingstuffs set by EU legislation (EC, 2002b; EC 2006f) which may pose a health risk to animals and may indirectly affect humans through the consumption of animal products. Moreover, while both ryegrass and Italian ryegrass samples showed an ability to actively reduce the uptake of elements such as Cu at the highest contamination levels, this ability was not observed for other toxic contaminants like Hg. Given the well known potential for bioconcentration along the food chain for this metal (Reis et al., 2009) the impact of soil contamination in feed and food supply chains in Portugal should be further investigated.

Both empirical STP models (using reactive soil pools and soil properties) and the $CaCl_2$ soil test were able to explain between 40 and 90 % of the variability in levels of Cd, Zn, Pb, Cu, Hg, As, Sb and Ba in crops. For Co, U and Ni only a few relationships of poor quality could be derived given the low soil contamination levels and plant contents for these elements. Further studies on plant uptake processes for these PTE’s should include wider ranges of contamination particularly for U and Co, for which little information is available. For the remaining elements including Cr, Mo, Se and B no apparent relationship between the availability in soil and levels in crops could be found at these contamination levels indicating that the plant levels observed were independent from the quality of the soil. In this case, the ingestion of contaminated soil (particularly with Cr) can be a more important pathway for the intake of contaminants by cattle compared to the ingestion of feed crops.

The example with Hg and other PTE's showed that the STP model allowed to identifying the most relevant variables controlling elements' availability and influencing the accumulation of inorganic contaminants in arable crops. Italian ryegrass proved to be able to actively mobilize elements from soils and in that case plant contents could be predicted well both from 0.01 M $CaCl_2$ and from 0.43 M HNO_3 extracts alone. For other crops such as ryegrass, the analysis of the factors controlling PTE's uptake by ryegrass revealed that when the levels of Org C and Al_{ox} or pH increase, the uptake of certain PTE's decreases. The content of Org C influenced SPT of Cd, Zn, Cu and Co while pH inversely affected the uptake of Cd, Pb, As, Co, Zn, U and Cu. Soil contents of Al_{ox} played a significant role in the retention of Hg, Pb, As and U in the solid phase, reducing their availability for root uptake. Hence, soil characteristics must be taken into account when defining management practices for these agricultural soils and when evaluating possible changes in land use. At the mining areas where the formation of acid mine drainage (AMD) is known to occur (Luís et al., 2009; Rodrigues et al., 2010b), attention should be given to the impact of AMD in the acidification of soils surrounding the water streams and to potential effects of flooding events in these areas since a decrease in soil pH will lead to an increase in the soil availability of toxic elements for feed crops. The influence of soil properties in SPT must also be taken into consideration if attempting to apply clean-up techniques (such as phytoextraction) at the contaminated areas (Clemente et al., 2005; Koopmans et al., 2008).

Our findings showed that the $CaCl_2$ soil test and the empirical STP models can provide useful information for both site-specific and regional assessments of soil quality. At the local scale, the $CaCl_2$ extraction (a fast and simple soil analysis) can be used to assess the availability of contaminants to plants and to determine whether or not a crop can be grown at a specific site, not only for commonly studied elements (Cd, Zn, Cu and Pb) but also for other contaminants such as Hg, As, Sb and Ba. In addition, the STP model also allows to perform regional assessments of the impact of soil contamination in the quality of feed and food on the basis of existing monitoring data for soil properties including pH, Org C and metal contents. These models provide a better basis for defining threshold values of dangerous element concentrations in soil and regional risk maps than that of linear models or fixed soil quality standards and therefore provide a substantial increase in risk estimation accuracy. This approach can form the basis of a more harmonised strategy for risk assessment methodologies across Europe.

In the future, the application of STP functions to additional food crops (vegetables) should be tested. Possible errors in measurements used to calibrate the regression models as well as uncertainties associated with such models (e.g. variation between cultivars as well as crops; differences associated to different degrees of pollution or climate regimes) must also be further studied.

Chapter 8

Impacts of soil contamination on feed and food supply chains in Portugal

8.1 Introduction

The EU Thematic Strategy for Soil Protection has clearly identified food and biomass production has one of the key soil functions since the quality and safety of feed and food that are traded freely within the internal market can influence animal and human health throughout Europe (EC, 2006a). Cases of contamination of animal feeding stuffs have been reported in the past, as for example when contaminated cattle feed was imported into the UK in 1989-1990 and resulted in Pb, As, Cd and Hg toxicity in some animals (Crews et al., 1992; Beresford et al., 1999; Beresford et al., 2001). Hence, it is crucial to develop strategies to characterize the links between soil contamination, plant uptake, dietary transfer of contaminants to animals and finally consumer exposure from dietary intake of plant and animal products as well as to determine critical concentrations of contaminants in soils in order to deliver safe and high-quality products (de Vries et al., 2007; Franz et al., 2008).

Recently, the HERACLES expert network on Human and Ecological Risk Assessment for Contaminated Land in Europe (initiated in 2005) performed a review of risk assessment tools and concluded that soil quality standards adopted in EU countries are widely variable in multiple aspects (Carlon, 2007). Namely, large uncertainties in risk assessment relate to transfer and exposure modelling and to the selection of exposure pathways. The inclusion/ exclusion of exposure sources such as the consumption of home-grown vegetables and the uncertainties in their characterization have a striking impact in the human health risk assessment across Europe (Carlon, 2007).

An overview of the pathways of contaminants in terrestrial ecosystems that may contribute for animal and human exposure is shown in Figure 8.1. As of now it is believed that plants readily take up elements that are in the soil solution (available pool) in either free ionic or complexed forms and that the available pool of PTE's in soils is in equilibrium with the sorbed or reactive element content in the soil (Kabata-Pendias, 2004; Kalis et al., 2007; Römkens et al., 2009b; Rodrigues et al., 2010b). The degree to which PTE's are available for plant uptake and further accumulation in edible plant parts depends strongly on the degree of pollution and soil properties (Franz et al., 2008; Römkens et al., 2009b; Rodrigues et al., 2010b). Empirical models can be used as an approximation of soil to plant transfer (STP) mechanisms and to correlate the metal availability to plants with common soil properties (Efroymson et al., 2001; Krauss et al., 2002; Römkens et al., 2009b; Rodrigues et al., 2010b).

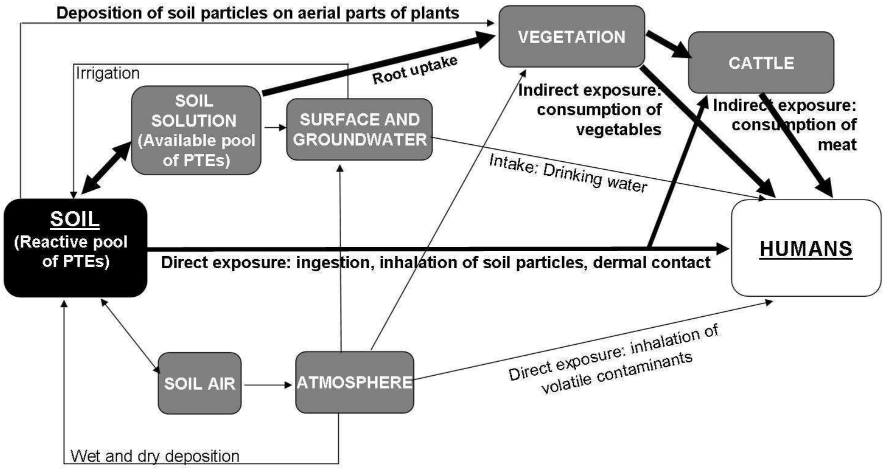


Figure 8.1: Pathways of human exposure to soil contaminants

Since plants constitute the foundation of the terrestrial food chain (Peralta-Videa et al., 2009) toxic elements absorbed from soils can be transferred to higher strata of the food chain and accumulate in specific animal and human organs (Beresford et al., 1999; Lopez-Alonso et al., 2000; Sedki et al., 2003; Prankel et al., 2005; de Vries et al., 2007; Nriagu, et al 2009; Reis et al., 2009). As illustrated in Figure 8.1, grazing animals also involuntarily ingest soil along with grass, particularly in areas where soils are contaminated with or contain high geogenic concentrations of PTE's (Thornton and Abrahams, 1983; Smith et al., 2009).When acutely or chronically ingested by animals and humans some of these elements can impair kidney and liver functions, affect the central nervous or female reproductive system, promote cancer or even cause death (Beresford et al., 2001; Peralta-Videa et al., 2009). Previous studies have determined feed to animal bioaccumulation factors (BAF's) for specific elements as a tool to estimate the levels of contamination in animal tissues if exposed to a contaminated diet (van Hooft, 1995; Beresford et al., 2001; Sedki et al., 2003). For humans, most exposure assessments concerning the dietary intake of PTE's are based on measured concentrations in food products and analysis of consumption patterns (EC, 2004). However, for specific regions, monitoring data are not available (Franz et al., 2008) often due to constraints associated to the costs of monitoring programs. Hence, an approach to assess regional differences in PTE's levels and soil characteristics which may lead to differences in PTE's levels in feed and food crops and eventually to differences in human dietary exposure to these contaminants is necessary.

8.2 Aim, scope and objectives

Measured concentrations of eighteen metals and metalloids (Hg, As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Se, B and Mo) in a variety of contaminated and non-contaminated soils and their respective contents in field-grown feed and food crops will be used to assess the impacts of soil contamination in feed and food supply chains in Portugal. The relative importance of the soil-plant, soil-plant-animal and soil-animal pathways of PTE's to the health of livestock grazing at contaminated sites as well as to health of human populations will also be discussed.

8.3 Analysis of plant-animal and soil-animal pathways

To further analyse the impact of the observed concentrations of PTE's in feed crops on animal health, the daily intake (DI) for cows and sheep was determined following Eq.4.9 (Chapter 4) and based on measured soil and feed PTE's concentrations at each sampling site. The following assumptions were made for the calculation of DI's:

- dry mass intake for cows: 16.9 kg d^{-1} of grass; and 0.41 kg d^{-1} of soil (de Vries et al., 2007);
- dry mass intake for sheep: 2.5 kg d^{-1} of grass; and 0.10 kg d^{-1} of soil (de Vries et al., 2007);
- worst case scenario in which the animals graze at the field all the time and always at the same sites.

Animal DI's of PTE's are given in Table 8.1. The principal source of intake of Cd, Zn, Cr, Se, Mo, B and to a lesser extent Ni, Ba, Cu and Hg was found to be by the ingestion of grass which accounted for a median of 70-98 % of DI of these elements for both cow and sheep (Table 8.1). By contrary, around half of the median intake of Pb, Co, As, U and Sb across all sites was via direct ingestion of soil (Table 8.1). Smith et al. (2009) calculated the daily intake of Cu, Zn and Pb by sheep grazing at contaminated soils and obtained results indicating that most Pb intake is generally in the form of soil.

Animal acceptable daily intake (ADI) for various PTE's (Table 8.2) was calculated according to Eq.4.11 based on limit concentrations for animal organs (food safety and animal health) and the plant-animal bioaccumulation factors given in Table 8.2.

Concentrations of As, Cd, Hg, Pb, Cu and Zn in cow organs (kidney, liver and muscle) estimated on the basis of levels of PTE's in soil and feed ate each sampling site are shown in Figure 8.2a. The food safety and animal health criteria were also included for assessment of potential risks. The estimated levels of As, Cd and Hg in sheep kidney, liver and muscle are shown in Figure 8.2b.

Table 8.1: Calculated daily intake (DI) of PTE's by cow and sheep

		Cow				Sheep			
		DI as grass	**DI as soil**	**Total DI**	**% ingested as grass**	**DI as grass**	**DI as soil**	**Total DI**	**% ingested as grass**
Hg	**mg d^{-1}**	0.15-**92** (0.67)	0.005-**40** (0.17)	0.18-**132** (0.86)	2.7-98 (81) %	0.022-**14** (0.10)	0.001-**9.8** (0.041)	0.028-**23** (0.14)	1.7-98 (72) %
Cd	**mg d^{-1}**	0.17-**84** (4.3)	0.041-1.5 (0.16)	0.21-**85** (4.6)	76-100 (96) %	0.025-**12** (0.64)	0.010-0.37 (0.040)	0.035-**13** (**0.72**)	65-100 (93) %
Zn	**mg d^{-1}**	211-**6686** (1636)	7.0-490 (70)	226-**7175** (1722)	78-99 (95) %	31-**989** (**242**)	1.7-119 (17)	35-**1108** (**264**)	68-98 (92) %
Cu	**mg d^{-1}**	54-**937** (202)	3.0-**362** (40)	65-**1133** (262)	48-98 (81) %	8.0-139 (30)	0.74-88 (9.9)	11-186 (42)	36-96 (72) %
Pb	**mg d^{-1}**	1.4-**9353** (32)	4.2-**2604** (27)	9.0-**10496** (70)	7.2-91 (48) %	0.20-**1384** (4.8)	1.0-**635** (6.5)	1.7-**1685** (13)	4.5-86 (36) %
Ni	**mg d^{-1}**	8.4-806 (55)	1.8-16 (6.6)	13-810 (64)	55-100 (89) %	1.2-119 (8.1)	0.45-3.9 (1.6)	2.2-120 (10)	42-99 (84) %
Co	**mg d^{-1}**	0.85-58 (5.1)	0.21-20 (3.5)	1.2-68 (11)	19-92 (59) %	0.13-8.6 (0.75)	0.050-4.9 (0.85)	0.20-11 (2.1)	12-88 (47) %
Mn	**mg d^{-1}**	186-15802 (1631)	32-1000 (124)	247-16213 (1993)	71-99 (92) %	28-2338 (241)	7.7-244 (30)	42-2438 (305)	60-98 (87) %
Fe	**g d^{-1}**	1.7-52 (7.4)	1.3-42 (9.1)	3.3-70 (18)	18-87 (44) %	0.25-7.6 (1.1)	0.32-10 (2.2)	0.62-13 (3.5)	12-87 (32) %
As	**mg d^{-1}**	1.7-**941** (39)	2.6-**838** (39)	4.5-**1408** (83)	4.8-91 (50) %	0.25-139 (5.8)	0.63-**204** (9.5)	0.93-**253** (16)	3.0-85 (38) %
U	**mg d^{-1}**	0.17-3.4 (0.51)	0.12-5.3 (0.57)	0.29-7.0 (1.2)	14-92 (44) %	0.025-0.50 (0.075)	0.030-1.3 (0.14)	0.055-1.6 (0.23)	9.1-88 (33) %
Cr	**mg d^{-1}**	14-1854 (103)	0.41-29 (7.6)	18-1858 (121)	52-100 (93) %	2.0-274 (15)	0.10-7.0 (1.8)	3.2-275 (19)	39-100 (89) %
Ba	**mg d^{-1}**	24-1355 (217)	6.6-199 (25)	42-1374 (251)	46-99 (89) %	3.5-200 (32)	1.6-49 (6.0)	7.9-205 (41)	34-98 (83) %
Al	**g d^{-1}**	1.7-22 (3.4)	1.7-14 (4.8)	3.4-31 (10)	20-84 (47) %	0.25-3.2 (0.50)	0.42-3.4 (1.2)	0.67-5.9 (2.0)	13-76 (35) %
Sb	**mg d^{-1}**	0.34-249 (0.51)	0.041-64 (0.62)	0.38-313 (2.0)	5.1-94 (58) %	0.050-37 (0.075)	0.010-16 (0.15)	0.060-52 (0.34)	3.1-90 (45) %
Se	**mg d^{-1}**	1.7-470 (5.9)	0.21-8.0 (0.49)	1.9-472 (7.1)	23-99 (92) %	0.25-70 (0.88)	0.050-1.9 (0.12)	0.31-70 (1.1)	15-99 (88) %
Mo	**mg d^{-1}**	1.4-85 (14)	0.082-2.8 (0.33)	1.9-85 (14)	67-100 (98) %	0.20-13 (2.0)	0.020-0.68 (0.080)	0.30-13 (2.1)	56-99 (96) %
B	**mg d^{-1}**	17-338 (34)	1.2-3.0 (1.9)	17-338 (36)	88-99 (96) %	2.5-50 (5.0)	0.31-0.72 (0.47)	2.5-50 (5.4)	82-99 (94) %

Note: Values of daily intake above ADI$_{animal}$ values given in Table 8.2 are shown in bold and underlined

Table 8.2: Limit concentrations of PTE's in crops and animal products, bioaccumulation factors between plant and animal organs ($BAF_{plant-animal}$) and calculated acceptable daily intakes for animals (ADI_{animal})

Plant/ criteria	**Element**	**Limit concentration** (mg kg^{-1} d.w.)	**Animal**	**Element**	**$BAF_{plant-animal}$** (mg kg^{-1} f.w./mg kg^{-1} d.w.)			**Limit concentration** (food safety) (mg kg^{-1} f.w.)			**Limit concentration** (animal health) (mg kg^{-1}f.w.)			**ADI_{animal}** (food safety) (mg d^{-1})			**ADI_{animal}** (animal health) (mg d^{-1})			
					kidney	liver	muscle	kidney	liver	muscle	kidney	liver	muscle	kidney	liver	muscle	kidney	liver	muscle	unspecific
Green fodder (animal health)	Pb	34^{a}	**Cow**	Pb	0.086^{g}	0.0404^{g}	0.0013^{g}	0.50^{f}	0.50^{f}	0.10^{f}	3^{g}	2^{g}		**101**	214	1332	604	857		
	Cd	1.1^{a}		Cd	2.99^{g}	0.554^{g}	0.0033^{g}	1.0^{f}	0.50^{f}	0.050^{f}	5^{g}	1.4^{g}	0.02^{g}	**5.8**	16	262	29	44	105	
	As	2.3^{a}		As	0.0692^{h}	0.0387^{h}	0.016^{h}	2^{k}	2^{k}		14^{l}	14^{l}		**500**	895		3502	6262		
	Hg	0.11^{a}		Hg	0.638^{g}	0.158^{g}	0.00092^{g}	$0.05*^{g}$	$0.05*^{g}$	$0.05*^{g}$	1.4^{g}	2^{g}		**1.4**	5.5	941	38	219		
	Zn	150^{b}		Zn	0.3^{i}	0.5^{i}	0.4^{i}	150^{k}	150^{k}		$135\text{-}175^{l}$	600^{l}		8655	**5193**		7790	20772		
	Cu	$15^{b,c}$		Cu	0.8^{i}	2.8^{i}	0.1^{i}	100^{k}	100^{k}		15^{l}	100^{m}		2164	618		**325**	618		
	Co	10^{d}																		
	Mo	10^{e}																		
Leaf vegetables (food safety)	Pb	2^{f}	**Sheep**	Pb				0.50^{f}	0.50^{f}	0.10^{f}	5^{g}	5^{g}	0.1^{g}							**60**-100^{n}
	Cd	1.3^{f}		Cd	2.08^{g}	1.85^{g}	0.0029^{g}	1.0^{f}	0.50^{f}	0.050^{f}	4^{g}	2^{g}		1.25	**0.7**	45	5	2.8		
	As			As	0.0286^{j}	0.0237^{j}	0.0065^{j}	$2**^{k}$	$2**^{k}$					**182**	219					
	Hg	$0.2*^{g}$		Hg	0.468^{g}	0.0572^{g}	0.00094^{g}	$0.05*^{g}$	$0.05*^{g}$	$0.05*^{g}$	1^{g}	4^{g}		**0.28**	2.3	138	5.6	182		
	Zn			Zn				$150**^{k}$	$150**^{k}$											**150^{n}**
	Cu			Cu				$100**^{k}$	$100**^{k}$											

Note 1: green fodder criteria is originally given as 30, 1, 2 and 0.1 for Pb, Cd, As and Hg on the basis of 12% moisture content (EC, 2002b)

Note 2: leaf vegetables quality criteria is originally given as 0.30, 0.20 and 0.03 for Pb, Cd and Hg f.w. (EC, 2006e; de Vries et al., 2007) For d.w. Calculations a moisture content of 85% was considered (Brassica vegetables, this study)

* currently not applicable

**assumed to be equal to bovine organs

a) Undesirable substances in animal feed (EC, 2002b)
b) Additives in Feedingstuffs (EC, 2006f)
c) Advisory Committee on Animal Feedingstuffs (ACAF, 2000)
d) Gal et al., 2008
e) O'Connor et al., 2001
f) Maximum levels in foodstuffs (EC, 2006e)
g) de Vries et al., 2007
h) van Hooft, 1995
i) Sedki et al., 2003
j) Beresford et al., 2001
k) Nriagu et al., 2009
l) López Alonso et al., 2000
m) Miranda et al., 2009
n) Smith et al., 2009

The lowest ADI value of Pb for cows (101 mg d^{-1}) was determined from the safety threshold limit in kidney regarding food safety (Table 8.2). Although the median Pb total DI for cows across the various sampling sites was below this limit, there is a risk that at the most contaminated sites Pb animal intake will be higher than this ADI (maximum total DI=10496 mg kg^{-1}, Table 8.1). The same situation was observed for sheep (ADI=60-100 mg d^{-1}, maximum total DI=1685 mg kg^{-1}, Table 8.1). In fact, should the animals graze always at the same areas and at the most contaminated sites and the ADI values for Pb regarding both food safety and animal health would be surpassed. This would also lead to an exceedance of limit Pb concentrations in cow kidney, liver and muscle regarding both animal health and food safety in at least 8 sites (Figure 8.2a).

The total ingestion of Hg and Cd at the most contaminated sites which can be as high as 132 and 85 mg d^{-1}, respectively for cows and as high as 23 and 13 mg d^{-1}, for Hg and Cd respectively for sheep (Table 8.1) may surpass the ADI values for these elements regarding animal health (cow: 38 mg d^{-1} for Hg and 29 mg d^{-1} for Cd; sheep: 5.6 mg d^{-1} for Hg and 5 mg d^{-1} for Cd). Such ingestion of Hg and Cd by cows and sheep may also pose food safety risks in the case of consumption of kidney and liver from these animals since ADI values obtained from the critical limits in organs given in Table 8.2 can also be surpassed for livestock grazing exclusively at the contaminated areas (Figure 8.2a,b).

Although median As animal DI values were always below food safety ADI's, the toxicity associated with As may be a problem at the contaminated areas since the ingestion of both contaminated grass and soil leads to a total maximum DI of 1408 mg d^{-1} for cow and 253 mg d^{-1} for sheep (Table 8.1) which are associated with As contents in kidney and liver higher then the food safety limits (Figure 8.2a,b). The As ADI calculated for cows regarding animal health (3502 mg d^{-1}) was never surpassed by the estimated DI of this element, not even at the most contaminated sites (Table 8.1).

Despite general ruminant endurance to Cu and Zn intake (Smith et al., 2009) excessive levels of Cu and Zn in cow kidney and liver regarding food safety limits (Table 8.2) may result from animal diet at the most contaminated sites (maximum DI for cows: 1133 and 7175 mg d^{-1}, for Cu and Zn respectively).

The ADI values for the remaining elements, particularly for Ni, Cr, Co, U, Ba, Sb, Se, Mo and B, could not be calculated due to the lack of $BAF_{plant\text{-}animal}$ from the available literature. Nevertheless, for example in the cases of Ni and Cr, the estimated maximum DI could reach values of 810 and 1858 mg d^{-1}, respectively for cows and of 120 and 275 mg d^{-1} for sheep (Table 8.1) for livestock grazing always at same contaminated sites and possible adverse effects associated to such levels of element intake should be further investigated.

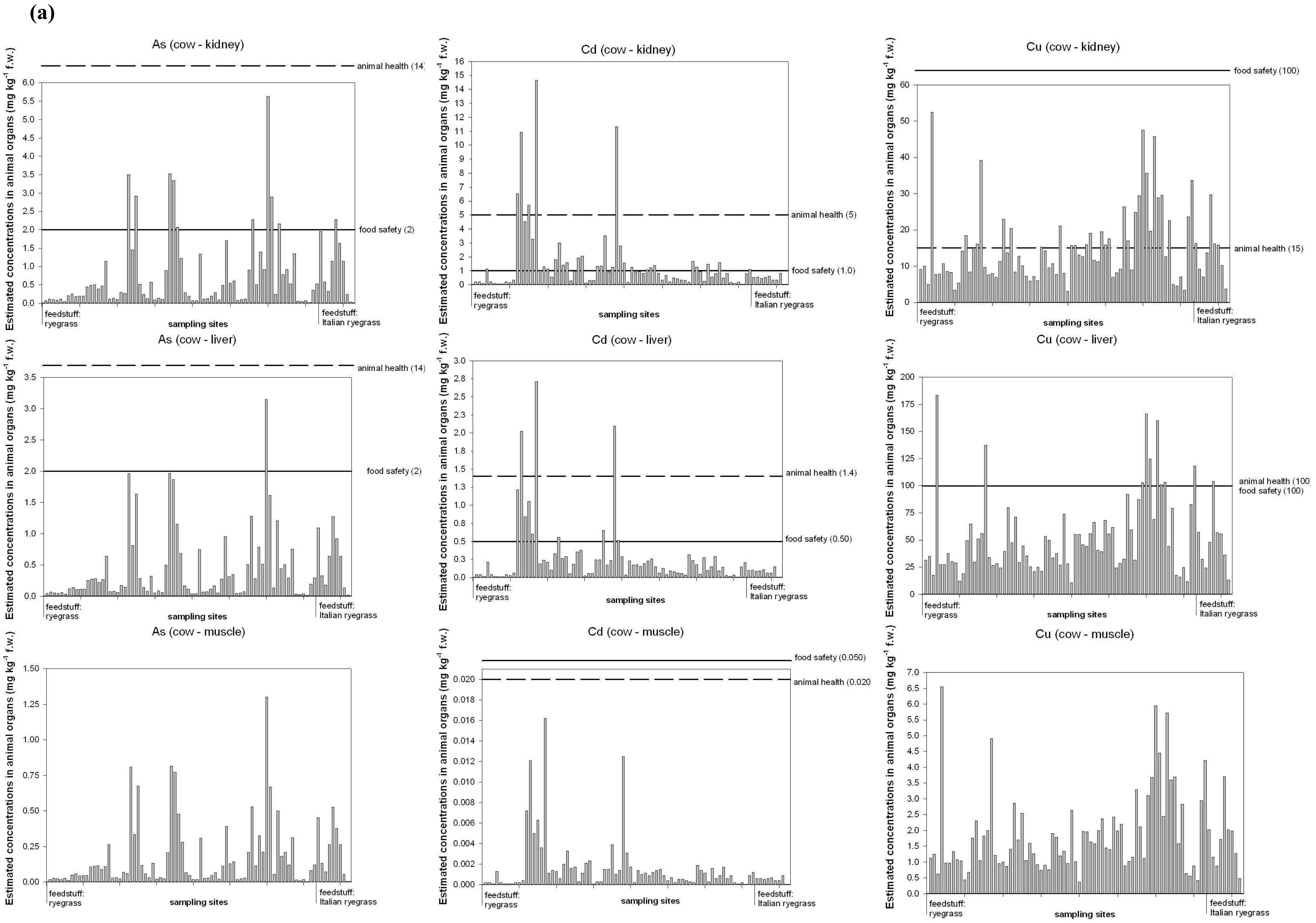


Figure 8.2: levels of PTE's in cow (a) and sheep (b) tissues

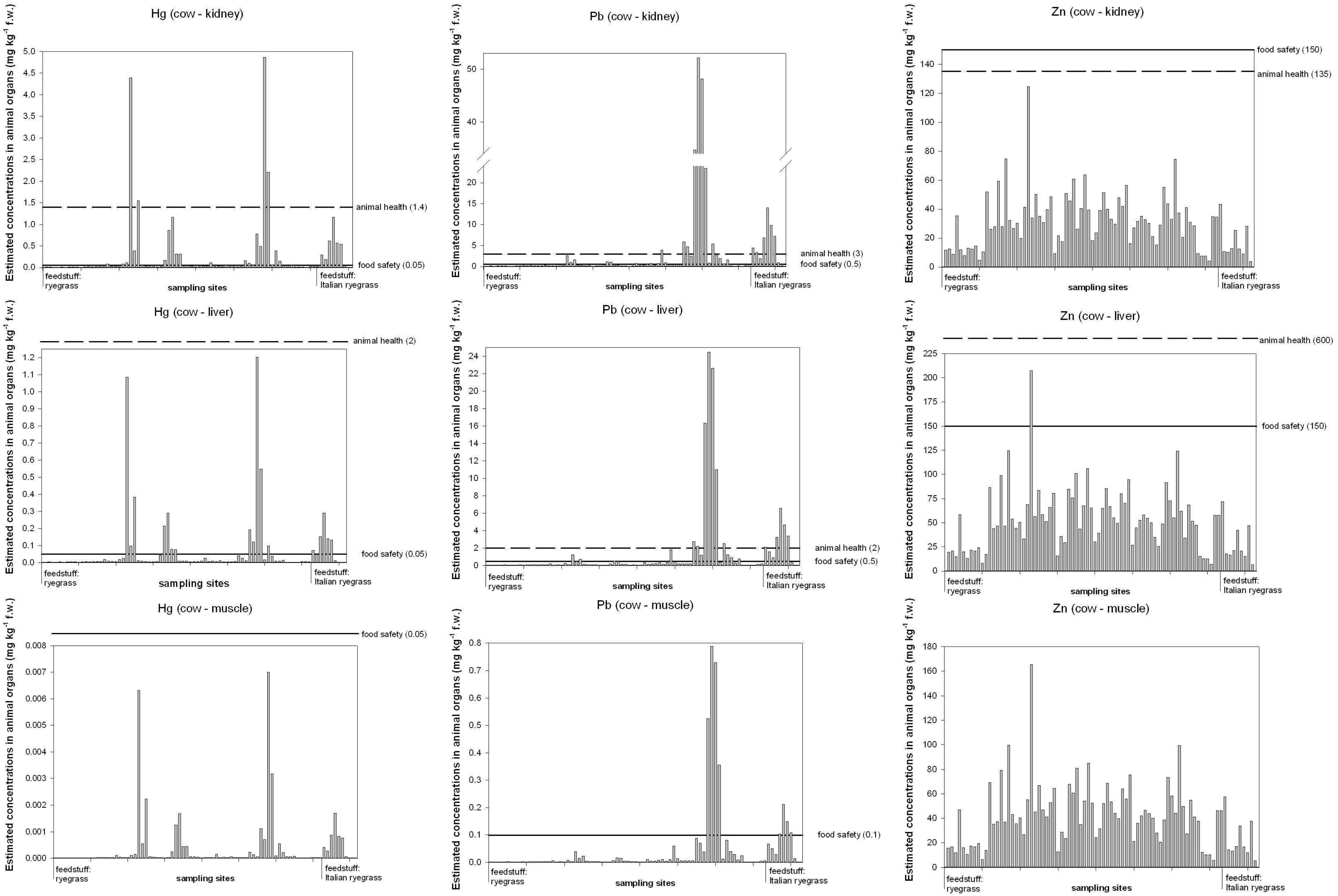


Figure 8.2 (cont.)

(b)

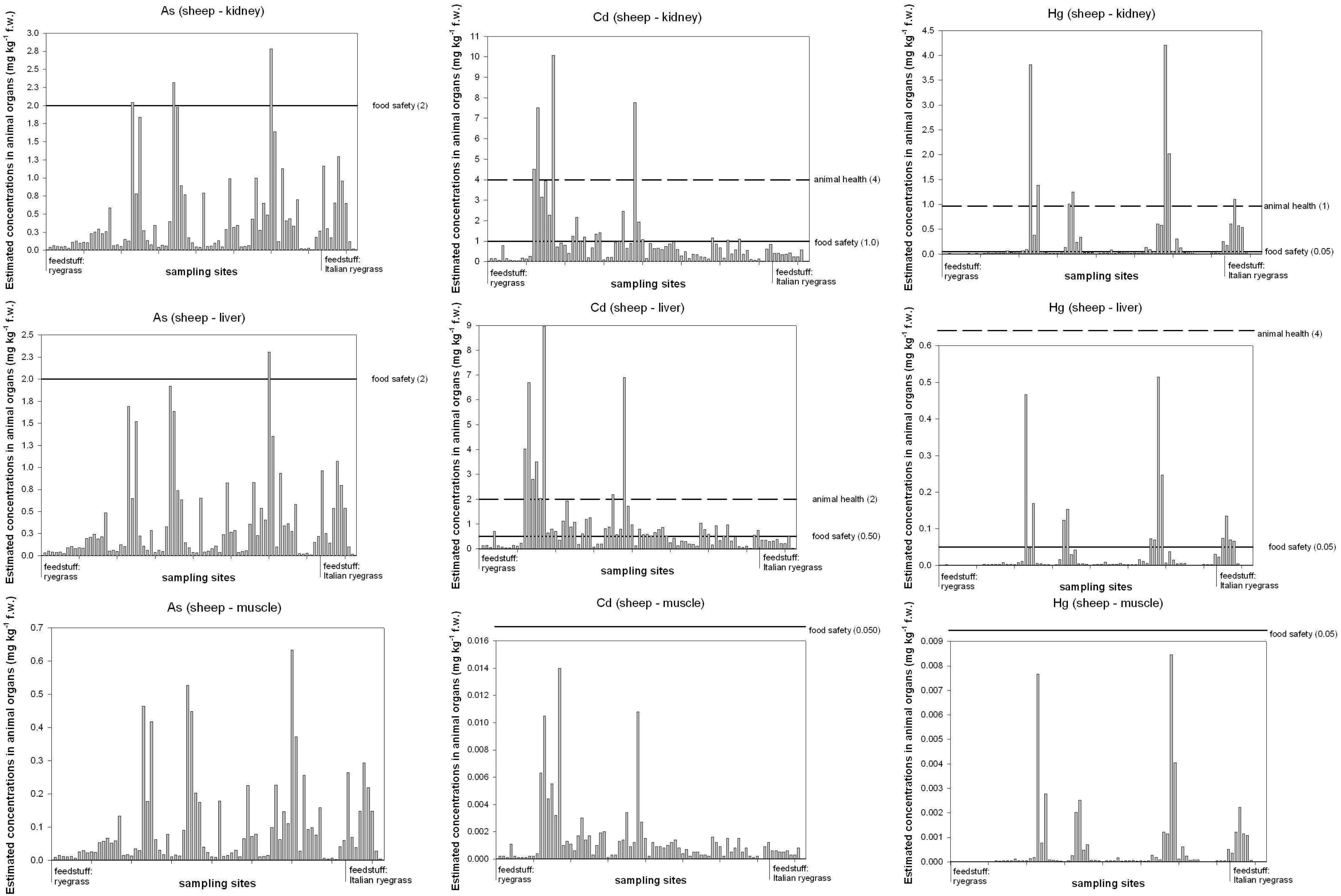


Figure 8.2 (cont.)

8.4 Analysis of soil-plant-human, soil-human and soil-plant-animal-human pathways

I have selected four most toxic elements (As, Cd, Hg and Pb) from the group of PTE's analysed to assess the impacts of soil contamination on human exposure and have calculated the respective human DI's according to Eq.4.12 (Chapter 4). Since limits for human tolerable intakes for inorganic contaminants available from literature are generally given on a weekly basis (Provisional Tolerable Weekly Intake, PTWI) I have multiplied calculated DI's by seven to obtain the respective weekly intakes. Estimated weekly intake of As, Cd, Hg and Pb for humans based on levels of the contaminants in soils, measured levels in *Brassica oleracea* and estimated concentrations in animal (cow and sheep) organs (kidney, liver and muscle) as well as food consumption patterns for the Portuguese population are given in Figure 8.3(i).

For As, the estimated weekly intake at the various study sites varied between 0.01 and 3.5 mg w^{-1} (mean=0.20 mg w^{-1}; median= 0.06 mg w^{-1}) (Figure 8.3(i)). A PTWI has been established for As in drinking water in the form of inorganic arsenic (PTWI=0.015 mg kg^{-1} bodyweight, corresponding to 1.05 mg inorganic arsenic w^{-1} for a 70 kg adult), but not for other foodstuffs (EC, 2004). Although in general the estimated weekly intakes from our study were below 1.05 mg w^{-1} these is a risk that this PTWI is exceeded at the most contaminated sites which may lead to values of human dietary As intakes up to 3.5 mg w^{-1} (Figure 8.3(i)). It should be noticed though that our estimates referred to total As (inorganic and organic) and since As detected in foodstuffs is generally mainly found in the organic form which is less toxic (Bocio et al., 2005), taking into account only inorganic As, the intake would decrease.

The analysis of the contribution of the different pathways for the intake of As by humans shown in Figure 8.3(i) allowed to conclude that the median contribution of the soil ingestion pathway was 37 %, the consumption of meat (cow and sheep combined) contributed for a median of 25 % of the As intake while offal (cow kidney and liver) had a median contribution of 34 %. The removal of offal from the food chain has been recommended as an option to reduce human dietary contaminants intake from animal origin (Prankel et al., 2005). In the case of As, we have estimated that the removal of offal from diet would in fact contribute to reduce exposure but there were still 3 sites at which the PTWI could be exceed (Figure 8.3(ii)) mainly due to elevated soil levels.

For Cd, the estimated weekly intake at the various study sites varied between 0.01 and 1.0 mg w^{-1} (mean=0.09 mg w^{-1}; median= 0.05 mg w^{-1}) (Figure 8.3(i)). A PTWI of 0.49 mg w^{-1} for a person weighing 70 kg has been recommended for Cd by the WHO (EC, 2004). According to our estimates only 3 of the studied sites pose potential risks of leading to an exceedance of the PTWI (Figure 8.3(i)). Furthermore, the human exposure to Cd can be substantially reduced by the removal of offal from diet as shown in Figure 8.3(ii) since we estimate that a median of 76% of the Cd dietary intake derives from this source. Removing the intake of Cd associated to offal, the weekly intake of this contaminant varied between 0.001 and 0.03 mg w^{-1} which is considerably lower than the PTWI of 0.49 mg w^{-1} (Figure 8.3(ii)).

Estimated weekly intake of Pb varied between 0.01 and 6.5 mg w^{-1} (mean=0.3 mg w^{-1}; median= 0.06 mg w^{-1}) (Figure 8.3(i)). Since the PTWI suggested for Pb is 1.75 mg w^{-1} for a person weighing 70 kg there is a risk of exceedance of this limit at contaminated sites from the sampling area identified as (d) in (Figure 8.3(i)). The analysis of the contribution of the different pathways for the intake of Pb by humans calculated in the present study allowed to conclude that the median contribution of the soil ingestion pathway was 42 %, the consumption of meat (cow and sheep combined) contributed for a median of 2 % of the Pb intake while offal (cow kidney and liver) had a median contribution of 48 %. In the case of Pb, we have estimated that the removal of offal from diet would contribute to reduce exposure but there were still 2 sites at which there is a risk of exceedance of PTWI (Figure 8.3(ii)) mainly due to elevated soil levels.

Finally, for Hg, the estimated weekly intake at the various study sites varied between 0.0004 and 0.4 mg w^{-1} (mean=0.02 mg w^{-1}; median= 0.003 mg w^{-1}) (Figure 3(i)). A PTWI of 0.35 mg w^{-1} for a person weighing 70 kg has been recommended for Hg (EC, 2004). According to our estimates there is risk of exceeding the PTWI only at 2 sites (Figure 8.3(i)). Furthermore, the human exposure to Hg can also be substantially reduced by the removal of offal from diet as shown in Figure 8.3(ii) since estimates indicate a median of 93 % of the Hg dietary intake derives from this source. Removing the intake of Hg associated to offal, the weekly intake of this contaminant is expected to be lower than 0.04 mg w^{-1} at al sites which is considerably lower than the PTWI of 0.35 mg w^{-1} (Figure 8.3(ii)).

(i)

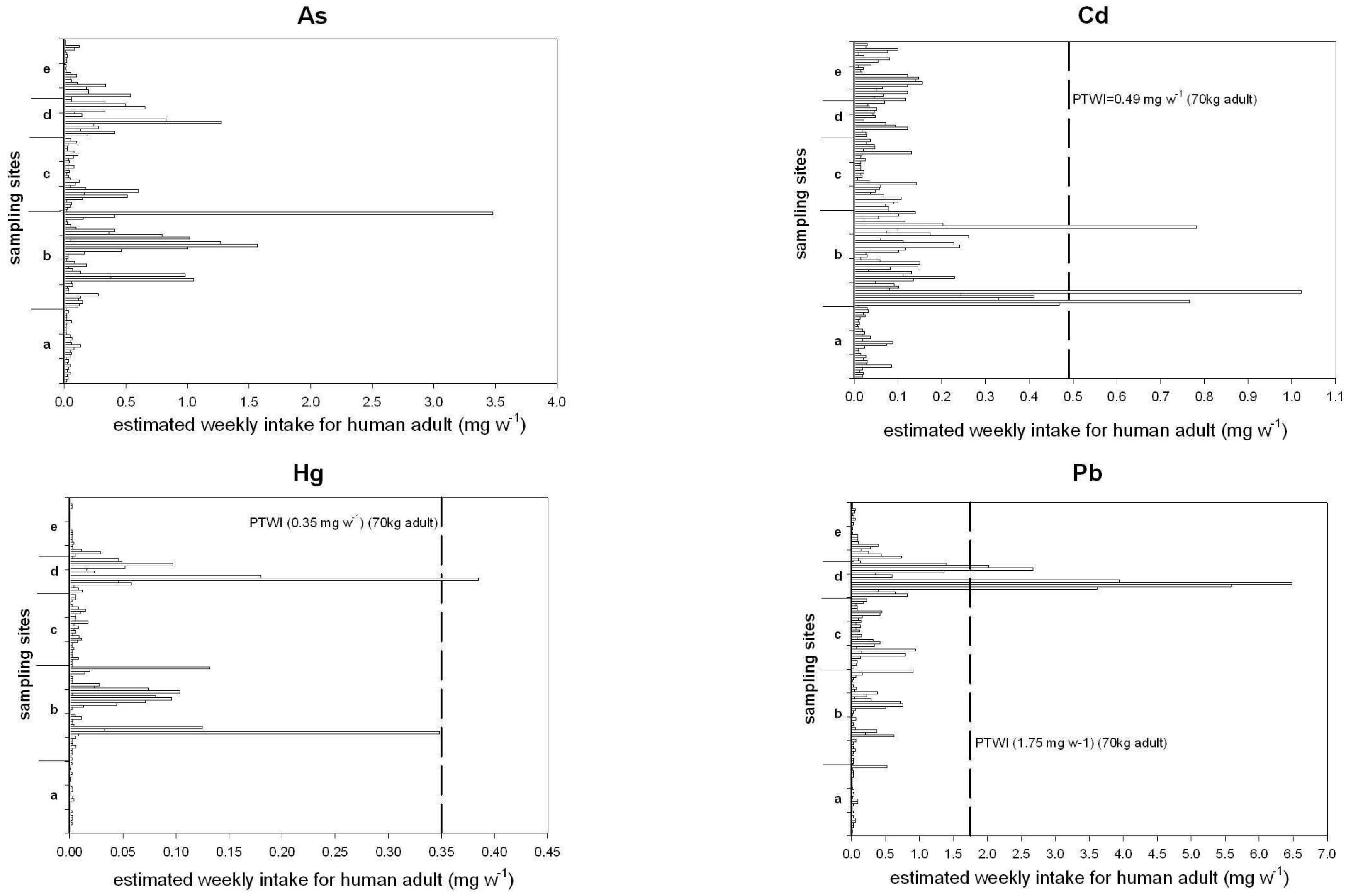


Figure 8.3: Calculated weekly intakes of Cd, As, Pb and Hg on the basis of measured data on soils and Brassica oleracea and estimated values in animal products, including offal (i) and excluding offal (ii) from calculations. Sampling sites include the areas of Esposende (a), Estarreja (b), Lousal (c), Caveira (d) and Aljustrel (e) in Portugal

(ii)

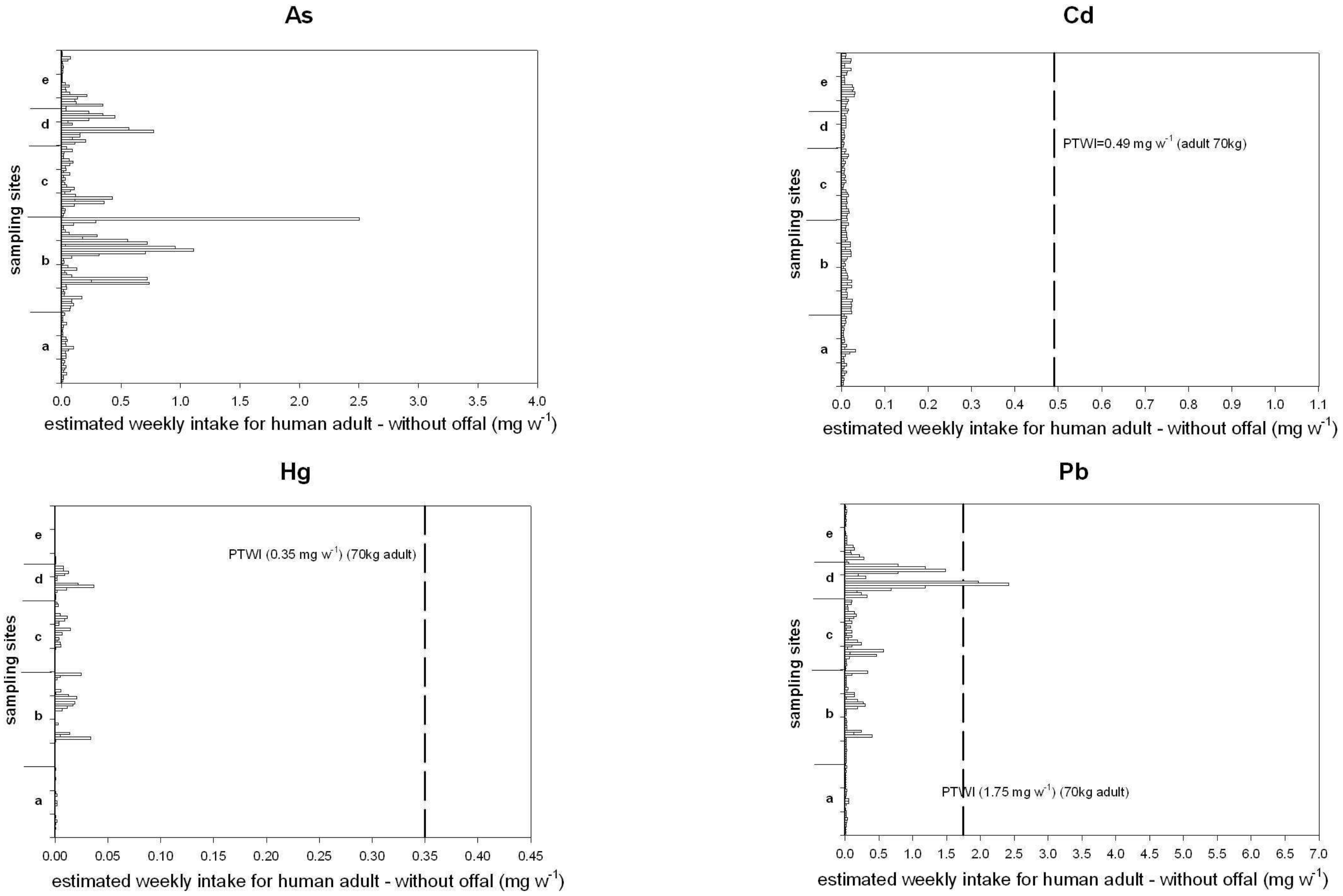


Figure 8.3 (cont.)

8.5 Summary and conclusions

In this Chapter a chain model approach was tested to assess the transfer of inorganic contaminants from soils to feed and food crops; from crops to grazing animals (cow and sheep); and finally for humans. By the calculation of dietary intakes, it was observed that the levels of soil contamination at polluted sites may lead to intakes of Pb, Hg, Cd, As, Cu and Zn that exceed animal ADI's posing potential risks to animal health and food safety.

For animals, the ingestion of grass was the most relevant pathway for the intake of Cd, Zn, Cr, Se, Mo, B, Ni, Ba, Cu and Hg while for Pb, Co, As, U and Sb the direct ingestion of soils accounts for around half of the element intake. The analysis of the pathways for human exposure of the toxic elements Cd, Pb, Hg and As showed that the removal of animal (cow) liver and kidney from the food chain is an option to reduce human dietary Cd and Hg intake. For As and Pb the contribution of offal for human exposure is relatively smaller compared to Cd and Hg and after the removal of these products from diet, elevated soil levels may still lead to elevated exposure due to soil ingestion.

The *Brassica oleracea* was the only food crop included in this study and it didn't significantly contribute to human exposure to PTE's from soils. In the future, additional vegetables should be taken into consideration. This will allow to better evaluate the relative contribution of different food crops for human dietary exposure to inorganic contaminants.

The direct measurement of levels of PTE's in animal organs (kidney, liver and muscle) would also improve the accuracy of the calculations of human dietary intakes. Animal ADI's regarding both food safety and animal health were calculated based on BAFplant-animal from literature but these are available only for a limited number of contaminants. Further research on the actual availability of PTE's present in feed to animals as well as on the accumulation of contaminants in animal organ is needed.

Chapter 9

The accumulation of soil contaminants in crops: analysis of European human exposure models and suggestions for model development in Portugal

9.1 Introduction

The previous Chapters have addressed the analysis of health risks due to uptake of potentially toxic elements (PTE's) in crops from contaminated soil. In this Chapter, an approach for the derivation of soil quality criteria for agricultural soils in Portugal in view of animal and human health will be evaluated. To further analyse the validity of the approach, this will be compared with exposure models for risk assessment of soil contamination available from literature.

Although no general human exposure model is used in the EU, this tool is in widespread use in several European countries and has been applied both to derive soil and groundwater quality criteria (e.g. soil screening levels) and to assess site-specific exposure. Exposure and risk assessment tools for contaminated soils developed in European countries during the last two decades include the Contaminated Land Exposure Assessment tool CLEA (United Kingdom), CSOIL (The Netherlands), CETOX (Denmark), Vlier-humaan (Flanders, Belgium), ROME 2.1 (Italy) and unnamed models from Sweden and France. The majority of these models include a crop consumption exposure pathway although using different approaches for calculating the accumulation of soil contaminants in plants and for assessing human exposure to these contaminants due to the intake of plant material (Swartjes, 2002).

In general, the calculation of human exposure to soil contaminants via crop consumption depends on three critical factors (Paustenbach, 2000; DEFRA and Environment Agency, 2002b):

- the concentration of the contaminant accumulated in crops from the surrounding soil;
- the amount of crop consumption and the fraction of total vegetation that comes from a contaminated soil; and,
- the fraction of the contaminant in vegetables that is absorbed by the human body.

The concentration of a PTE in crops and the amount of crop consumed per day will relate to the human daily intake of the contaminant. The intake of a contaminant is the amount of a chemical entering or contacting the human body at the point of entry (that is mouth, nose, or skin) by ingestion, inhalation or skin contact and is generally expressed by human exposure models in terms of mass of contaminant per kg body weight over a period of time (for example mg kg^{-1} bw day^{-1}) (DEFRA and Environment Agency, 2002b). Not the entire intake is absorbed by the human body and the amount of contaminant that reaches the circulating blood having been absorbed by the body through the gastrointestinal system, the pulmonary system, and the skin is often referred to as human uptake (DEFRA and

Environment Agency, 2002b). The uptake is commonly related to intake by the bioavailability of the contaminant in the human body (Paustenbach, 2000). Most human exposure models estimate human intake only without assessing the uptake of contamination in food since it is difficult to estimate bioavailability and its practical use is limited to exposure to a limited number of contaminants (for example Pb). Even so, estimating factors determining contaminants intake is still a highly complex assessment with many areas of variability to be considered such as the characterisation of the routes of exposure to PTE's via crop consumption (Paustenbach, 2000).

The routes of exposure to PTE's (particularly metals) commonly included in modelling human exposure via crop consumption are shown in Figure 9.1. As shown in Figure 9.1, plants can accumulate contaminants from soils via a number of different pathways namely, by re-suspension/ deposition of soil dust/ rain splash and by uptake by plant roots (and transport to the leaves of the plants).

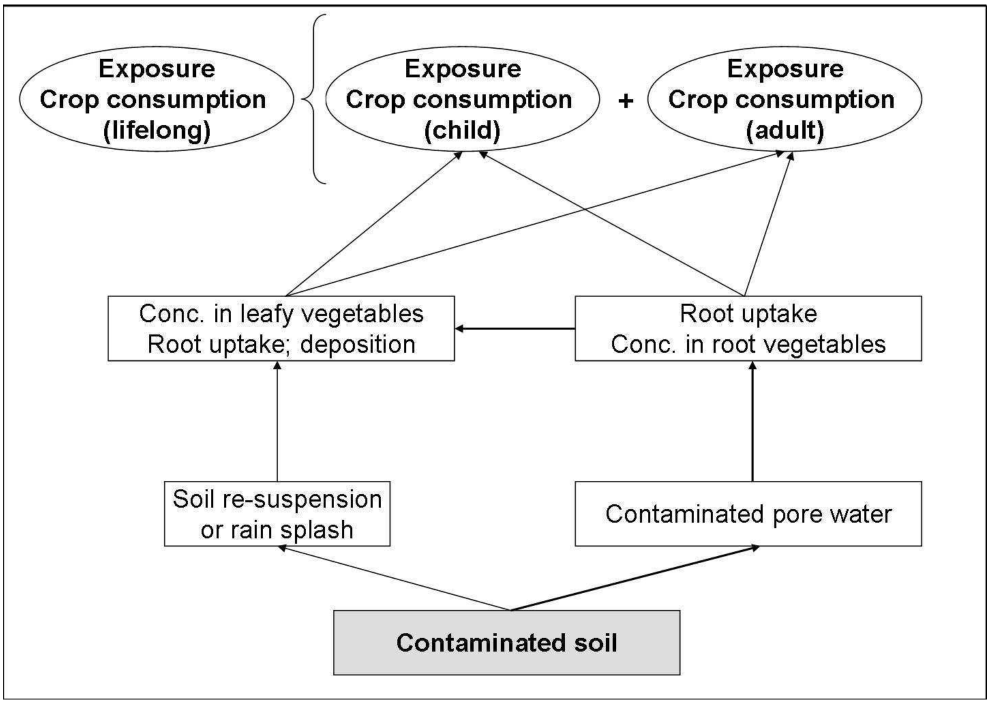


Figure 9.1: Routes of human exposure to soil contaminants via crop consumption (Brand et al., 2007; Swartjes, 2009)

For many contaminants, the uptake into the root via the transpiration stream is the most important route contributing to soil-to-plant transfer (Kabata-Pendias, 2001). Route uptake is also the pathway most commonly assessed by exposure models although the dry deposition of particles of soil has been considered in certain cases (Versluijs et al., 1998). In the context of soil contamination, other processes such as leaf uptake through adsorption of vapours are generally considered relevant only for volatile organic compounds (DEFRA and Environment Agency, 2002b).

Root uptake and transport within plant tissues and organs involve many processes that depend on plant species and individual cultivars and vary with specific contaminants. Ideally, because of the inherent complexity of the soil-plant system, the concentration of soil-derived contaminants in vegetables would be based on measured data (DEFRA and Environment Agency, 2002b). However, in deriving generic assessment criteria, this is impracticable and therefore human exposure modelling generally adopts one the following approaches from scientific literature:

- Simple empirical relationships – the use of soil-plant bioconcentration factors (BCFs) derived from literature (e.g. Travis and Arms, 1988; Kabata Pendias, 2001);
- Complex empirical relationships that use observed experimental or field data to relate the contaminant concentration, soil properties and the types of plant to one or more regression equations (e.g. Baes et al., 1984; Andersen and Christensen, 1988; Efroymson et al., 2001);
- A semi-empirical and mechanistic approach that includes complex models to describe the geochemistry of the soil and the biochemistry of the plant from fundamental scientific principles (Allison et al., 1991; Department of the Environment, 1992).

The use of soil-plant empirical BCFs is the most commonly approach followed by human exposure models for metal contaminants although given the large variations in reported uptake of metal contaminants by different plants under various soil conditions and lack of data for certain contaminants, cautious approaches are generally adopted and plant uptake data are usually analysed on a substance-by-substance basis (Versluijs et al., 1998; DEFRA and Environment Agency, 2002b; Swartjes, 2002). Commonly, human exposure models do not include the possibility to use BCFs that are dependent on soil properties and therefore do not allow to calculate the site-specific uptake/accumulation in crops as function of soil characteristics and quantify the influence of relevant soil properties like pH (Swartjes, 2002).

9.2 Aim, objectives and scope

The main objectives of this Chapter are to review human exposure models that include the crop consumption pathway and to make suggestions for model development in Portugal. The possibilities of use of the approach for derivation of soil quality criteria in Portugal and the potential for an extension to site-specific studies will be evaluated.

This Chapter will focus on the possible accumulation of harmful concentrations of PTE's in crops grown on contaminated soils and not so much on the dynamics of the process of accumulation in the crops as it was the case of Chapter 7. It deals particularly with high concentration ranges of PTE's in which the elements are considered contaminants and not with concentration ranges in which these are considered essential to plants.

It should be noticed that the main aim of this Chapter is to support decision making in Portugal and that to reach a stage in which decisions can be made on the risk of contaminated soils, a pragmatic approach is necessary.

9.3 Human exposure models from other European countries

9.3.1 The Contaminated Land Exposure Assessment Model (CLEA) (UK)

The CLEA model is used in the UK in the calculation of both Soil Guideline Values (SGVs) and Site-Specific Criteria. The derivation of SGVs and Site-

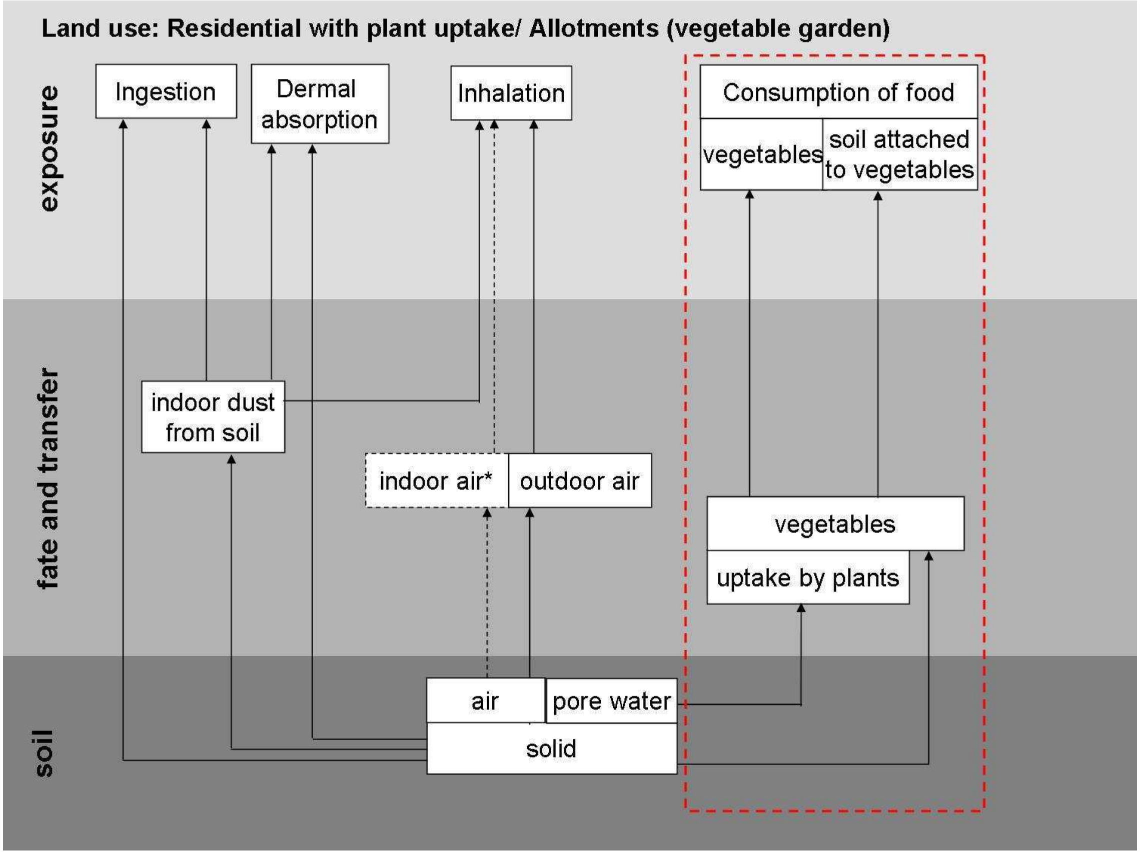
Specific Criteria follow a similar approach with the difference that in the calculation of SGVs, human exposure characteristics are taken from generic datasets and contaminant fate and transport parameters as well as algorithms are based on generic site conditions and simplifications of real behaviour. The SGVs have been developed so that they apply to a range of different sites and are appropriately protective to be used as generic assessment criteria (DEFRA and Environment Agency, 2002b). When deriving Site-Specific Criteria, generic datasets are used in conjunction with site-specific information. For example, rather than modelling plant uptake of contaminants it is possible to measure the values (DEFRA and Environment Agency, 2002b).

The CLEA model has the following characteristics:

- Considers two compartments: soil and groundwater (only in the unsaturated zone when in equilibrium with the soil);
- Considers the following land-uses and respective critical receptors (in brackets): residential-with-plant-uptake (female child receptor of 0 to 6 years); residential-without-plant-uptake (female child receptor of 0 to 6 years); commercial/ industrial (female adult worker of 16-59 years); vegetable gardens, called allotments (female child receptor of 0 to 6 years);
- It has been developed as a probabilistic model: many of the input parameters for calculating exposure (such as body weight) have an input range of values with a probability density function (PDF) populated with data from the UK population and appropriate for each variable;
- It calculates exposure using the PDF for each age class and for the selected critical receptor: for example, if the child of age class 0-6 years is the critical receptor, than the model calculates exposure using the PDF for each year; the 95th percentile of the exposure is then selected from the exposure PDF of each year and these are then averaged to calculate an overall exposure for this critical receptor;

- The distribution of contaminants into the soil phases is incorporated in the model by using partition coefficients (K_d, K_{oc}, K_{henry}) although some of the algorithms (e.g. dermal, plant uptake) use the fugacity theory;
- It is possible to calculate the site-specific uptake/accumulation in crops as function of soil characteristics, namely as a function of pH (for Cd) and % soil organic matter (for Hg and organic contaminants);
- Commonly home-grown vegetables in the UK are considered in the model: namely potatoes, carrots, onions and leeks (treated as one category), brussels sprouts, cabbage, lettuce;
- Phytotoxicity is generally not included although the model is able to incorporate limits to vegetable concentration due to phytotoxicity.

An overview of fate and transfer processes and of the exposure pathways considered in the application of CLEA model for two land uses including exposure via crop consumption – residential with plant uptake and allotments is shown in Figure 9.2. The dashed line box indicates the crop consumption pathway.



*not included in the default conceptual model for allotments

Figure 9.2: Fate, transfer and exposure pathways for two land uses including exposure via crop consumption – residential with plant uptake and allotments (CLEA)

Fate and transport models are used by CLEA to generate the chemical exposure rates such as those for:

- Volatilisation of contaminants to outdoor air;
- Migration and subsequent concentration of contaminants to indoor air;
- Uptake of contaminant into vegetables.

When deriving SGVs, the CLEA model is used to estimate average daily exposure (ADE) to soil contamination based on the conceptual exposure models for each land use. The ADE is calculated according to Eq. 9.1.

$$ADE = \frac{(IR_{inh} x EF_{inh} x ED_{inh})}{BWxAT} + \frac{(IR_{oral} x EF_{oral} x ED_{oral})}{BWxAT} + \frac{(IR_{dermal} x EF_{dermal} x ED_{dermal})}{BWxAT} \qquad \textbf{9.1}$$

Where,

ADE= average daily human exposure to a chemical from soil (mg kg^{-1} bw d^{-1})

IR = intake/ chemical exposure rate (mg d^{-1}); the subscripts inh, oral and dermal apply to inhalation, ingestion, and dermal contact routes, respectively. IR_{oral} and IR_{inh} are normally measures as intakes; IR_{dermal} is normally measured as uptake.

EF= exposure frequency (d y^{-1})

ED= exposure duration (y)

BW= human body weight (kg)

AT= averaging time (d)

The key features for evaluating uptake of contaminants into fruits/ vegetables using CLEA model are:

- Plant absorb contaminants from the soil mainly via soil solution into the root systems (root uptake);
- Contaminants move around the plant to the edible portions via transpiration (translocation);
- Vegetables are harvested with contaminants in the plant tissue and in soil grains trapped in the skin and between leaves;
- Preparation and cooking can reduce contaminant concentrations (for example pealing potatoes);
- Contaminants enter the body in food.

The CLEA model estimates the chemical exposure rate of soil contaminants from the consumption of fruits and vegetable gardens only. Fruits include herbaceous fruits (e.g. cucumber, tomato, strawberries), shrub fruits (soft fruits such as blackcurrant, raspberry) and tree fruits (apples, pears and fresh stone fruits). Six groups of vegetables were included in the model: Brussels sprouts; cabbage; carrots; leafy salads; onion (including shallots and leeks) and potato. These vegetables were selected because they are some of the more commonly consumed vegetables that are also grown in gardens and allotments. The model does not take into account of other possible intakes from contaminated food such meat and dairy produce. However many of these factors are considered as background intakes when deriving the Tolerable Daily Soil Intake (TDSI). The TDSI is the fraction of Tolerable Daily Intake (TDI) allocated to tolerable exposure from contaminants in soil and that takes into account the general level of background exposure and it is the value that is used as the health criteria value for the derivation of the SGVs. TDIs are derived for threshold contaminants. For non-threshold contaminants, Index Doses are derived and they convey minimal risk levels, with the additional requirement to keep any intake as low as reasonably practicable (ALARP).

The chemical exposure rate for the consumption of homegrown garden vegetables is calculated by CLEA using Eq. 9.2:

$$IR_{fruit/vegetable\_consumption} = C_{soil} x \sum_{fruit/vegetable\_type} (CR_{fruit/vegetable} xBWxHF_{fruit/vegetable} xCF_{fruit/vegetable}) \qquad \textbf{9.2}$$

Where,

$IR_{\text{fruit/vegetable consumption}}$= intake rate of a contaminant from consumption of garden vegetables (mg d^{-1})

C_{soil}= concentration of a contaminant in soil (mg g^{-1} dw)

$CR_{\text{fruit/ vegetable}}$= total daily consumption rate of each vegetable (g fw kg^{-1} bw d^{-1})

BW= body weight (kg)

$HF_{\text{fruit/vegetable}}$= fraction of each vegetable consumed that is assumed to be homegrown

$CF_{\text{fruit/vegetable}}$= calculated soil-to-plant concentration factor (mg g^{-1} fw plant over mg g^{-1} dw of soil).

The CLEA model uses data from the UK National Diet and Nutrition Surveys to derive the PDFs for consumption rate for each fruit and vegetable ($CR_{fruit/\ vegetable}$) covering the age ranges of interest. The fraction that is assumed to be homegrown ($HF_{fruit/\ vegetable}$) is modelled probabilistically using a beta-shaped PDF that is defined by: the fraction of the total consumption rate from the UK National Food Survey that is assumed to be the average consumption rate for homegrown vegetables based on percentage of "non-purchased" produce; and the fraction of the total consumption rate that is considered to be a reasonable upper limit for the consumption of homegrown vegetables.

In the CLEA model, the soil-to-plant concentration factors (CF) are required in units of fresh weight (fw) plant concentrations to compare with the fresh weight fruit/ vegetables consumption data and therefore is generally given by:

$$CF = \frac{C^{*}_{plant} x DW_c}{C_{soil}} \qquad \textbf{9.3}$$

Where:

CF= soil-to-plant concentration factor (mg g^{-1} fw plant over mg g^{-1} dw of soil);

C^{*}_{plant}= chemical concentration in edible plant tissues (mg g^{-1} dw)

C_{soil}= concentration of a contaminant in soil (mg g^{-1} dw)

DW_c= dry weight to fresh weight conversion factor (g dw g^{-1} fw)

The soil-to-plant concentration factors used by CLEA are estimated on a substance-by-substance basis. It is important that the concentration of the contaminant in the edible parts of the relevant fruits and vegetables are estimated rather than the concentration in the whole plant. For this purpose the CLEA model divides the vegetables in two groups: root vegetables (root zone accumulation of soil contaminants using data derived from studies on roots and tubers); and leafy vegetables (concentration factors based on stem and leaf accumulation of soil contaminants with observed data derived from studies on leaves).

The procedure adopted in the CLEA model to determine soil-to-plant concentration factors for metal contaminants included:

- review of literature on soil chemistry and plant uptake to identify trends in behaviour related to soil properties;
- review of recognised soil-to-plant concentration factors for the contaminant of concern; and
- review of primary literature on the uptake of contaminants by the fruits and vegetables of interest in order to calibrate any generalised soil-plant relationships.

Collins et al. (2006) set out guidelines for evaluating plant uptake studies from the scientific literature for inorganic and organic chemicals that include the article quality, scale of the study, and other factors that might affect the usability of data (source of contamination, soil type, plant type).

In 2009 the Environment Agency (2009a,b,c,d,e) has published SGVs for generic site assessment for Se, Cd, As, Hg, and Ni on the basis of a review of soil-to-plant concentration factors available from literature according to produce categories. In circumstances where these SGVs are exceeded, assessors are advised to consider contaminants phytoavailability on a site-specific basis (including the sampling and chemical analysis of edible parts of fruits and vegetables) and establish site-specific soil-to-plant concentration factors.

The extent to which the CLEA model considers the impact of soil properties on the plant uptake of metal contaminants depends critically on the available scientific literature for each contaminant of interest. Based on literature available on Cd (which is considerably more extensive than that of most metal) an approach to modelling soil-to-plant concentration factors using soil properties has been developed for CLEA. An extensive literature review was used to collect a range of observed uptakes for fruit, leaf and root vegetables included in CLEA across the pH range. Regression analysis was used to determine the linear relationship between uptake and soil pH for this focused dataset. The respective soil-to-plant concentration factors for can be modelled in CLEA with changes to the soil pH.

Recent developments of the CLEA model (Environment Agency, 2009f) have included the definition of an alternative approach for estimating soil-to-plant concentration factors for inorganic contaminants that is consistent with the approach used by the Food Standards Agency (FSA) within PRISM Version 2.0 (Thorne et al., 2005). Further considerations on the PRISM model will be given on section 9.4.

9.3.2 CSOIL (the Netherlands)

The CSOIL model calculates the risks that humans are exposed to if they come into contact with soil contamination via different exposure routes: soil, air, water and crops. CSOIL also calculates the maximum concentration of a contaminant in the soil at which it is still safe for humans. This maximum concentration determines the level of the so-called Dutch "Intervention Value". Intervention Values are generic soil quality standards that are based on the potential risk for both humans and ecosystems (Brand et al., 2007). In soil contamination the Intervention Value differentiates between lightly and seriously contaminated soils and the urgency of remediation is determined by the level at which soil contamination exceeds the intervention value (Brand et al., 2007; Carlon, 2007).

In addition to the Intervention Values, the Dutch regulatory framework for soil quality assessment also included the derivation of "Target Values" for soil which were related to the negligible risks to ecosystems. Recently, the publication of the "Soil Quality Decree" implied the abandonment of the toxicological definition of the "Target Value". The "Background Values" are currently in place and were derived from the study of national background concentrations in the Netherlands (95th percentile of the distribution of background concentrations in the top soil). In addition to the Background value, two new values were introduced in the new Soil Quality Decree which relate the quality of the soil to its function: maximum concentrations for "Residence" land use and maximum concentrations for "Industrial" land use. Revised Intervention Values still exist in the Dutch Soil Protection Act.

The CSOIL human exposure model has the following characteristics:

- it can be used for the derivation of soil quality standards (Intervention Values) in the framework of the Dutch Soil Protection Act; the derivation of remediation objectives; determination of the urgency of remediation; calculation of site-specific exposure;
- considers the following compartments: soil and groundwater (unsaturated zone);
- uses the following exposure scenarios: residential with vegetable garden; residential with normal garden (standard scenario); residential without garden; industrial; infrastructure; recreational areas; parks; social-cultural areas;
- for the derivation of Intervention Values uses the standard scenario "residential with normal garden";
- considers the following standard age ranges: 0 – 6 years (children) and 6 - 70 years (adults) which can be calculated separately or summed up;
- The distribution of contaminants into the soil phases is incorporated in the model using the fugacity theory and using partition coefficients (K_d, K_{oc}, K_{henry});
- Uses generic BCFs - a model for site-specific calculation of the uptake/accumulation in crops (as function of soil characteristics) is in progress.

An overview of fate and transfer processes and of the exposure pathways considered in the application of CSOIL model for residential with vegetable garden land use are shown in Figure 9.3. The dashed line box indicates the crop consumption pathway.

In general terms, the CSOIL model concept consists of three parts:

1 – the description of the behaviour of the compound in the soil and the partitioning over the soil phases;

2 – the transfer processes and parameterisation of the exposure routes (direct and indirect);

3 – the quantification of the lifetime exposure (Otte et al., 2001).

From the exposure routes described in Figure 9.3, the following three are generally responsible for at least 90 % of the total exposure:

- the human exposure via the ingestion of contaminated soil particles;
- the human exposure to volatile compounds in the indoor air;
- the human exposure via the consumption of contaminated crops (Otte et al., 2001).

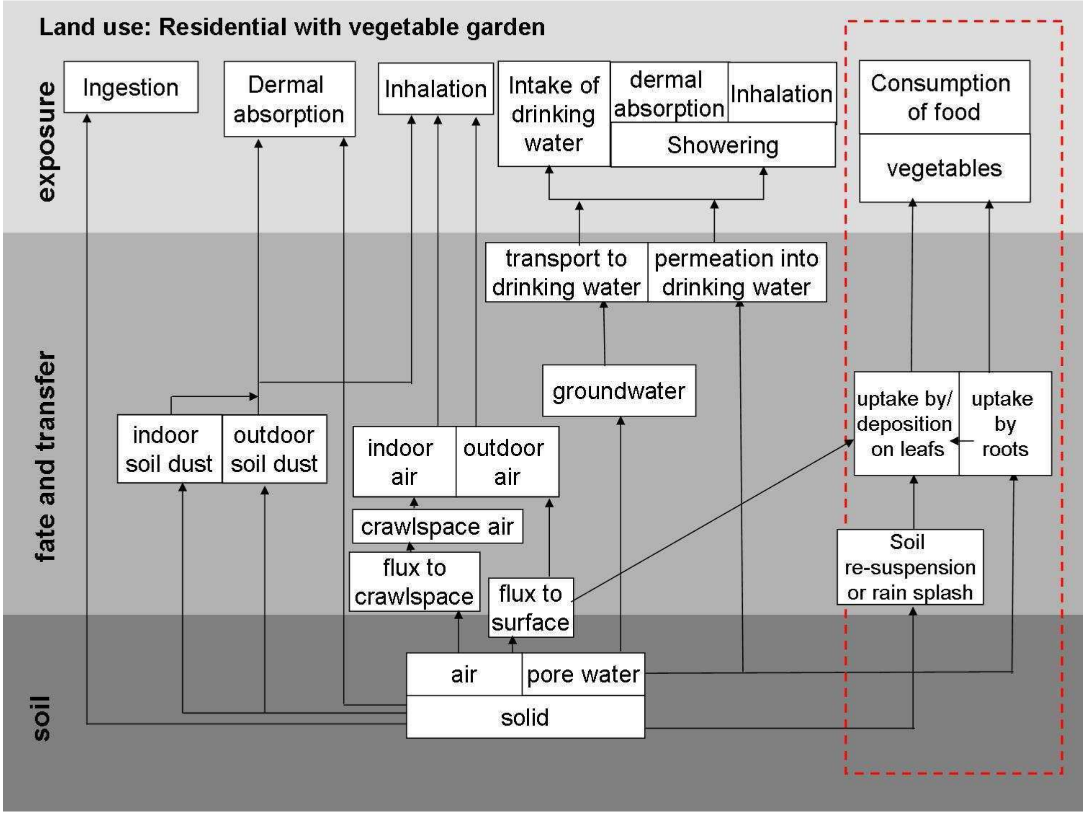


Figure 9.3: Fate, transfer and exposure pathways for residential with vegetable garden land use (CSOIL)

Regarding the crop consumption pathway there are two main exposure routes for vegetation included in the CSOIL model: via the air (deposition of soil dust/ soil re-suspension/ rain splash and deposition of local volatized contaminant) and via uptake by plant roots (Figure 9.3). From every plant different parts are eaten by humans and therefore a difference is made between roots and leaves of the plants. The concentration in the plant is calculated by adding up the concentrations resulting from deposition on leaves and the accumulation in the plant roots. CSOIl also calculates the transport of a contaminant from the roots to the leaves of the plant. The human exposure depends on the concentration in the crops, the amount of consumption and the fraction of the total vegetation that comes from a contaminated soil (Rikken et al., 2001).

In CSOIL, the uptake of a contaminant by the roots of the plant is modelled differently for organic compounds, metals and other inorganic compounds. For metals and empirical approach is used in which the uptake by the plant is within the use of a bioconcentration factor (BCF). To obtain the BCF, available experimental data are used for which it is supposed that the contribution due to local deposition is included.

The CSOIL model is applied to calculate the human toxicological risk limit (MPR) which in combination with the ecotoxicological risk limit is used to derive the Intervention Value. The human toxicological definition of serious soil contamination is "the soil quality resulting in exceeding of the Maximum Permissible Risk for intake (MPR_{human})" (Brand et al., 2007). The MPR_{human} can be defined as the amount of substance that any individual can be exposed to in a daily basis, during a full lifetime without significant health risk. The MPR_{human} is expressed as a tolerable daily intake (TDI) or an excess carcinogenic risk via intake (CR_{oral}), both covering exposure by oral ingestion and dermal contact. For non-genotoxic carcinorgens and non-carcinogenic contaminants (threshold contaminants) the toxicological TDI is derived on the basis of effect data, usually on test animals, and extrapolation factors, in analogy with the procedure used by the World Health Organisation (WHO). For genotoxic contaminants (non-threshold contaminants) even the lowest exposure rate results in an increased chance of adverse effects for humans. The MPR_{human} is defined as the dose of a contaminant (based on body weight for oral intake) which forms a risk of one additional case of lethal tumour in 9,000 lifelong exposed individuals; this definition is based on a political decision (Carlon, 2007). The MPR_{human} can also be expressed as tolerable concentration in the air (TCA) or an excess carcinogenic risk via air (CR_{inhal}). To derive human toxicological risks limits the oral and inhalative exposure are calculated under standardized conditions (potential exposure), separately. Besides, the MPR_{human} is derived for oral exposure and for inhalative exposure. The human toxicological risk limit is defined as the concentration of a contaminant in the soil for which the sum of oral (including dermal) and inhalative risk indexes equal 1:

$$\left(\frac{\sum oral\ \exp osure}{MPR_{human\_oral}}\right)+\left(\frac{\sum inhalative\ \exp osure}{MPR_{human\_inhalative}}\right)=1 \qquad \textbf{9.4}$$

The algorithms of the CSOIL model are also part of a protocol to assess site-specific human exposure due to the consumption of homegrown vegetables (Swartjes et al., 2007). The site-specific calculation of the contaminant concentration in vegetables differs for metals, other inorganic contaminants and organic compounds.

In the framework of the revision of the Dutch Intervention Values an improved procedure for the assessment of the accumulated concentration in vegetables was developed (Swartjes et al., 2007). For metals Freundlich-type soil-plant relations are combined with geometric means of BCFs available. The Freundlich-type soil-plant relations were derived for calculation of the accumulated concentration in vegetables, as a function of total concentration and the major soil properties. For each vegetable with sufficient and proper data available, the following equation was derived:

$$\log\left[M_{vegetable}\right] = a + b\log\left[M_{soil}\right] + cpH_{soil} + d\log\left[\%Clay\right] + e\log\left[\%OC\right] + f\left[other\_factors\right] \quad \mathbf{9.5}$$

Where:

$M_{vegetable}$= metal concentration in the edible part of the vegetable (mg kg^{-1} dw)
M_{soil}= total metal concentration in the soil (mg kg^{-1} dw)
pH= pH KCl
%Clay= clay content of the soil (%)
%OC= organic carbon content of the soil (%)
a, b, c, d, e, f= empirical parameters

The resulting soil-plant relationships enable site-specific calculation of the accumulated concentration in specific vegetables and the derivation of vegetables-specific BCFs for a specific metal concentration and specific soil properties. Versluijs and Otte (2001) proposed that the application range for the derived soil-plant relations is within the 5th and 95th percentiles of the underlying data for soil concentrations, pH, organic matter and clay content. When for a specific vegetable no valid soil-plant relationship could be derived the geometric mean of the BCFs available were used. The approach followed to derive vegetable-specific BCFs is shown in Figure 9.4.

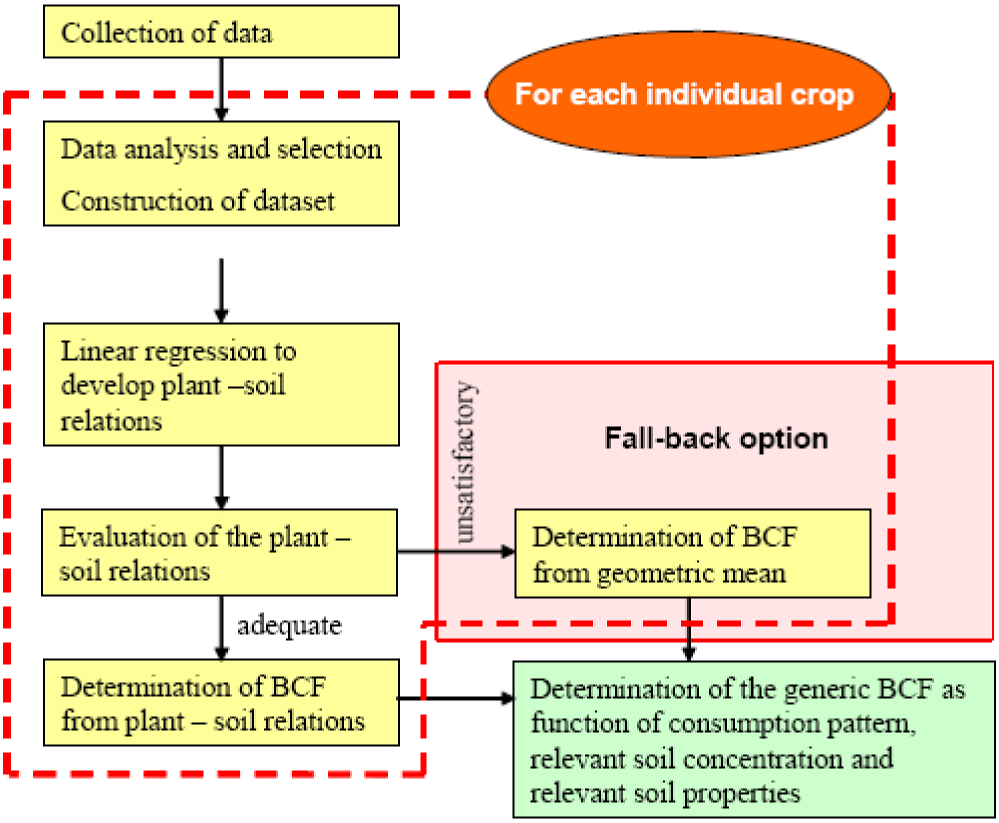


Figure 9.4: Procedure to derive vegetable-specific BCFs (Swartjes et al., 2007)

To account for the influence of differences in soil type on the BCFs, metal-specific soil type correction for BCFs has been proposed, related to the medium values of organic matter and clay contents of the Dutch RIVM plant - soil database. As a consequence, the soil type correction factor for the BCF, $STcf_{BCF}$, is:

$$STcf_{BCF} = \frac{A + (Bx\%Clay) + (Cx\%OM)}{A + (Bx\%Clay_{average}) + (Cx\%OM_{average})} \qquad \textbf{9.6}$$

Where:

% Clay= actual clay matter content (%)

%OM= actual organic matter content (%)

% $Clay_{average}$= average clay matter content of the of the RIVM plant – soil database (%)

$\%OM_{average}$= average organic matter content of the RIVM plant – soil database (%)

The soil type correction factor for the BCF for cadmium, for example, ranges from 0.61 for a sandy soil with no organic matter up to 1.56 for clay soils with a high organic matter content. To apply this correction the geometric values for the BCF must be divided by the soil type correction factor. This soil type correction, that is performed to correct the geometric means of the BCFs for actual soil properties, rather concerns a practical approach. However, although the scientific foundation is limited, Swartjes et al., 2007 reported that it has improved the strength for site-specific application. According to the same authors, another improvement of the use of the soil-plant relations would be to test the calculated vegetable concentrations against criteria for phytotoxicity, i.e. levels in vegetables that will damage plant tissue and reduce growth.

9.3.3 Vlier-humaan (Flanders, Belgium)

In Flanders (Belgium) the derivation of human health based values for soils is performed by the Vlier-humaan model that is similar to CSOIL (the Netherlands) conceptual approach but accounts for the typical Flemish conditions and policy decisions. The calculations follow a land-use dependent approach.

The Vlier-humaan human exposure model has the following characteristics:

- It is used to derive soil clean-up standards and it can also be used for human health risk assessment;
- Considers the following environmental compartments: soil, air, groundwater;
- Considers the following land-use/ exposure scenarios: nature/ agriculture; residential; recreation; industry
- Considers two human exposure scenarios in which exposure represents an average daily dose: 0 – 6 years (children) and 6 - 70

years (adults); a lifetime average is only calculated for non-threshold contaminants (genotoxic carcinogens); in the industrial scenario, exposure is only calculated for adults;

- In the case of threshold contaminants (noncarcinogens and nongenotoxic carcinogens) background exposure is added;
- The distribution of contaminants into the soil phases is incorporated in the model using the fugacity theory and using partition coefficients (K_d, K_{oc}, K_{henry});
- BCFs available are not dependent on soil characteristics although it is possible to use measured crop concentrations or measured BCFs;
- Phytotoxicity is included on an ad-hoc basis.

An overview of fate and transfer processes and of the exposure pathways considered in the application of Vlier-humaan exposure model for agricultural land use are shown in Figure 9.5. The dashed line box indicates the crop and meat/ dairy products consumption pathways.

Differently from both the CLEA and CSOIL approach, the Vlier-humaan model includes the soil-fodder-animal-meat/ dairy products pathway in its human exposure assessment. There are also standards incorporated in the model that limit the maximum concentration in contact media: drinking water concentration, crop concentration, air concentration (indoor and outdoor), and meat concentration.

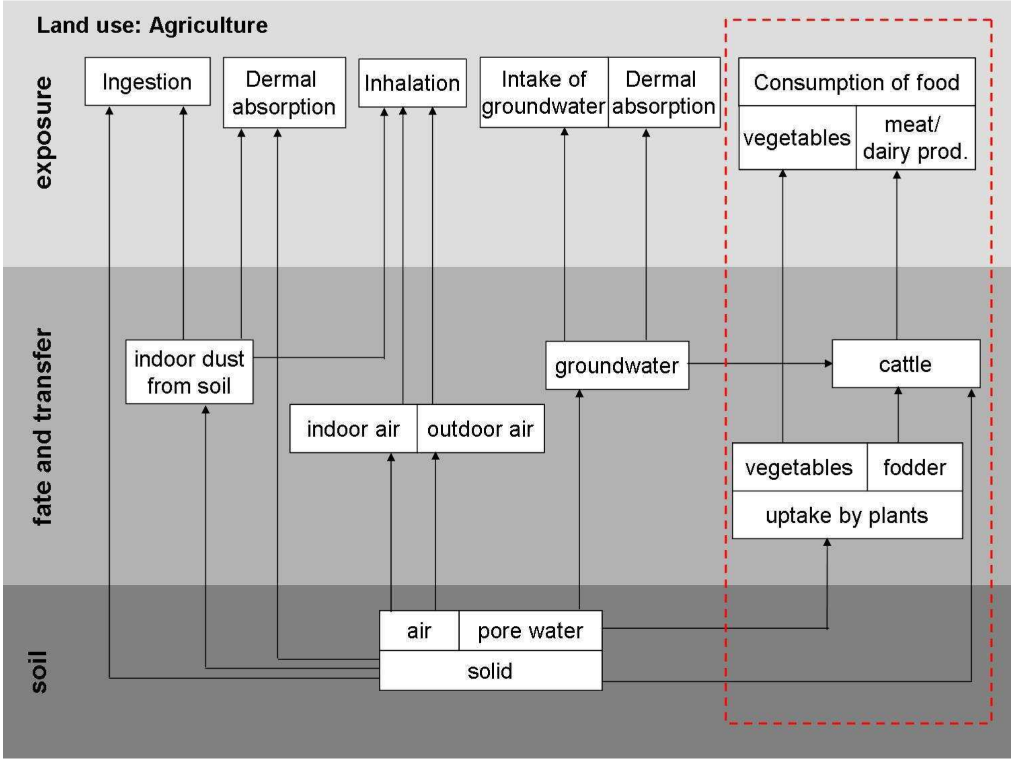


Figure 9.5: Fate, transfer and exposure pathways for agricultural land use (Vlier-humaan)

When assessing exposure to threshold contaminants using the Vlier-humaan model, a risk index (RI) is calculated separately for children and adults according to:

$$RI = \frac{I_{oral} + I_{dermal} + I_{oral}^{background}}{TDI_{oral}} + \frac{I_{inhalation} + I_{inhalation}^{background}}{TDI_{inhalation}} \qquad 9.7$$

Where *I* is the intake dose and *TDI* is the tolerable daily intake (toxicological reference value taken from existing databases in order of preference: EU, WHO, US-EPA IRIS, RIVM, others). The first condition for calculation of soil clean-up standards using this approach is that the concentration in soil corresponds with a RI≤1.

9.3.4 Other countries

Models to assess human exposure from soil contaminants from other European countries include:

- CETOX-human (Denmark);
- ReasOnable Maximum Exposure, ROME 2.1 (Italy);
- an unnamed model from Sweden;
- an unnamed model from France;
- LUR exposure model (Basque Country, Spain).

All these models include the crop consumption human exposure pathway except ROME 2.1. In general the models include specific (National) data on root and leafy vegetable consumption and fraction of root and leafy vegetables that is homegrown which affect the variation in human exposure via crop consumption (Swartjes, 2002). This variation is also affected by the approach selected for accounting for soil-plant relations although the extent to which the variability in BCFs affects the calculation of human exposure by the different models has not been assessed (Swartjes, 2002).

The Swedish, French and LUR human exposure models use generic soil-plant BCFs available from literature. In the case of CETOX-human (Denmark) a dietary model composed of different vegetables and fruits is included and it is possible to calculate the site-specific uptake/accumulation in crops as function of soil characteristics (Swartjes, 2002). A BCF for each crop and chemical has been determined. No further details on the calculation of BCFs included in this model could be found though since the model is not available for third parties.

9.4 Generic models for predicting plant uptake of chemicals from soil

9.4.1 The PRISM foodchain modelling software

The PRISM software models radionuclide transport in terrestrial foodchains following accidental or planned releases of radioactivity to the atmosphere developed under the auspices of the UK Food Standards Agency (FSA). The currently available version of the code provides capability for undertaking deterministic and probabilistic foodchain calculations, including the representation of correlated model parameters. It uses models for radionuclide metabolism by animals and also the soil and plant parts of the terrestial foodchain. PRISM includes models for trace contaminants, covering 40 elements and 127 associated radionuclides. As described above, the CLEA model has also included certain aspects of the PRISM models in its approach.

The PRISM model comprises interlinked soil, plant and animal modules (Thorne et al., 2005). This soil-plant model includes the calculation of soil-to-root concentration factors (CR) as shown by Eq.9.8.

$$CR = \frac{\delta}{(\theta_w + \rho_s K_d)} \qquad \textbf{9.8}$$

Where:
CR= soil-to-root concentration factor, Bq kg^{-1} fw plant per Bq kg^{-1} dw soil
δ= soil-plant availability correction, dimensionless
θ_w= water-filled soil porosity, cm^3 cm^{-3}
ρ_s= dry soil bulk density, g cm^3
K_d= soil-water partition coefficient, cm^3 g^{-1}

Note that when applied in the scope of the CLEA model CR is given mg g^{-1} fw plant per mg g^{-1} dw soil.

According to Eq.9.8 the chemical concentration in soil solution is determined by the soil-water partition coefficient (K_d) and depends on soil type and pH. The proportionality constant (δ) accounts for a number of factors found in plant uptake pot experiments and includes the total plant density (including roots), the depth of the pot soil, the duration of the experiment, and an empirical calibration parameter.

In the development of the PRISM model Thorne et al. (2005) separated 17 elements including Pb, Ni and Se into so-called groups of "consistent behaviour" based on considerations of soil chemistry, plant kinetics and uncertainty and proposed guidelines for determining the value of δ for these 17 elements. The CLEA model has also adopted these guidelines. The values of δ proposed by Thorne et al. (2005) are:

- very low uptake potential elements (for example lanthanides and higher actinides): $\delta=0.5$;
- Essential to metabolic plant processes or chemically similar to these elements (for example As, Cd, Pb, Hg and Ni): $\delta=5$;
- Unusually high uptake potential elements (for example Se): $\delta=50$.

Generic CR values are adopted for each inorganic element across all crops of interest, on the basis that the range of uncertainty in the CR value for a particular plant is not significantly different from the total range of uncertainty across all plant (Thorne et al., 2005).

Thorne et al. (2005) also considered the transport of inorganic elements within the plant from the root zone to edible fruits, leaves, root storage organs and tubers. They concluded that elements transported within the xylem though passive transpiration should be treated separately from those transported by the phloem (which interacts with organs such as fruits and tubers). The CR values calculated in Eq.9.8 is then corrected to account for the fraction reaching the internal plant system (f_{int}) to derive the soil-to-plant concentration factor representative of edible plant parts as shown in Eq.9.9.

$$CF = CRxf_{\text{int}} \qquad \textbf{9.9}$$

Where f_{int} is the fraction of chemical in the root system reaching edible plant parts including root store, tubers, fruits and shoots (between 0 and 1).

For example, for elements transported by the xylem such as Pb and Hg, f_{int} used by CLEA model was based on a review of the available uptake literature and has been categorised for each produce group: green vegetables (root to shoot); root vegetables (root to root store); tuber vegetables (root to tuber); herbaceous, shrub and tree fruits (root to fruit). For elements transported by the phloem such as As and Cd, f_{int} was assumed to be 0.5 for any internal plant compartment, to account for their rapid and highly distributed behaviour (Thorne et al., 2005)

9.4.2 Gay and Korre (2009)

Gay and Korre (2009) published a methodology for the probabilistic calculation of intake of Cd by individuals using an adaptation of the CLEA model which includes pH variability in the calculation of the soil-to-plant concentration factor (CF_{veg}).

Gay and Korre (2009) used an equation similar to a combination of Eq.9.1 and Eq.9.2 to estimate the intake route for Cd absorbed into vegetables:

$$ADE_{vegetables} = (C_{soil} x CF_{vegetable}) x (\frac{EF_{oral} x ED_{oral}}{AT} x \sum_{vegetable\_type} (CR_{vegetable} x HF_{vegetable})) \qquad \textbf{9.10}$$

In the original CLEA model, C_{soil} is treated deterministically, however in the Gay and Korre (2009) methodology, C_{soil} is treated spatially and probabilistically, so the intake equation has been rearranged to separate C_{soil} (whose values are derived in the geostatistical exposure assessment step) from person-specific elements. The model also uses an average CF_{veg} across all vegetable types. This CF_{veg} is given by:

$$\log(CF_{veg}) = a - b \times pH \qquad \textbf{9.11}$$

Where *a* and *b* are constants.

The results obtained by Gay and Korre (2009) showed that taking pH into account can influence the outcome of the risk assessment greatly. It has been proposed by the authors that a similar adaptation could be used for other combinations of soil variables which influence CF_{veg}.

9.4.3 Franz et al. (2008)

Franz et al. (2008) proposed a chain modelling approach to estimate the impact of Cd pollution on human dietary exposure. This study developed a model to estimate exposure from soil characteristics that consisted in: calculation of plant Cd levels from soil contamination levels and soil properties; calculation of animal transfer from consumption and contamination levels; and human exposure from both plant and animal products.

Franz et al. (2008) assumed that root uptake is the dominant process for chemical accumulation by plants from soil. They used a Freundlich-type soil-plant relation for calculation of the accumulated Cd concentration in grass, maize and vegetables, as a function of total concentration and the major soil properties. The following regression equation was applied:

$$\log(Cd_{plant}) = INT + \alpha \log(\%OM) + \beta \log(\%Clay) + \gamma \log(Cd_{soil}) + \sigma pH \qquad \textbf{9.12}$$

The regression parameters INT (intercept) and α, β, γ and δ were estimated by regression analysis (stepwise multiple regression) with two Dutch national and one local (Kempen area) datasets (Franz et al., 2008).

Animal exposure and transfer to cattle kidneys, livers and meat were calculated using a consumption database and a parameterized linear simulation model. Transfer of Cd to animal organs was calculated using linear biotransfer rate, assuming an irreversible Cd accumulation without excretion from the target organ (worst-case scenario). Human exposure (long-term, chronic) was estimated in a probabilistic approach using Monte Carlo simulation using a consumption database.

The validation of models obtained for the Dutch Kempen area showed that the results based on predicted contamination levels reflect the results based on measured data (Franz et al., 2008).

The model by Franz et al. (2008) is a relatively simple and fast approach to evaluate Cd exposure for specific regions accounting for the variability of both Cd soil levels and soil properties. The key aspects of such approach in determining the validity of results produced are the calibration of soil-plant relationships, the definition of Cd biotransfer rates between feed and animal organs and the definition of human food consumption patterns.

9.4.4 Brus et al. (2005)

Brus et al. (2005) developed an approach to derive probabilistic quality standards for Cd in soil derived from quality standards in wheat, in the Netherlands. The transfer of Cd in the soil to the crop was modelled by a regression model:

$$\log(Cd_{wheat}) = a_0 + a_1 pH + a_2 \log(\%OM) + a_3 \log(\%Clay) + b\log(Cd_{soil}) + (\varepsilon_{\mathrm{mod}} + \varepsilon_{ms}) \qquad \mathbf{9.13}$$

The coefficients a_0, a_1, a_2, a_3 and b are regression coefficients. The Eq. 9.13 also includes an error term ($\varepsilon_{mod}+\varepsilon_{ms}$). The variance of ε_{mod} (τ^2) is assumed to be constant and includes for example variation associated with different cultivars or with the Cd-Zn interaction during the plant uptake process. The second error term, ε_{ms} accounts for error in the measurement of the Cd concentration in wheat. The model was calibrated for Cd concentration in wheat using a Dutch national survey data and local data (Meuse) and applying both Ordinary Least Squares (OLS) fitting (assuming that the variance of the error in measurement was 0) and Weighted Least Squares fitting (with weights equal to the inverse of the variance of the measurement error).

Given the food quality standard and a maximum acceptable probability of exceeding this standard, the Cd concentration in soil at which the probability of excess Cd in wheat equals this maximum (critical threshold) was calculated by the inverse use of the regression model.

The study by Brus et al. (2005) showed that when determining probabilistic soil quality standards it is important to have reliable estimates of the variance of regression residuals and that neglecting error in the measurements leads to more conservative estimates of the p% critical threshold for $p < 50$. Another key aspect of this approach is the calibration of the regression models. The domain of a regression model is limited to the extension of the calibration data and in order to be able to estimate the critical threshold for a wide range of arable soils calibration data must include a wide range of soil types/ conditions. Furthermore, when a national model is used for local estimation of the critical threshold, this may lead to biased estimates, even at probability levels where the model need not be extrapolated (Brus et al., 2005).

9.4.5 Hough et al. (2004)

Hough et al. (2004) tested an approach to assessing potential risk of metal exposure from consumption of home-produced vegetables by urban populations. These authors developed models of uptake of Cd, Cu, Ni, Pb, and Zn for a selection of commonly grown allotment and garden vegetables from the UK using the following approach:

$$\log\left[M_{plant}\right] = C + \beta_1 pH + \beta_2 \log\left[M_c\right] \tag{9.14}$$

Where:

$[M_{plant}]$= concentration of the metal in the plant (mg kg^{-1})

$[M_C]$= soil metal concentration which is assumed to be adsorbed on organic carbon (mg of a specific metal kg^{-1} of soil organic carbon)

C, β_1, and β_2 are empiric metal- and vegetable-specific coefficients.

Regression analysis of [M_{plant}] against pH and [M_C] (Eq.9.14) provided good estimates of uptake for Cd, Cu, Ni, and Zn by all vegetables, with less satisfactory results for Pb (Hough et al., 2004). The reason for this may be that uptake of Pb by vegetables is relatively small compared with Pb concentrations in the local soil and dust; a significant source of Pb contamination of vegetables particularly in urban areas is atmospheric deposition (Hough et al., 2004). This study showed that the environmental behaviour of the different contaminants should be carefully considered when applying such empirical plant uptake models.

9.5 Contaminated soil exposure assessment in Portugal

9.5.1 Conceptual approach for exposure assessment

The conceptual approach for contaminated soil exposure pathways in Portugal evaluated in this study is shown in Figure 9.6.

As shown in Figure 9.6 this work focused on two main pathways of human exposure: consumption of food (vegetables and animal products) and soil ingestion. Although no groundwater samples were collected and analysed during this study, the soil: soil solution partition relationships derived in Chapter 6 proved most relevant insight on the potential for mobility of contaminants from Portuguese soils with different characteristics which in the future may contribute for the evaluation of human exposure via the intake of groundwater.

The conceptual framework for analysis of contaminated soil exposure pathways developed in this study relates to oral intake (ingestion through the mouth) of contaminants associated to a specific land use: agriculture. The framework can also be applied in the assessment of human exposure to soil contaminants from vegetable gardens. It should be noticed that this study focused on the analysis of contamination through the terrestrial food chain including both the consumption of vegetables grown at contaminated sites and the consumption of animal products. Although the ingestion of vegetables was the pathway more commonly included in exposure models, the later exposure route was also part of the Vlier-humaan (Flanders, Belgium) model as described in section 9.3.3.

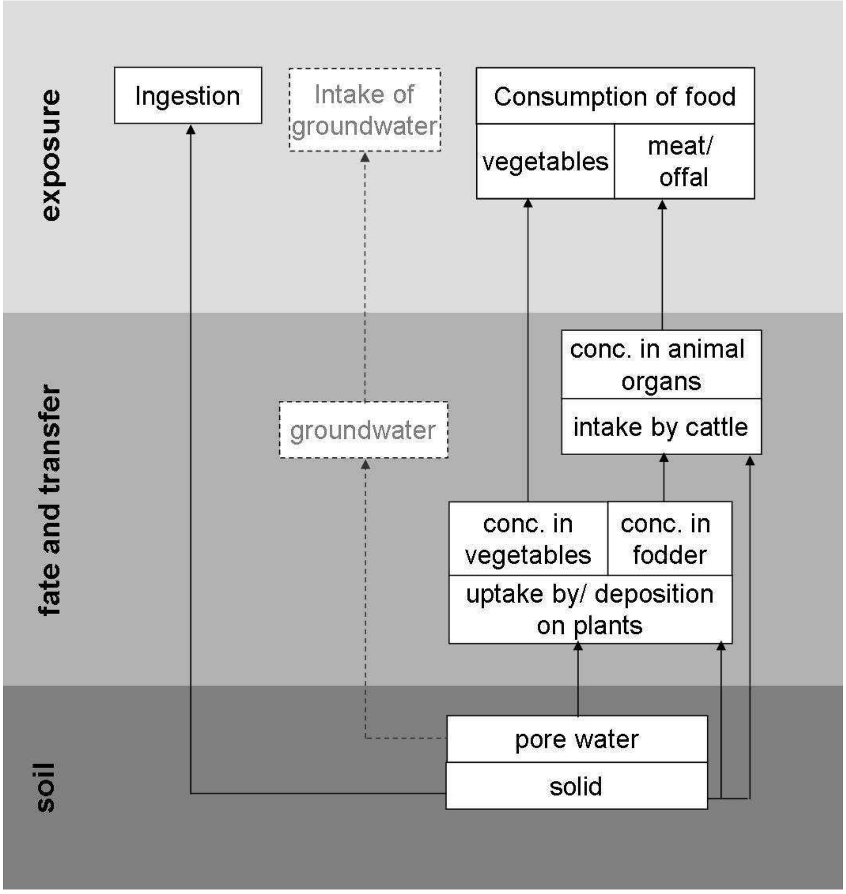


Figure 9.6: Conceptual approach for analysis of contaminated soil exposure pathways developed in this study (agricultural soils in Portugal)

There are other exposure pathways such as dermal contact and inhalation of contaminants that were not covered by this work but that should be subject of future investigation regarding the definition of a strategy for contaminated soil exposure assessment in Portugal regarding other land uses such as recreational, residential, commercial or industrial:

- Inhalation of soil dust;
- Volatilization to indoor/ outdoor air and inhalation of vapours;

- Dermal adsorption from soil/ dust;

There are also other potential health risks from soil contamination that were not evaluated during this study but that may be critical on a site-specific assessment such as:

- Sources of contamination other than surface soils and groundwater including drinking water and surface waters such as lakes;
- Off-site migration and potential impacts on human health;
- Short-term exposure resulting in human health risk such as poisoning or by directly bodily contact.

Similarly to exposure models developed in other countries described in the previous sections of this Chapter, the work developed in the course of this investigation can support two main activities:

- The development of site-specific exposure assessments to PTE's: it requires the assessment of soil properties and analysis of concentrations of contaminants in soils and other matrices (such as vegetables, fodder and eventually animal organs and groundwater) as well as the determination of local food consumption habits and the assessment of the origin of consumed products;
- The derivation of soil quality criteria for agricultural soils in Portugal: threshold concentrations of PTE's can be derived from quality criteria for plants, foodstuff, fodder, animal organs or water quality using generic assumptions about the characteristics and behaviour of contaminants in soils (as a function of soil properties), pathways of exposure (soil to animal transfer) as well as animal diet and human food consumption patterns.

The framework for exposure assessment and calculation of threshold soil concentrations in Portugal developed in this study is shown in Figure 9.7.

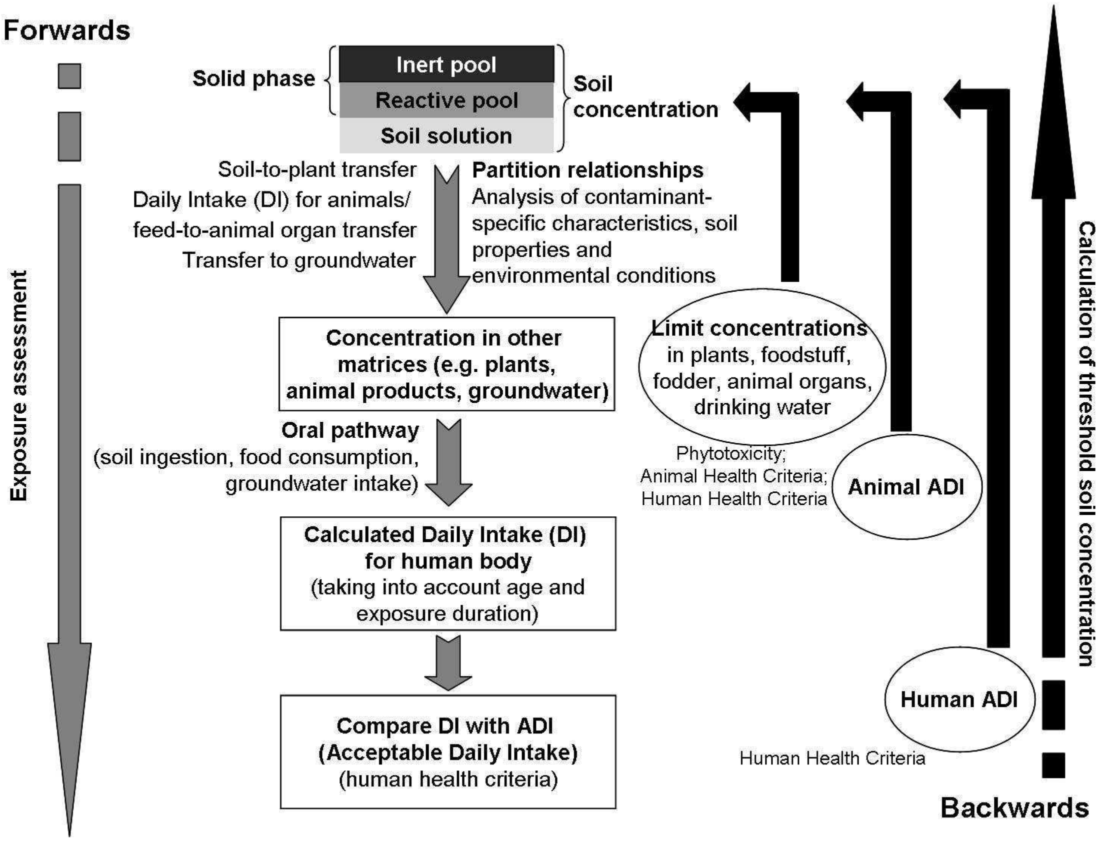


Figure 9.7: Framework for exposure assessment and calculation of threshold soil concentrations in Portugal (this study)

Site-specific exposure assessments in agricultural fields/ vegetable gardens can be derived using detailed site-specific information:

- Origin, identification and spatial (horizontal and vertical) distribution of contaminants in soil;
- Partition of contaminants in soils: analysis of key soil properties (such as pH, organic carbon, clay, metal oxides); evaluation of reactive pools of contaminants; evaluation of available pools of contaminants (soil solution concentrations); assessment of loss mechanisms including physical transport, dilution and degradation;
- Fate and transfer of contaminants from soils to other environmental media: analysis of concentrations in plants, food crops, fodder, grazing cattle organs and groundwater; assessment of contaminant intake characteristics for animals and calculation of site-specific soil-to-plant transfer functions and feed-to-animal-organs BAFs;

- Evaluation of additional exposure pathways: of concluded that further pathways may be relevant for on-site human exposure (e.g. inhalation or dermal absorption) these must be assessment and the chemical intake associated to these routes must be included;
- Local background exposure may be further assessed;
- Site investigation should better define site users and duration of exposure as well as the fate of produced foodstuffs, fodder and animal products; this information may be based on surveys;
- Intake characteristics for humans: soil ingestion; groundwater intake patterns; specific food consumption patterns and identification of cases where generic assumptions may not apply (for example self-sufficient families growing a significant proportion of their own fruit, vegetables and animal products).

In Chapter 7 relationships between concentrations of eighteen metals and metalloids (Hg, As, Cu, Pb, Zn, Cd, Ni, Cr, Co, Ba, U, Fe, Mn, Al, Sb, Se, B and Mo) in a variety of contaminated and non-contaminated soils in Portugal and their contents in field-grown feed and food crops were derived. The approach developed for the definition of soil-to-plant transfer functions can be followed in site-specific exposure assessments as well.

In Chapter 8 I have also tested an approach for the calculation of cattle (cow and sheep) Daily Intake of contaminants as well as for estimating concentrations of PTE's in animal organs and human Daily Intake of PTE's. This approach may also be followed in site-specific exposure assessments using site-specific information.

As commonly applied by exposure models for assessment of site-specific human exposure, the calculated Daily Intake of a contaminant is divided by the human health criteria (Acceptable Daily Intake) to obtain a Risk Index (RI). If RI>1 there is a risk to human health associated with the oral pathway of exposure.

These calculations are rather straightforward as shown by Eq.9.15 and 9.16:

$$DI_{oral} = \frac{\sum_{vegetables}[PTE]_{veg} \times I_{veg} \times f_{locally\_grown}}{BW} + \frac{\sum_{animal\_prod}[PTE]_{animal\_prod} \times I_{animal\_prod} \times f_{locally\_graz}}{BW} + \frac{[PTE]_{soil} \times I_{soil}}{BW} + \frac{[PTE]_{gw} \times I_{gw}}{BW} \quad \mathbf{9.15}$$

Where:

DI_{oral}= oral daily intake of a PTE for humans in mg kg BW^{-1} d^{-1}

BW= body weight (kg)

$[PTE]_{veg}$ = concentration of a PTE in vegetables, in mg kg^{-1} f.w.

$[PTE]_{animal\_prod}$ = concentration of a PTE in animal products, in mg kg^{-1} f.w.

$[PTE]_{soil}$ = concentration of a PTE in soil, in mg kg^{-1} d.w.

$[PTE]_{gw}$ = concentration of a PTE in groundwater, in mg l^{-1}

I_{veg}, I_{animal_prod} = daily intake of vegetables and animal organs (kidney, liver and muscle/ meat), respectively, by humans in kg d^{-1} f.w.

I_{soil}= daily ingestion of soil by humans in kg d^{-1} d.w.

I_{gw}= daily intake of groundwater by humans in kg l^{-1}

$f_{locally\_grown}$= fraction of vegetables consumed that is grown at the contaminated site

$f_{locally\_graz}$= fraction of animal products consumed that originate from animals grazing at the contaminated site

$$RI_{oral} = \frac{DI_{oral}}{ADI} \quad \mathbf{9.16}$$

Where:

RI= risk index (oral intake of contaminants)

ADI= acceptable daily intake in mg kg BW^{-1} d^{-1}

Regarding the intake of contaminants via vegetables and animal products consumption, a bioavailability correction factor may also be considered when available to express the relative bioavailability of a contaminant in the human body.

As described by Eq.9.16 the RI is a relationship between the calculated intake of contaminant and the human health criteria value, as for example the ADI. Since all chemicals have the potential to cause harm to human health depending on the duration and level of exposure, the definition of a health criteria value such as the ADI is the approach commonly followed to describe the level of exposure to a chemical derived from toxicity data with the purpose of safeguarding human health (Environment Agency, 2009f). Human health criteria are available from variable international toxicological data sources (such as the World Health Organisation, WHO, within the International Program on Chemical Safety, IPCS) (Carlon, 2007). It is common that when defining their contaminated land management strategies, countries selected national committees or expert groups to evaluate toxicological data from variable data sources and select the health criteria values for the various contaminants, particularly for threshold contaminants. In Chapter 8 of this work, values of Provisional Tolerable Weekly Intake (PTWI) available from the WHO database (EC, 2004) for Cd, Pb, Hg and As were used. The oral intake of each of these contaminants was calculated on a weekly basis and compared to PTWI values to evaluate risks to human health.

9.5.2 Derivation of soil quality criteria for agricultural soils in Portugal

In this section, Freundlich-type soil-to-plant-transfer functions derived in Chapter 7 will be applied to calculate soil quality criteria for agricultural soils in Portugal. Threshold concentrations of Cd, Hg, Pb, As, Cu and Zn in soils were back-calculated from:

- Limit concentrations in feed materials (green fodder);
- ADI’s for grazing animals calculated in view of both food safety and animal health criteria.

Threshold concentrations of Cd, Hg, Pb, As, Cu and Zn in soils (both total and available contents) were back-calculated from green fodder limits presented in Chapter 8 (Table 8.2) and according to Eq.9.17 and are shown in Table 9.1.

$$\log[PTE]_{soil\_threshold} = \frac{\log[PTE]_{\lim it\_plant} - \log(a) - (b...i) \cdot \log[soil\_properties]}{n} \qquad \mathbf{9.17}$$

Furthermore, threshold values of PTE's in soils can be back-calculated from ADI's for grazing animals. These threshold values are the maximum soil concentrations at which the contents of contaminants in animal organs (kidney, liver and muscle/ meat) will not surpass food safety and/or animal health criteria (de Vries et al., 2007) as given by:

$$ADI_{animals} = \left(k_{sp} x[PTE]^{n}_{soil\_threshold}\right) xI_{feed} + [PTE]_{soil\_threshold} xI_{soil} \qquad \mathbf{9.18}$$

The threshold concentration of a PTE in soils can be calculated iteratively from Eq. 9.18 given an ADI and using K_{sp} and n values from derived SPT functions.

The ADI$_{\text{animal}}$ of Cd, Hg, Pb, As, Cu and Zn for cows also calculated in Chapter 8 (Table 8.2) were used to calculate the threshold soil total concentrations in view of food safety and animal protection according to Eq. 9.18 and the results are shown in Table 9.1 as well.

From Table 9.1 it is clear that for elements such as Cd, Zn, Cu and Pb the characteristics of soil significantly affect the soil level at which the contaminant will exceed limit levels in crops and may pose risks in terms of food safety and animal health. For instance with a variation in pH from 4 to 6, the soil total Cd concentrations at which levels in green fodder may exceed the EC quality standards vary from 2.1 to 4.8 (Table 9.1). This shows that the inclusion of SPT functions in the calculations of threshold soil concentrations allows accounting for the lower availability of Cd to plants at higher pH values. As shown in Table 9.1, for Cd, Cu and Zn both soil pH and

Org C% affect the green fodder levels of the contaminants while for Pb it is important to consider Al_{am_ox} when evaluating risks associated to the transfer of this element into the food chain.

Table 9.1: Calculated threshold concentrations for PTE's in Portuguese soils and soil quality criteria available from Portugal and other countries

	Threshold soil total concentrations* (mg kg^{-1} d.w.) - This study													
	Cd			**Hg**	**Pb**			**As**	**Cu**			**Zn**		
	pH=4; Org C=3%	pH=5; Org C=3%	pH=6; Org C=3%		Al_{am_ox}=50 mmol kg^{-1}	Al_{am_ox}=100 mmol kg^{-1}	Al_{am_ox}=150 mmol kg^{-1}		pH=4; Org C=3%	pH=5; Org C=3%	pH=6; Org C=3%	pH=4; Org C=3%	pH=5; Org C=3%	pH=6; Org C=3%
Green fodder production *(Lolium perenne)*	2.1	3.1	4.8	1.9	411	618	789	85	87	168	324	367	592	955
Food safety (cow-kidney)	0.5	0.7	1.0	0.7	51	70	82	775	3177	3536	3845	2837	4028	5488
Food safety (cow-liver)	1.6	2.4	3.6	5.6	113	152	179	1483	437	570	703	1181	1735	2459
Animal health (cow-kidney)	3.4	4.9	7.1	65	332	446	524	6561	115	171	237	2374	3396	4666
	Threshold soil available concentrations (mg kg^{-1} d.w.) - This study**													
	Cd			**Hg**	**Pb**			**As**	**Cu**			**Zn**		
Green fodder production *(Lolium perenne)*	0.3			0.004	71			0.53	1.9			21		
	Soil quality criteria other countries (soil total concentrations, mg kg^{-1} d.w.) (source: Carlon, 2007)													
	Cd			**Hg**	**Pb**			**As**	**Cu**			**Zn**		
Portugal (agricultural soils amended with sewage sludge)***	1 (pH<5.5) 3 (5.5<pH<7.0) 4 (pH>7.0)			1 (pH<5.5) 1.5 (5.5<pH<7.0) 2 (pH>7.0)	50 (pH<5.5) 300 (5.5<pH<7.0) 450 (pH>7.0)			n.a.	50 (pH<5.5) 100 (5.5<pH<7.0) 200 (pH>7.0)			150 (pH<5.5) 300 (5.5<pH<7.0) 450 (pH>7.0)		
UK (soil guideline values: allotments) (based on a sandy loam soil with 6% organic matter content)	1.8			80 (inorganic Hg)	n.a.			43	n.a.			n.a.		
the Netherlands (generic target values - intervention values) (standard soil 10 %OM; 25 % clay)	0.8 - 12			0.3 - 10	85 - 530			29 - 55	36 - 190			140 - 720		
Flanders, Belgium (clean-up values; agricultural areas)	2			10	200			45	200			600		

* soil total concentrations = *aqua regia* extraction for Cd, Pb, As, Cu and Zn; total contents for Hg

** soil available concentrations = 0.01M $CaCl_2$ extraction

*** Decreto-Lei 276/2009, from 2nd October 2009

n.a.= not available

With the exception of Cu, threshold soil concentrations derived for the various elements from animal health criteria are above the respective thresholds derived from food safety criteria (Chapter 8, Table 8.2), which means that by defining soil quality criteria to protect human health, the animal health protection is also assured. For Cu, the limit concentration in cow kidney in view of animal health found in literature was more stringent than the limit content defined for food safety (Chapter 8, Table 8.2) which explains why Cu determined threshold soil total concentrations were of 115-237 mg kg^{-1} d.w. regarding animal health protection and were over the 400 mg kg^{-1} d.w. derived concerning food safety.

The Portuguese legislation (Decreto-Lei 276/2009, 2009) includes soil quality standards only for agricultural soils which are subject to sewage sludge application. The following metals were included in Decreto-Lei 276/2009 Cd, Cu, Ni, Pb, Zn, Hg and Cr (at $pH \leq 5.5$; $5.5 < pH \leq 7.0$; and $pH > 7.0$). The standards were established with the purpose of protecting water, vegetation, animals and human health from soil contamination associated with the practice of agricultural soil amendment with sewage sludge. No information on the approach followed to derive these soil quality standards could be found which makes difficult the comparison with the values derived in this study. Nevertheless it should be noticed that the soil quality standards established by Decreto-Lei 276/2009 take into account one of the key soil properties also considered in this study (soil pH). Moreover, both threshold soil concentrations (total) calculated in the present study and those available from the Portuguese legislation have the same order of magnitude. In general, our approach produced threshold values that are somewhat less conservative compared to the soil quality standards established by Decreto-Lei 276/2009 this can be associated for example with the fact that only the oral intake pathway was considered in our study.

Threshold soil concentrations (which are named differently by the different countries) currently in use at the UK (soil guideline values), at the

Netherlands (Target/ Intervention Values), and at Flanders, Belgium (clean-up values) were also included in Table 9.1 for comparison. Despite the disparities among the various threshold soil concentrations for the various countries it is clear that the soil total concentrations derived in the present study are of the same order of magnitude of soil quality criteria currently in use at other countries (Table 9.1).

The differences between our values and those from the UK, Netherlands, and Belgium (Flanders) as well as those among the criteria defined by these three countries relate to differences in the approaches used for the derivation of threshold soil concentrations. These differences can be associated to three main factors:

- variability of results associated with observed differences attributable to true heterogeneity or diversity in a population (USEPA, 1997);
- variability of results associated with differences in the methodology defined to assess exposure and evaluate risks such as the selection of specific pathways of exposure, selection of exposure scenarios, protected receptors and levels of “acceptable” risks (toxicological data) that have been included in the calculations by each approach;
- uncertainty, or the lack of knowledge about specific factors in a risk or exposure assessment.

Unlike the methodological variability and the uncertainty, the variability of results associated with the heterogeneity in a population cannot be reduced by further study only better described or understood (Environment Agency, 2009f). In fact this type of variability is controlled mainly by geographical and biological factors and is expected to occur when defining contaminated soil exposure assessment approaches for populations with variation in terms food consumption habits or body weights associated with gender and age.

The methodological variability is associated with the technical aspects of the approach and with the decision making process and is controlled mainly by regulatory and political factors. Although these factors are not generally included in the derivation of threshold values for contaminated land exposure assessment, they are implicit in basic assumptions, methodological choices and default input values. As described in sections 9.3.1, 9.3.2 and 9.3.3 the specific pathways of human exposure vary between the three exposure models. Moreover, when defining Target and Intervention Values in the Netherlands both human exposure and ecotoxicological risk assessment (toxicity to terrestrial ecosystems) are taken into account – information relative to both sensitive receptors is integrated to yield the overall soil quality criteria (Carlon, 2007). For the UK and Flanders, the soil quality criteria presented here relate to human receptors only. The approach followed in the present study considers both human and animal health as protected receptors in the definition of threshold soil concentrations for agriculture land use. Differences in exposure duration for human receptors as well as age of receptors considered sensitive in the calculation of generic threshold soil concentrations also contribute to the variability of results.

The uncertainty associated with exposure models can be of three main types (Environment Agency, 2009f; Baveye et al., 2009):

- Parameter uncertainty: relates to measurement errors, including sampling, analysis and systematic errors – the "reality" cannot be apprehended directly and has to be quantified through a measurement process;
- Model uncertainty: relates to limitations in the way that the model represents "reality" – a model encompasses various levels of detail of the "reality" to be described which will then be translated using one of a number of mathematical algorithms; there are many situations in exposure assessment where the scientific understanding is still restricted to simplistic observed relationships between parameters;
- Scenario uncertainty: associated with limitations in the model for the exposure assessment and its suitability to actual depict a defined

"reality" and relates to calibration, sensitivity and validation of the exposure model; scenario uncertainty is an inevitable consequence of designing exposure scenarios that are intended to be appropriate for screening purposes and that use generic assumptions, in these cases validation is limited to confirm that assumptions, choices and hypothetical relationships are reasonable.

The uncertainty gives rise to built-in error within exposure models (Environment Agency, 2009f). Many of the algorithms used in exposure models are semi-empirical in nature and draw on limited results of laboratory and filed trials. Although more complex and mechanistic approaches to modelling soil systems have been particularly by the scientific community, there is often a paucity of data to parameterize such approaches (Environment Agency, 2009f). Inevitably, many semi-empirical approaches are based on observations from a small number of real world situations and consider very few combinations of environmental conditions and other variables. In principle, such errors can be corrected by greater understanding of how systems work and with improved input data.

As discussed in sections 9.3.1, 9.3.2 and 9.3.3, the scientific basis for the derivation of soil quality criteria using human exposure models varies from country to country. In particular, differences among the calculations of accumulation of soil contaminants in plants associated to the selection of soil-to-plant transfer BCFs may contribute to differences in the calculation of threshold soil concentrations.

When selecting BCFs for metal accumulation in plants from available studies it must be taken into account that these:

- Can be subject to measurement uncertainty;
- Are crop specific;
- Are subject to soil type, properties and matrix effects;

- Are subject to concentration effects – and it should be used to infer on contaminants accumulation that are within the range for which they were derived;
- Can also be subject to influence from environmental conditions and climate (Otte et al., 2001).

The use Freundlich-type soil-to-plant-transfer functions derived by multiple linear regression from field data as applied in the approach developed in the current study to assess human exposure from soil contaminants in Portugal can contribute to reduce the sources of uncertainty in the assessment of PTE's accumulation in plants.

9.6 Summary and conclusions

This study showed that the use of SPT functions in the definition of threshold levels of PTE's at which functions of soil are safeguarded (namely production of high quality feed and food) allowed to account for the effect of soil properties on the availability of soil contaminants to crops increasing risk estimation accuracy. In the cases of Cd, Cu and Zn the variability of pH and Org C significantly affect elements' levels in green fodder, while for Pb the Al_{am_ox} is a relevant limiting factor on the availability of this element to crops analysed. Hence, this approach provides a better basis for defining threshold soil values of dangerous element concentrations at national/ regional level based on the regional variability of soil properties and degrees of pollution as well as for defining risk maps than that of linear models based on constant soil-plant transfer factors.

This study also showed that the $CaCl_2$ extraction (a fast and simple soil analysis) can be used to assess the availability of contaminants in soils and to determine whether or not a crop can be grown at a specific site by back-calculating soil threshold concentrations from EC feed/ food crop quality standards which can be useful particularly at the local scale.

Further advances on the use of this approach may be achieved by the analysis of uncertainties associated to the SPT functions namely the impacts of different plant species on model performance and uncertainties associated to model coefficients. Brus et al. (2005) reported on the analysis of the regression residuals and provided relevant insight on the effects of the variance of regression residuals on the assessment of the probability of exceeding Cd EC quality standard in wheat in the Netherlands.

Finally, this study has focused on the quality of soils for agriculture production as a key factor for feed and food production and showed that this approach has a potential for EU-wide improvement of soil quality criteria in agricultural areas. Further advances on the use of this approach for calculation of threshold soil concentrations may be achieved if other pathways of human exposure such as groundwater drinking, dermal contact with soils and inhalation are included and if lifelong exposure to contaminants is considered in the definition of risk levels. The threshold soil concentrations derived on the basis of feed and food protection should also be analysed in combination with thresholds for the protection of other sensitive receptors such as terrestrial ecosystems.

Chapter 10

General Discussion and Conclusions

The most relevant achievements of this PhD program will be discussed according to the list of milestones defined in Chapter 1 (Introduction).

M1- Review of contaminated land management strategies from Portugal and other EU countries

An EU Thematic Strategy for Soil Protection has been launched in 2006. It is important to emphasize that several threats (including erosion, loss of organic matter, compaction, salinisation, landslides, sealing and contamination) to soil functions have been taken into account in the definition of such strategy and that the development of a Soil Framework Directive has been proposed. Regarding soil contamination, this Directive would provide grounds for the development of a common risk-based strategy to manage contaminated sites within the EU. Nevertheless, to date no final agreement regarding the contents of such Directive has been reached by Member States.

During the last three decades, several EU Member States including Austria, Belgium, Italy, France, Germany, the Netherlands, Spain and the UK have developed and implemented national soil protection strategies. This PhD program included the revision of such strategies, with particular emphasis on aspects related with the management of contaminated sites. In most countries the strategy to deal with soil contamination includes the development of an approach for exposure modelling and risk characterisation. It was clear from this study that the definition of such strategy is far from being a consolidated and settled process and that several scientific, technical and regulatory aspects as well as political approaches vary from country to country.

The policy and regulatory frameworks for soil protection in Portugal were also reviewed. Documents containing provisions regarding the assessment, management, remediation and/ or prevention of soil contamination were analysed. It was observed that apart from the Decree-Law nº 118/2006, regulating the use of sewage sludge in agriculture to prevent harmful effects on soil, plants and humans (in D.R. 1ª Série nº118, 21-06-2006), amended in 2009 by the Decree-Law nº 276/2009 (in D.R. 1ª Série nº192, 2-10-2009) there are no legislative instruments in Portugal that distinctively address soil contamination aspects. To date, legal or technical issues related to soil contamination problems are dealt with within the scope of a more general Environmental Framework Law or within the scope of specific provisions included in waste management, water, integrated pollution prevention and control, environmental liability or environmental impact assessment regulations. The development of a Portuguese National Strategy on Soil Protection integrated with the EU Thematic Strategy on Soil Protection has been foreseen within the National Program of the Physical Planning Politics was introduced in September 2007 (Law n.º 58/2007, "Programa Nacional da Política de Ordenamento do Território (PNPOT)" in D.R. 1ª Série, nº170, 04-09-2007) but has not yet been implemented.

The analysis of almost three decades of experience in dealing with soil protection aspects in Portugal and in other EU countries showed that:

- The development of a National Soil Protection Strategy for Portugal integrated with the EU Thematic Strategy for Soil Protection is not only necessary but also urgent;

- The development of a Portuguese National Soil Protection Strategy must also comprise the development of a Contaminated Land Legal Framework setting guidelines for exposure modelling and risk characterisation/ management.

Such Contaminated Land Legal Framework should as far as possible take into account the specificity of Portuguese soils and exposure scenarios while considering the needs and possibilities of harmonization of risk assessment procedures for contaminated soils in Europe.

The development of guidelines for exposure modelling and risk characterisation/ management in Portugal that takes into account the specificity of Portuguese soils and exposure scenarios requires the development of a national database that described the variability of key soil characteristics across the country, the distribution of geogenic and anthropogenic chemicals in Portuguese soils as well as the characterisation of pathways of exposure of sensitive receptors to soil contaminants.

The “Soil Geochemical Atlas of Continental Portugal” (Inácio et al., 2008) (which relates to the information included in the FOREGS Geochemical Atlas of Europe) provides most useful information regarding the characterisation and distribution of background levels of inorganic elements in Portuguese soils, crucial for the interpretation of monitoring data and identification of areas with anthropogenic soil contamination. Information regarding the variability of generic soil properties such as pH and total organic carbon has also been produced in the scope of the FOREGS Geochemical Atlas of Europe and of the Portuguese Atlas of Environment. Nevertheless, the information necessary for the characterisation of exposure pathways to soil contaminants at a national/ regional level as well as at a local level is generally not available.

M2- Collection of soils and plant samples from agricultural fields from different areas in Portugal.

Samples were collected from geographically distributed areas with the aim of studying soils from agricultural fields with different characteristics and a wide range of contamination levels for the various elements. Crops growing at those areas were also collected. This study included the collection of 136 soil samples and 128 crop (ryegrass, Italian ryegrass, collard greens, orchard grass and rye) samples from different areas in Portugal.

The approach followed to select sampling areas as well as the sampling methodology allowed to obtain the necessary information on:

- Regional variability of soil properties;
- Variability of total pools of PTE's and identification of origin and magnitude of anthropogenic contamination;
- Variability of total concentrations of PTE's in plant samples;
- Variability of reactive and available pools of PTE's and its relation with the variability of several contaminants in plant samples;
- Characterisation of soil-to-plant relationships;
- Calculation of estimated daily intakes of PTE's by grazing animals and humans;
- Back-calculation of threshold values of Cd, Hg, Pb, As, Cu and Zn from limit concentrations in feed materials (green fodder) and ADI's for grazing animals calculated in view of both food safety and animal health criteria.

This information allowed to characterise the pathway of exposure of grazing animals and humans to soil PTE's (and particularly Cd, Hg, Pb, As, Cu and Zn) via crop consumption. Although a significant number of samples of feed crops were sampled which allowed the characterisation of the soil-feed-animal exposure route, the number of food crops samples collected in the scope of this study was limited. Regarding vegetables, only collard greens were found and sampled at the selected sampling areas. Therefore, only a limited characterisation of the soil-vegetables-humans exposure route was possible. In the future further vegetables, as well as fruits should be sampled and analysed. Particularly, potatoes, roots, tubers, other types of leaf vegetables (cabbages, lettuce, spinach), stem and stalk vegetables, fruiting vegetables, shrub fruits and tree fruits should be collected in future studies.

Feed-to-animal organs BAFs available from literature were used to estimate the accumulation of PTE's in animal organs. In the future, the collection of animal products (kidney, livers and muscle samples) would allow the calculation of regional/ local specific BAFs.

The collection of groundwater samples in areas where such water is known to be used for irrigation and/or drinking purposes would also allow a more adequate characterisation of pathways of exposure to soil contaminants in agricultural areas.

Finally, the sampling strategy defined in this investigation can be considered appropriate for the characterisation of exposure pathways (soil ingestion and food consumption) associated with specific land uses (agricultural or vegetable gardens). When characterising exposure routes associated with other land uses (such as residential, recreational or industrial) such as inhalation of dusts, inhalation of vapours or dermal contact, the sampling strategy must include the collection of additional types of samples as for example dusts and indoor/ outdoor air.

M3- Analysis of soil and plant samples: chemical characterisation of all samples collected

The dataset produced by this study generated relevant information on the variability of key soil properties in different areas in Portugal. The pH values varied from very acidic (pH~3, samples from mining areas subject to the influence of acid mine drainage) to neutral, with soil pH generally increasing from North to South. Soil textures varied from sandy loam (mostly in the northern sampling areas) to silty clay loam, with higher clay contents being observed in the country southern areas. Wide ranges of amorphous metal oxides contents were observed, with amorphous Al oxides being particularly abundant in the North of the country and Fe oxides showing higher contents in soils from Centre and South areas. Organic C contents were generally medium to low, with the exception of a few samples from more intensive cultivated areas and DOC contents in $CaCl_2$-extracts were for most samples extremely low. The low DOC contents can be related to differences in the mineralization of soil organic carbon to CO_2 but further investigation on dissolved organic matter contents in soil solution from Portuguese soils preferably in combination with a quantification of the humic fractions should be developed in the future.

Three areas were found to be particularly impacted by anthropogenic soil contamination (particularly with Cu, Pb, Hg, As, Zn and to a lesser extent Se, Sb and Ba): Estarreja (industrial); Lousal and Caveira (mining). High contents of certain geogenic contaminants were also observed in certain areas. Nickel, Co, Cr, Mn and Fe were particularly abundant in soils from South-West areas of Portugal around mining sites (Lousal & Caveira and Aljustrel) while Al and U were the most abundant elements in soils from the North-West area of Portugal (Esposende) and were associated to soil type and bedrock lithology.

Very large reactive pools of particularly Zn, Cu, Pb, As and Ba were observed in certain areas with samples showing HNO_3-extractable concentrations of these five elements up to 519, 1492, 581, 384 and 208 mg kg^{-1}. Highest HNO_3 extractability ratios were obtained for Cd which agrees with other studies from the Netherlands and Belgium (Römkens et al., 2004; Meers et al., 2007a). Similarly to findings by other authors Zn and Ni showed relatively low HNO_3: aqua regia extraction ratios which has been associated to metal fixation and long-term immobilization by clay minerals

(Buekers et al., 2008; Römkens et al., 2009a). Relatively low reactivity was also observed for Cr, As, Se and Mo which can be strongly retained in soils through binding mechanisms with Fe, Al and Mn oxides (McLean and Bledsoe, 1992). The lowest HNO_3-extractability ratio was observed for Hg. Further assessments of the reactivity of the several Hg species in soils are needed.

Large $CaCl_2$-extractable pools were observed for contaminants including Zn, Cu, Pb, As, Mn, Ba and to a less extent, Ni, Co, Cr and Sb. The largest available pools of Cd, Zn, Hg, As, U, Al, Be and relatively high $CaCl_2$-extractable concentrations of Pb, Cu, Cr and Se were observed in soils from the industrially contaminated area (Estarreja). Soils from the mining areas (Aljustrel and Lousal & Caveira) showed the highest available pools of Cu, Pb, Ni, Co, Mn, Ba, Sb and Li. The smallest available pools of most PTE's were observed in soils from the non-contaminated northern area of the country (Esposende).

The EU green fodder limits for Hg, Pb, As and Cu contents were exceeded in shoots of both ryegrass and Italian ryegrass samples in our dataset. Cadmium and Zn contents in ryegrass shoots were also above green fodder quality criteria. Phytotoxicity limits for both Hg and Pb were exceeded in ryegrass shoots. Concentrations of Pb in certain rye samples were above the food safety limit for cereals. The exceedance of limits for feedstuffs and food crops (rye samples) was observed predominantly in the mining areas although ryegrass contamination was also observed in samples from the surroundings of the industrial site of Estarreja.

These results showed that there are problems of soil contamination with PTE's in Portugal related with anthropogenic sources. When compared to geogenic elements, anthropogenic contaminants (particularly As, Ba, Cd, Cu, Hg, Pb, Sb) showed a relatively higher reactivity and availability in soils. The higher availability of contaminants in soils was reflected by contamination of plant samples. It was clear from this study that there are potential risks to animal and human health deriving from crops grown at specific sites in the surrounding of mining and industrial facilities. An important outcome of this PhD program was that it provided insight into the magnitude of problems of soil contamination with PTE's as well as into their consequences in terms of crop quality (particularly feed crops). From this study such problems appear to be geographically limited to the vicinity of contamination sources (past effluent streams and solid wastes/ tailing deposits). Nevertheless, the actual geographical extent of soil-crop contamination problems must be evaluated in the future by more detailed site-specific investigations.

This study showed that soil quality chemical indicators contributed to effectively characterize specific soil functions namely the production of feed and fodder and the filtering and buffering of PTE's. The assessment of key soil properties such as pH, Org C, clay %, amorphous Fe and Al oxides and DOC (in soil solution) provided crucial information to explain the behaviour of contaminants in soils, namely it contributed to explain the reactivity and availability of soil contaminants. For example the potential to accumulate Cu, Pb, Zn, Cd, Ni, Co and Ba in the soil solid fraction (reactive pool) related to the organic carbon % while Hg HNO3-extractable contents were determined by the presence of amorphous Al oxides. The pH also proved to be a most important parameter in the definition of the available pools of most studied elements and consequently controlled their respective concentrations in vegetation. Therefore, the analysis of such parameters is important for the characterisation of pathways of exposure to soil contaminants. Commonly studied soil properties such as pH, organic carbon and clay can be useful to derive minimal empirical models that account for the effects of regional differences of soil characteristics in the identification of potential risks areas. Additional soil properties provide a more comprehensive understanding of the behaviour of contaminants in soils and allow to derive extended models which may be particularly useful in site-specific investigations.

The approach used here was based on the “reactive” and “available” fractions of PTE’s. The concept of “reactivity” in particular has been given increasing attention in the last few years. It is most useful in understanding the behaviour of PTE’s in soils and it proved to relate to the origin of elements in soils: geogenic chemicals tend to have lower reactive:total ratios than anthropogenic contaminants due to the fixation of geogenic elements in the mineral structure of clays (Römkens et al., 2004; Rodrigues et al., 2010a). When contaminants are introduced in soils by human activities their partition between the “reactive” solid fraction and soil solution will be determined by soil properties.

Two chemical extractions were used as approximations of the total reactive pools (0.43 M HNO_3) and of the soil solution (0.01 M $CaCl_2$) concentrations of PTE’s. Although more advanced techniques such as electron microprobe and synchrotron X-ray fluorescence (Jacobson et al., 2007) exist for the characterisation of the distribution of inorganic contaminants within the soil matrix, the use of chemical extraction proved to be a robust method to provide understanding on total:reactive and reactive:available partition relationships while allowing for cost-effective applications at local, regional and international scales. Other extractants (e.g. with EDTA or other types of diluted salts) have also been applied with similar purposes, nevertheless the methodologies followed here have been subject to increasing interest in the last few years which makes a strong point for their usefulness regarding comparability of results obtained.

M4- Derivation of soil-soil solution, soil-plant, and soil-plant-animal transfer functions for the different PTE’s

To date, empirical functions to characterise the behaviour of PTE’s within the soil matrix were based on data from soils in temperate regions of

Northern American and North-West Europe. Because the derived relations are empirical in nature differences may exist between soils from different climatic and geologic regions it was considered worthwhile to extend these studies using soils from Portugal.

Multiple regression empirical models provided an explanation for a significant part of the variance of the reactive pools of several contaminants studied in Portugal. For Cu and Pb, the reactive element pools could be almost entirely predicted from the aqua regia element pool although Org C (for Cu) and Org C in association with Fe, Al and Mn aqua regia contents (for Pb) also played a significant role. For elements such as Ni, Co, Mn and Ba, the inclusion of both pH and Org C, and to a less extent metal oxides, played a most relevant role in improving the reactivity predictions. Soil properties were particularly relevant for Ni and Ba reactive pools which could only be fairly predicted from total pools. The inclusion of pH, Org C and amorphous Fe oxides was also significant for Cd and Zn and Al oxides were particularly important for Cd. The inclusion of soil properties in the empirical model was also highly significant for anionic contaminants such as As, Cr and Se.

Multiple regression empirical models based on reactive element pools and soil properties (pH, organic carbon, clay, total Al, Fe and Mn) provided good estimations of available concentrations for a broad range of contaminants including As, Hg, Ba, Sb, Co, Se, Mo, Cd, Zn, Cu, Pb and Ni (r^2: 0.46-0.89). Furthermore, when applying empirical models from literature developed on the basis of a large array of soil conditions and variable environmental settings (Sauvé et al., 2000), similar model performances were obtained in particular, for Cd, Cu, Pb and Zn.

Compared to mechanistic models, empirical models require less input data and a less comprehensive process understanding which make them more suitable for national/ regional applications (Groenenberg et al., 2010a). But as observed in this study the complexity of the behaviour of certain elements cannot be fully captured by empirical relationships. This PhD program has led to recommendations for further research on the reactivity of Hg in soils, on the impact of clay on the solubility of As and Sb, on the mechanisms controlling U and Cr availability and on binding properties of dissolved soil organic matter from Portuguese soils. The better understanding of these processes is particularly important for future site-specific investigations.

Furthermore, aside from solid-solution partition, solution speciation also plays an important role in the availability of elements. For cationic metals, total dissolved concentrations include the free metal pool (Me^{n+}), as well as inorganic ion pairs and organic complexes (Sauvé et al., 2000). Free metal ion concentrations can sometimes be better predictors for adverse biological effects than total solution concentrations (Sauvé et al., 1998). Further investigation of mechanistically based speciation models (Groenenberg et al., 2010b) to predict the free metal ion activity in soil solution at contaminated soils in Portugal is also needed.

Regarding soil-plant relationships both empirical soil-to-plant transfer models (using total or reactive soil pools and soil properties) and the 0.01 M $CaCl_2$ soil extraction test were able to explain between 40 and 90 % of the variability in levels of Cd, Zn, Pb, Cu, Hg, As, Sb and Ba in crops, particularly in ryegrass and Italian ryegrass samples. For Co, U and Ni only a few relationships of poor quality could be derived given the low soil contamination levels and plant contents for these elements. Further studies on plant uptake processes for these PTE's should include wider ranges of contamination particularly for U and Co, for which little information is available. For the remaining elements including Cr, Mo, Se and B no apparent relationship between the availability in soil and levels in crops could be found at these contamination levels indicating that the plant levels observed were independent from the quality of the soil.

The derivation of these soil-to-plant transfer relationships was effective in the characterization of animal and human exposure to soil contaminants (particularly Cd, Hg, Pb, As, Cu and Zn) via the crop consumption pathway. These empirical functions can be included in a contaminated soil exposure assessment model for agricultural soils in Portugal since they can be applied in the calculation of threshold soil concentrations to be used in screening risk assessment procedures. Given that these relationships were derived on the basis of experimental data which covered a large range of Portuguese soils and soil conditions as well as field grown crops they can provide more useful information for contaminated soil exposure assessments in Portugal when compared with literature data. Nevertheless, a limited number of crop types were included in this study. Since soil-to-plant transfer functions are both element and plant specific, future studies should include additional crop types.

In this study I used literature feed-to-organ transfer relationships to determine the accumulation of ingested contaminants in animal organs but future studies should be developed in Portugal to characterize such relationships. Assessments of the bioavailability of soil contaminants within the animal body as well as the assessment of accumulation of ingested contaminants in animal organs are crucial to further evaluate risks to animal and human health associated with contaminants intake.

M5- Dietary exposure to PTE's for animals and humans: estimation of animal and human dietary exposure to PTEs on a regional level, using measured data (M3) and the predictive modelling approach taking into account the supply chain from soil to the consumer (M4).

For animals (cow and sheep), the ingestion of grass was the most relevant pathway for the intake of Cd, Zn, Cr, Se, Mo, B, Ni, Ba, Cu and Hg while for Pb, Co, As, U and Sb the direct ingestion of soils accounted for around half of the element intake. By the calculation of dietary intakes of the various elements, it was observed that the animal ADI's of Pb, Hg, Cd, As, Cu and

Zn were exceeded at the contaminated sites which indicates potential risks to animal health and food safety.

The pathways for human exposure to Cd, Pb, Hg and As were also evaluated using experimental data and estimated levels of PTE's in animal organs. For each site, the calculation of human daily intakes of these four elements included the following routes: soil ingestion, meat (ovine and bovine) consumption; offal (bovine) consumption; cabbage (collard greens) consumption as was performed considering the "worst-case scenario" (all food products were originated from the study site). Considering the contributions of these routes, Provisional Tolerable Weekly Intakes (PTWI) of these four elements were exceeded at a number of contaminated sites. The removal of animal (cow) offal (liver and kidney) from the food chain allowed to reduce human dietary intakes of Cd and Hg to levels significantly below the PTWI. These results suggest that animal offal should be removed from the human food chain in Portugal. Elevated intakes of As and Pb (due to soil ingestion) were estimated even after the removal of offal from diet. In future assessment additional sources of intake of PTE's, such as (ground-) water drinking, fruit and vegetables consumption as well as other sources of exposure not associated with the terrestrial food chain (e.g. fish consumption) should be considered to better understand the relative contribution of the various products.

M6- Threshold concentrations of PTE's in agricultural soils in Portugal.

The applicability of a chain model approach to estimate animal and human exposure was demonstrated successfully in this work. The approach tested can be used to assess whether or not a combination of soil properties and soil levels of PTE's in agricultural areas lead to unacceptable levels of grazing animals and human exposure. The conceptual approach can also be adapted for more detailed site-specific exposure assessments. The same approach can also be applied to calculate threshold concentrations of Cd, Hg, Pb, As, Cu and Zn in agricultural soils. These threshold concentrations are indicative values of potential risks to animal and human health that can be used for screening purposes in risk assessment procedures. The advantage of these threshold values if that they can be used for a relatively fast preliminary evaluation of Cd, Hg, Pb, As, Cu and Zn potential exposure risks for certain regions without the need for costly sampling programs. Another advantage is that soil conditions were taken into account for their derivation which increases the accuracy of the analysis of potential risks.

This study has focused on the quality of soils for agriculture production. Further advances on the use of this approach for calculation of threshold soil concentrations may be achieved if other pathways of human exposure such as groundwater drinking, dermal contact with soils and inhalation are included and if lifelong exposure to contaminants is considered in the definition of risk levels.

In the future a comprehensive validation study in which the results of PTE's concentrations in animal products are compared with those estimated by the studied approach is recommended. Additional validation of the derived soil-to-plant transfer relationships is also recommended. In particular, further advances on the use of this approach may be achieved by the analysis of uncertainties associated with the empirical models derived in the course of this investigation.

Finally, the threshold soil concentrations derived on the basis of feed and food protection should also be analysed in combination with thresholds for the protection of other sensitive receptors such as terrestrial ecosystems.

M7- Contaminated soil exposure assessment strategy for agricultural soils in Portugal.

The balance between scientific quality and practicability was sought throughout this work. This investigation provided a most valuable contribution for contaminated soil exposure assessment in Portugal. The approach developed has taken into consideration commonalities and differences in strategies implemented in other European countries which positively contributes for the alignment of exposure assessment procedures within the EU.

In the future, the approach developed in this investigation for contaminated soil exposure assessment in agricultural areas can be integrated in a Contaminated Land Legal Framework for Portugal. For this to be possible regulatory requirements such as integration with other existing laws as well as political aspects such as the prioritization of environmental and economical values need to be clarified. The integration of human health, groundwater and ecological protection objectives into soil protection objectives is particularly important.

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Annex I

Table 1: Overview of sampling locations, land uses and plant samples collected

	Location	Soil Sample Code	North	West	Land use	Sampling Depth	Plant Sample Code	Plant type	Plant species
North-West of Portugal - Agricultural area	Curvos (Esposende)	Curvos01	41°33.093	8°44.208	Grassland, horticulture	0-15cm	Curvos02	ryegrass	*Lolium perenne*
		Curvos03	41°33.093	8°44.218			Curvos04	ryegrass	*Lolium perenne*
		Curvos05	41°33.082	8°44.219			Curvos06	ryegrass	*Lolium perenne*
		Curvos07	41°33.072	8°44.224			Curvos08	collard greens	*Brassica oleracea*
	Curvos (Esposende)	Curvos09	41°33.126	8°44.171	Grassland, horticulture	0-15cm	CurvosFT	ryegrass	*Lolium perenne*
		Curvos10	41°33.172	8°43.967			Curvos11	collard greens	*Brassica oleracea*
		Curvos13	41°33.136	8°43.953			Curvos12	collard greens	*Brassica oleracea*
		Curvos15	41°33.139	8°43.963			Curvos14	ryegrass	*Lolium perenne*
	Abelheira (Esposende)	Abelheira-Curvos16	41°33.195	8°43.799	Grassland, horticulture	0-15cm	Abelheira-Curvos17	collard greens	*Brassica oleracea*
		Abelheira-Curvos19	41°33.198	8°43.791			Abelheira-Curvos18	ryegrass	*Lolium perenne*
	Palmeira (Esposende)	Palmeira20	41°32.768	8°44.540	Grassland	0-15cm	Palmeira21	ryegrass	*Lolium perenne*
		Palmeira22	41°32.768	8°44.560			Palmeira23	ryegrass	*Lolium perenne*
	Esposende (near Esposende-Barcelos road)	Esposende24	41°31.927	8°46.136	Grassland, horticulture	0-15cm	Esposende25	ryegrass	*Lolium perenne*
		Esposende33	41°31.967	8°46.138			Esposende26	collard greens	*Brassica oleracea*
		Esposende28	41°31.966	8°46.143			Esposende27	collard greens	*Brassica oleracea*
		Esposende29	41°31.965	8°46.149			Esposende30	ryegrass	*Lolium perenne*
		Esposende32	41°31.692	8°46.120			Esposende31	ryegrass	*Lolium perenne*
	Gandra (Esposende) - close to Gandra centre	Gandra35	41°31.430	8°45.417	Grassland, horticulture	0-15cm	Gandra34	collard greens	*Brassica oleracea*
		Gandra37	41°31.457	8°45.428			Gandra36	collard greens	*Brassica oleracea*
		Gandra38	41°31.455	8°45.395			Gandra39	orchard grass	*Dactylis glomerata*
		Gandra41	41°31.464	8°45.376			Gandra40	orchard grass	*Dactylis glomerata*
		Gandra42	41°31.474	8°45.357			Gandra43	orchard grass	*Dactylis glomerata*
		Gandra45	41°31.492	8°45.378			Gandra44	orchard grass	*Dactylis glomerata*
		Gandra47	41°31.492	8°45.400			Gandra46	orchard grass	*Dactylis glomerata*
		Gandra49	41°31.503	8°45.402			Gandra48	collard greens	*Brassica oleracea*
	Gandra	Gandra51	41°31.194	8°46.171	Grassland, horticulture	0-15cm	Gandra50	orchard grass	*Dactylis glomerata*
		Gandra53	41°31.183	8°46.156			Gandra52	orchard grass	*Dactylis glomerata*
		Gandra55	41°31.198	8°46.153			Gandra54	orchard grass	*Dactylis glomerata*
		Gandra57	41°31.189	8°46.198			Gandra56	collard greens	*Brassica oleracea*
		Gandra59	41°31.179	8°46.194			Gandra58	orchard grass	*Dactylis glomerata*

Table 1 (cont.)

	Location	Soil Sample Code	North	West	Land use	Sampling Depth	Plant Sample Code	Plant type	Plant species
Centre of Portugal - Industrial area	South Estarreja (close to town centre and near a road)	L1A2	40°45.206	8°33.824	Grassland	0-15cm	L1A1	ryegrass	*Lolium perenne*
		L2A1	40°45.176	8°33.779			L2A1	ryegrass	*Lolium perenne*
		L3A2	40°44.868	8°34.243			L3A2	ryegrass	*Lolium perenne*
	South Estarreja (close to town centre and near the river)	L4A3	40°44.780	8°34.219	Grassland	0-15cm	L4A3	ryegrass	*Lolium perenne*
		L5A2	40°44.611	8°34.516			L5A2	ryegrass	*Lolium perenne*
		L6A2	40°44.768	8°34.527			L6A2	ryegrass	*Lolium perenne*
	North Estarreja (around 500m south-west from a Chemical Complex, in the vicinity of an old effluent stream)	L7A3	40°46.074	8°34.870	Grassland, vineyard	0-15cm	L7A3	ryegrass	*Lolium perenne*
		L7A7	40°46.076	8°34.899			L7A7	ryegrass	*Lolium perenne*
		L7A9	40°46.066	8°34.911			L7A9	ryegrass	*Lolium perenne*
		L7A13	40°46.054	8°34.935			L7A13	ryegrass	*Lolium perenne*
		L7A14	40°46.048	8°34.956			L7A14	ryegrass	*Lolium perenne*
		L7A17	40°46.041	8°34.986			L7A17	ryegrass	*Lolium perenne*
		L7A18	40°46.047	8°34.993			L7A18	ryegrass	*Lolium perenne*
		L7A19	40°46.054	8°34.998			L7A19	ryegrass	*Lolium perenne*
		L7A21	40°46.040	8°35.012			L7A21	ryegrass	*Lolium perenne*
		L7A22	40°46.047	8°35.016			L7A22	ryegrass	*Lolium perenne*
		L7A25	40°46.037	8°35.049			L7A25	ryegrass	*Lolium perenne*
		L15A5	40°45.988	8°35.004					
		L16A1	40°46.008	8°34.965			L16A1	ryegrass	*Lolium perenne*
		L16A5	40°46.014	8°34.976					
	North Estarreja (around 1500m south-west from a Chemical Complex, around 700m from an old effluent stream)	L11A5	40°46.058	8°35.473	Grassland	0-15cm	L11A4	ryegrass	*Lolium perenne*
		L12A3	40°45.882	8°35.508			L12A2	ryegrass	*Lolium perenne*
		L12A8	40°45.843	8°35.536			L12A9	ryegrass	*Lolium perenne*
	Estarreja (around 200m south-west from a chemical complex, in the vicinity of an orl effluent stream)	L10A3	40°46.138	8°34.817	Grassland	0-15cm	L10A3	ryegrass	*Lolium perenne*
		L10A4	40°46.129	8°34.856					
		L14A2	40°46.088	8°35.027			L14A1	ryegrass	*Lolium perenne*
		L14A3	40°46.080	8°35.022					
		L14A5	40°46.082	8°35.004					

		L14A7	40°46.085	8°34.987					
		L14A9	40°46.090	8°34.971			L14A10	ryegrass	*Lolium perenne*
		L14A14	40°46.107	8°34.941					
		L14A15	40°46.099	8°34.938			L14A13	ryegrass	*Lolium perenne*
		L14A23	40°46.177	8°34.869					
		L14A28	40°46.140	8°34.821			L14A29	ryegrass	*Lolium perenne*
		L14A31	40°46.131	8°34.818			L14A30	ryegrass	*Lolium perenne*
		L14A42	40°46.154	8°34.862			L14A41	ryegrass	*Lolium perenne*
		L14A58	40°46.084	8°35.038			L14A57	ryegrass	*Lolium perenne*
		L14A64	40°46.182	8°35.189			L14A65	ryegrass	*Lolium perenne*
		L14A69	40°46.140	8°35.351			L14A70	ryegrass	*Lolium perenne*

Table 1 (cont.)

	Location	Soil Sample Code	North	West	Land use	Sampling Depth	Plant Sample Code	Plant type	Plant species
South of Portugal - mining areas	Lousal (inside mine perimeter, close to water stream)	Lousal01	38º01.964	008º25.253	Grassland	0-15cm	Lousal01	ryegrass	*Lolium perenne*
		Lousal02	38º01.964	008º25.257			Lousal02	ryegrass	*Lolium perenne*
		Lousal03	38º01.964	008º25.261			Lousal03	ryegrass	*Lolium perenne*
		Lousal04	38º01.965	008º25.262			Lousal04	ryegrass	*Lolium perenne*
		Lousal05	38º01.958	008º25.245			Lousal05	ryegrass	*Lolium perenne*
	Lousal (inside mine perimeter, close way out)	Lousal06	38º01.928	008º25.400	Grassland	0-15cm	Lousal06	ryegrass	*Lolium perenne*
		Lousal07	38º01.911	008º25.406			Lousal07	ryegrass	*Lolium perenne*
		Lousal08	38º01.919	008º25.407			Lousal08	ryegrass	*Lolium perenne*
		Lousal09	38º01.917	008º25.402			Lousal09	ryegrass	*Lolium multiflorum*
		Lousal10	38º01.920	008º25.400			Lousal10	ryegrass	*Lolium perenne*
	Lousal (small farm in the vicinity of mining site - southern area)	Lousal11	38º01.796	008º25.538	Horticulture	0-15cm	Lousal11	collard greens	*Brassica oleracea*
		Lousal12	38º01.796	008º25.535			Lousal12	collard greens	*Brassica oleracea*
		Lousal13	38º01.793	008º25.537			Lousal13	collard greens	*Brassica oleracea*
	Lousal (small farm ~500m of mining site - southern area)	Lousal14	38º01.710	008º25.640	Horticulture	0-15cm	Lousal14	collard greens	*Brassica oleracea*
		Lousal15	38º01.715	008º25.638			Lousal15	collard greens	*Brassica oleracea*
		Lousal16	38º01.720	008º25.641			Lousal16	orchard grass	*Dactylis glomerata*
							Lousal16cv	collard greens	*Brassica oleracea*
		Lousal17	38º01.715	008º25.644			Lousal17	orchard grass	*Dactylis glomerata*
							Lousal17cv	collard greens	*Brassica oleracea*
		Lousal18	38º01.714	008º25.642			Lousal18	orchard grass	*Dactylis glomerata*
							Lousal18cv	collard greens	*Brassica oleracea*
	Lousal (small farm ~500m of mining site - southern area)	Lousal19	38º01.695	008º25.631	Horticulture	0-15cm	Lousal19	collard greens	*Brassica oleracea*
		Lousal20	38º01.695	008º25.633			Lousal20	collard greens	*Brassica oleracea*
		Lousal21	38º02.262	008º25.635			Lousal21	collard greens	*Brassica oleracea*
		Lousal22	38º02.262	008º25.646			Lousal22	collard greens	*Brassica oleracea*
		Lousal23	38º02.241	008º25.619			Lousal23	ryegrass	*Lolium perenne*
		Lousal24	38º02.244	008º25.614			Lousal24	ryegrass	*Lolium perenne*
		Lousal25	38º02.241	008º25.614			Lousal25	ryegrass	*Lolium perenne*
	Lousal (vegetable	Lousal26	38º02.231	008º25.600	Horticulture	0-15cm	Lousal26	collard greens	*Brassica oleracea*

	garden ~2000m of mining site, northern area	Lousal27	38°02.239	008°25.581			Lousal27	collard greens	*Brassica oleracea*
	Caveira (inside mine perimeter)	Caveira28	38°07.135	008°29.984	Mining tailings deposit	0-15cm			
	Caveira (close to mining tailings deposit)	Caveira29	38°07.135	008°29.950	Grassland, pasture area	0-15cm	Caveira29	ryegrass	*Lolium perenne*
		Caveira30	38°07.126	008°29.951			Caveira30	ryegrass	*Lolium perenne*
		Caveira31	38°07.117	008°29.950			Caveira31	ryegrass	*Lolium perenne*
	Caveira (immediate vicinity of mining site)	Caveira32	38°07.209	008°29.892	Grassland, pasture area	0-15cm	Caveira32	ryegrass	*Lolium perenne*
		Caveira33	38°07.214	008°29.890			Caveira33	ryegrass	*Lolium perenne*
	Caveira (small farm, ~500 North from mining site)	Caveira34	38°07.457	008°29.960	Grassland	0-15cm	Caveira34	ryegrass	*Lolium perenne*
		Caveira35	38°07.468	008°29.960			Caveira35	ryegrass	*Lolium perenne*
	Caveira (inside mine perimeter, western area)	Caveira36	38°07.598	008°30.100	Mining tailings deposit	0-15cm			
	Caveira (hay production field, ~1000m North from mining site)	Caveira37	38°07.774	008°29.919	Grassland	0-15cm	Caveira37	ryegrass	*Lolium multiflorum*
		Caveira38	38°07.779	008°29.920			Caveira38	ryegrass	*Lolium multiflorum*
		Caveira39	38°07.789	008°29.936			Caveira39	ryegrass	*Lolium multiflorum*
		Caveira40	38°07.806	008°29.950			Caveira40	ryegrass	*Lolium multiflorum*
		Caveira41	38°07.828	008°29.970			Caveira41	ryegrass	*Lolium multiflorum*
		Caveira42	38°07.851	008°29.987			Caveira42	ryegrass	*Lolium multiflorum*
	Aljustrel (Ponte "Monte-Ruas" ~2000m from mining site)	Aljustrel01	38°07.849	008°29.990	Grassland	0-15cm	Aljustrel01	ryegrass	*Lolium multiflorum*
		Aljustrel02	37°52.389	008°08.980			Aljustrel02	ryegrass	*Lolium perenne*
	Aljustrel (Porto de Beja, "Água Forte" inside mine perimeter, close to Residual Waters Dam)	Aljustrel03	37°53.034	008°08.609	Grassland	0-15cm	Aljustrel03	ryegrass	*Lolium perenne*
		Aljustrel04	37°51.771	008°09.453			Aljustrel04	ryegrass	*Lolium perenne*
	Aljustrel (Ponte do Curval - close to river)	Aljustrel05	37°51.772	008°09.452	Grassland	0-15cm	Aljustrel05	ryegrass	*Lolium perenne*
		Aljustrel06	37°53.725	008°08.237			Aljustrel06	ryegrass	*Lolium perenne*
		Aljustrel07	37°53.702	008°08.235			Aljustrel07	ryegrass	*Lolium perenne*

	Aljustrel (Jungeiros)	Aljustrel08	37°55.932	008°12.488	Cropland	0-15cm	Aljustrel08	*rye*	*Secale cereale*
		Aljustrel09	37°55.940	008°12.495			Aljustrel09	*rye*	*Secale cereale*
		Aljustrel10	37°55.940	008°12.500			Aljustrel10	*rye*	*Secale cereale*
		Aljustrel11	37°55.955	008°12.510			Aljustrel11	*rye*	*Secale cereale*
		Aljustrel12	37°55.956	008°12.511			Aljustrel12	*rye*	*Secale cereale*
	Aljustrel (Pêro Bonito)	Aljustrel13	37°57.009	008°14.130	Grassland	0-15cm	Aljustrel13	ryegrass	*Lolium perenne*
		Aljustrel14	37°57.015	008°14.114			Aljustrel14	ryegrass	*Lolium perenne*
		Aljustrel15	37°57.021	008°14.100			Aljustrel15	ryegrass	*Lolium perenne*
	Aljustrel (Porto Ferreira)	Aljustrel16	37°56.781	008°09.876	Grassland	0-15cm	Aljustrel16	ryegrass	*Lolium perenne*
	Aljustrel (Ribeira do Rôxo)	Aljustrel17	37°56.689	008°08.847	Cropland	0-15cm	Aljustrel17	*rye*	*Secale cereale*
		Aljustrel18	37°56.687	008°08.843			Aljustrel18	*rye*	*Secale cereale*
		Aljustrel19	37°56.689	008°08.820			Aljustrel19	*rye*	*Secale cereale*
		Aljustrel20	37°56.688	008°08.790			Aljustrel20	*rye*	*Secale cereale*
		Aljustrel21	37°56.323	008°08.721			Aljustrel21	ryegrass	*Lolium multiflorum*
	Aljustrel (Rôxo, jusante)	Aljustrel22	37°56.848	008°14.467	Grassland	0-15cm	Aljustrel22	ryegrass	*Lolium perenne*
		Aljustrel23	37°56.889	008°14.502			Aljustrel23	ryegrass	*Lolium perenne*
	Aljustrel (Barranco do Xacafre)	Aljustrel24	37°57.032	008°12.294	Cropland	0-15cm	Aljustrel24	*rye*	*Secale cereale*
		Aljustrel25	37°57.025	008°12.314			Aljustrel25	*rye*	*Secale cereale*

Annex II

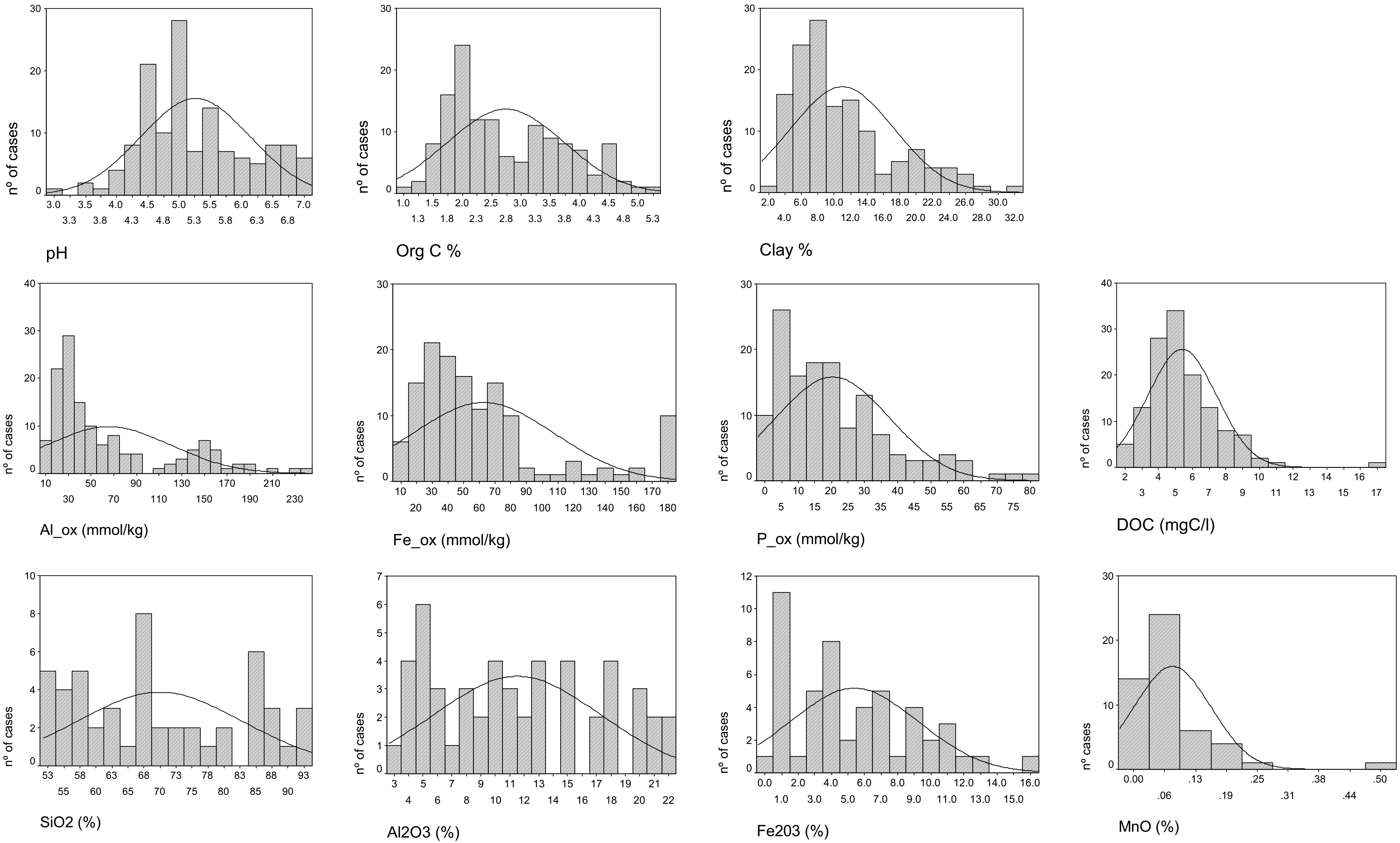


Figure 1: Distribution of key soil properties and soil chemical composition of samples from the entire dataset

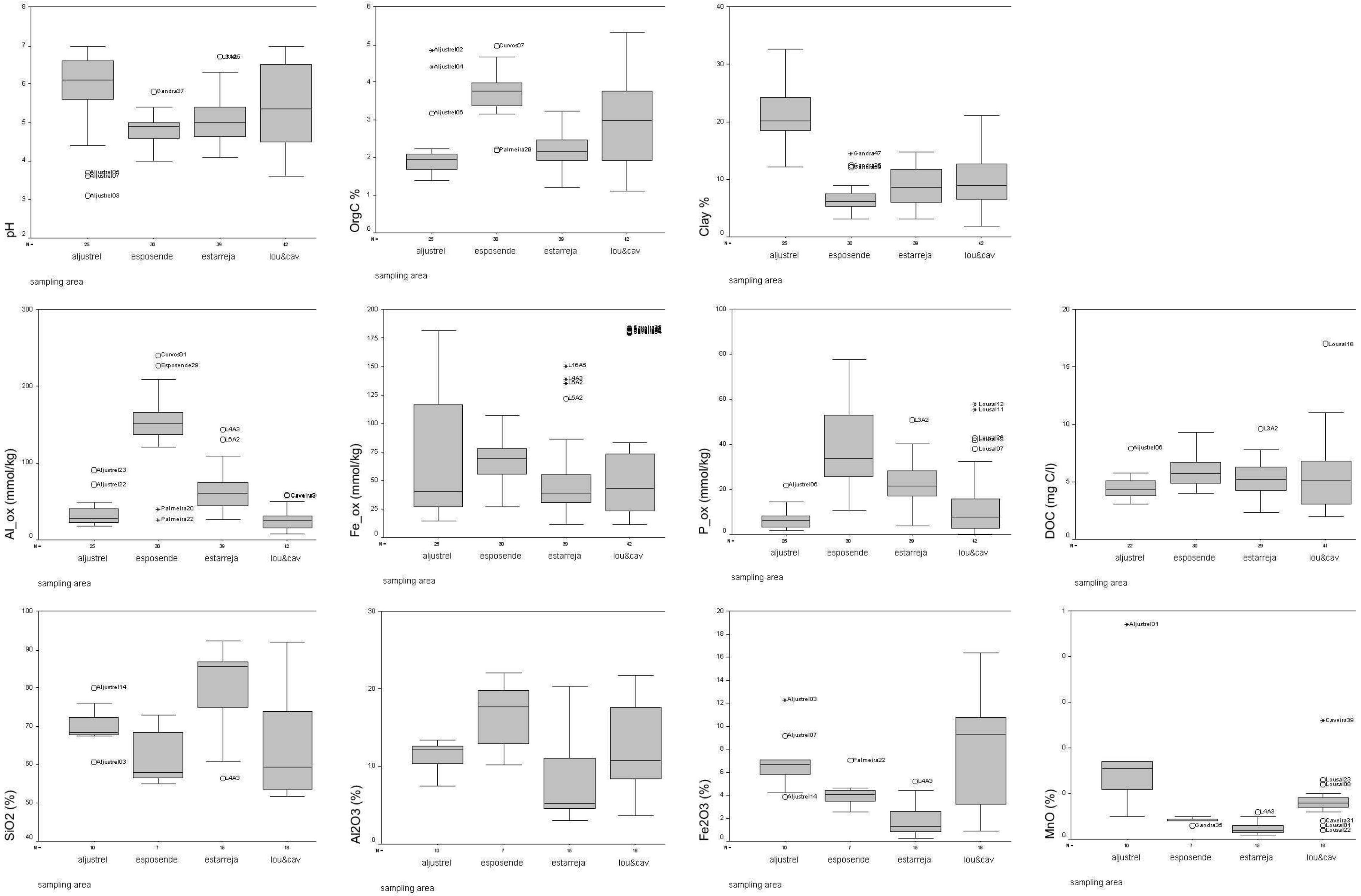


Figure 2: Boxplots of key soil properties and soil chemical composition of samples from the different sampling areas

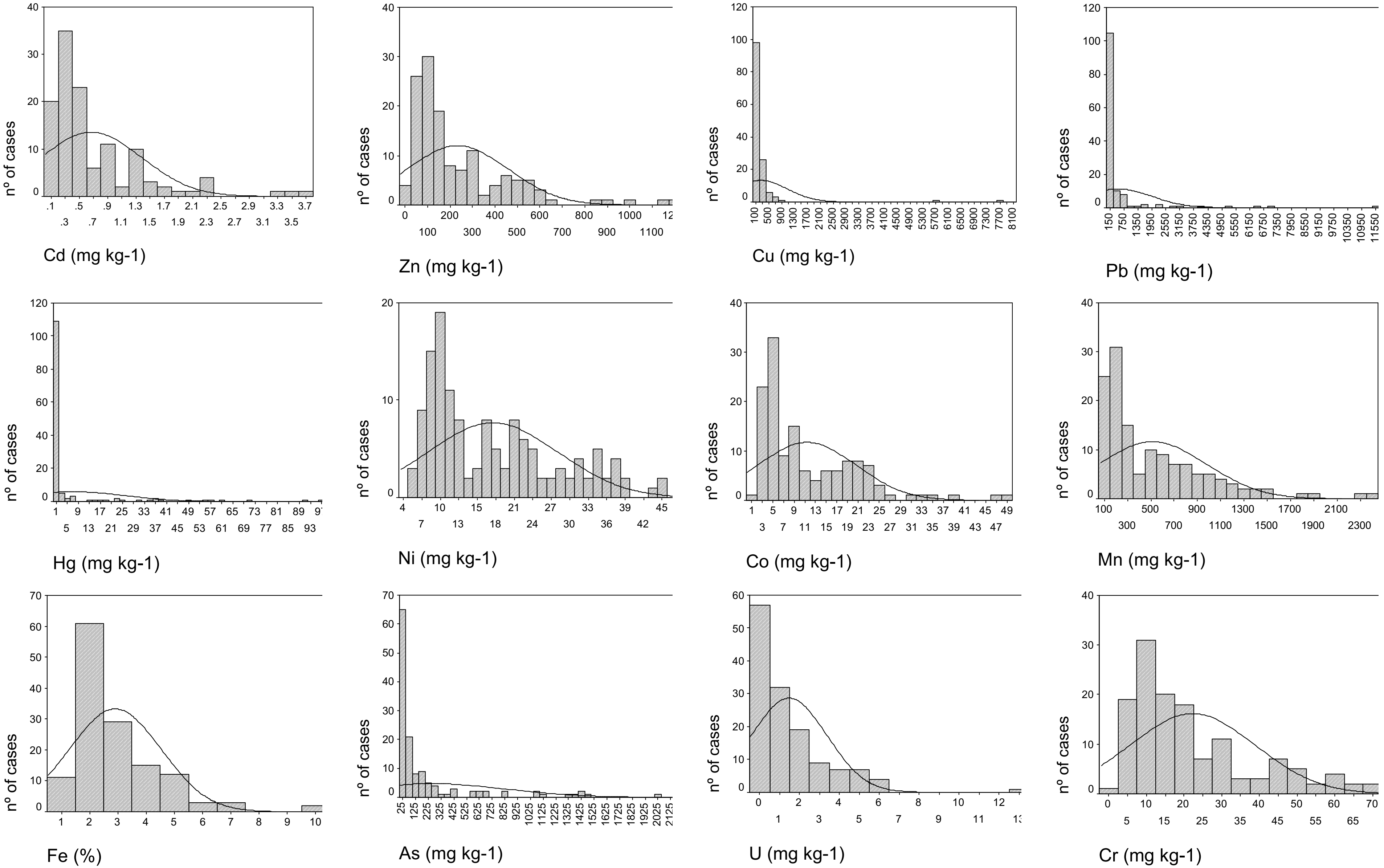


Figure 3: Distribution of elemental contents observed in this study (total concentrations for Hg and "pseudo-total" for other elements)

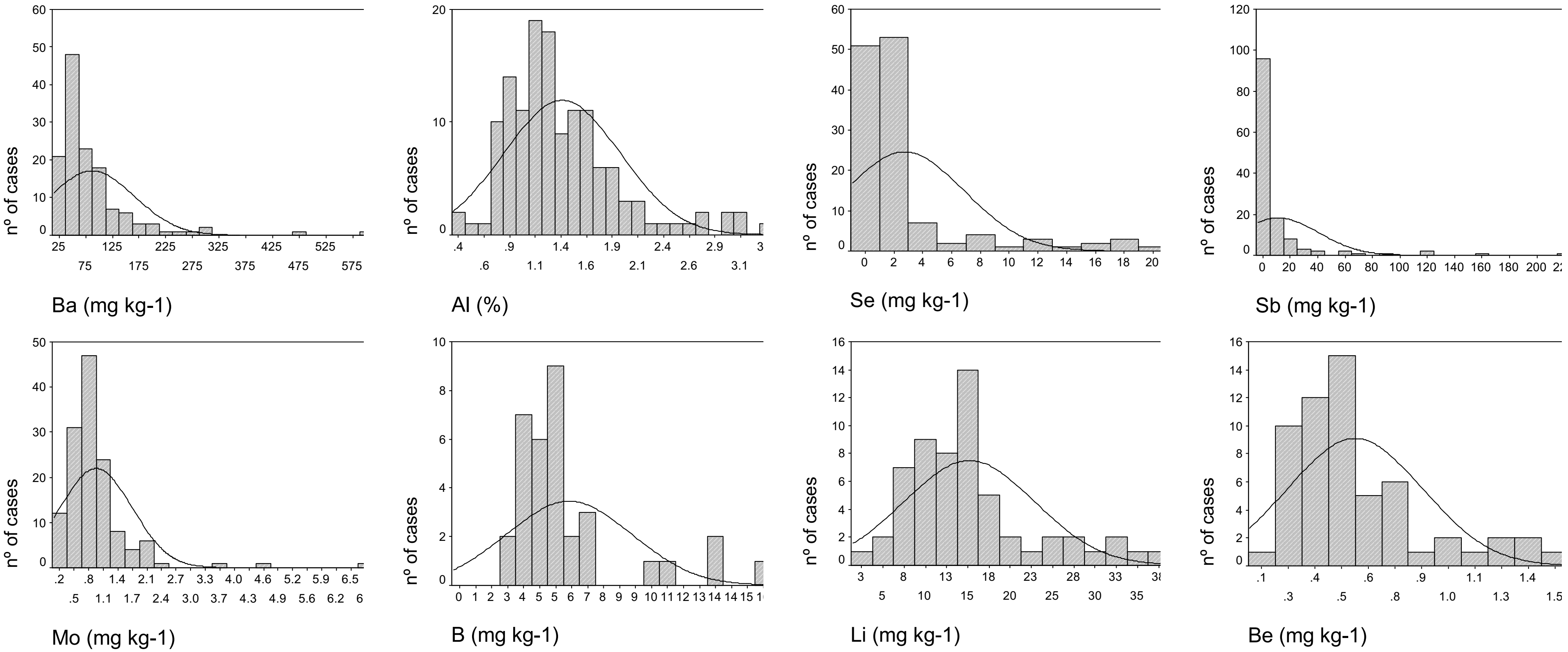


Figure 3 *(cont)*

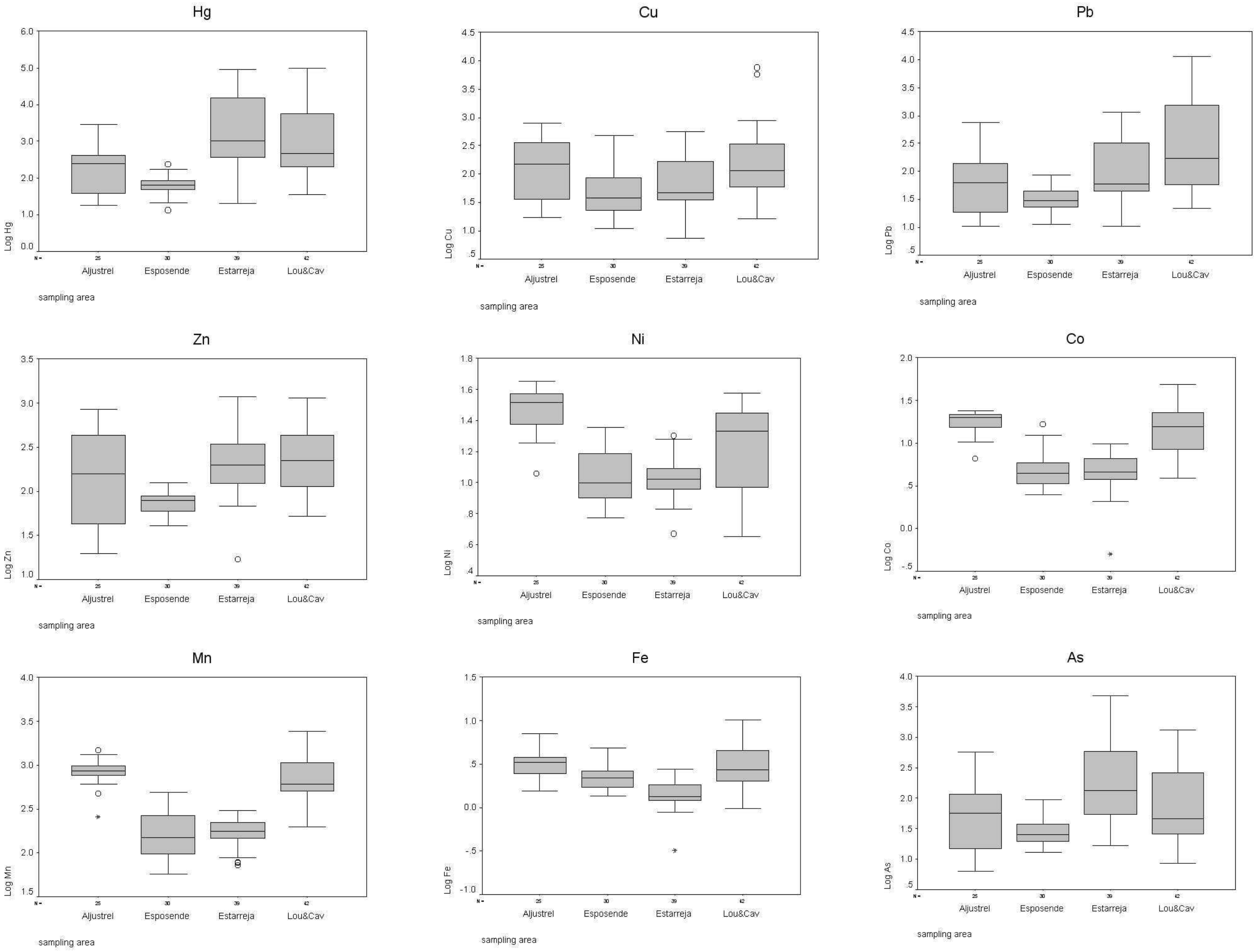


Figure 4: Boxplots of log transformed (Log_{10}) (pseudo-)total concentrations of PTEs (µg kg^{-1}, for Hg and mg kg^{-1} for other elements) in soils from the four sampling areas

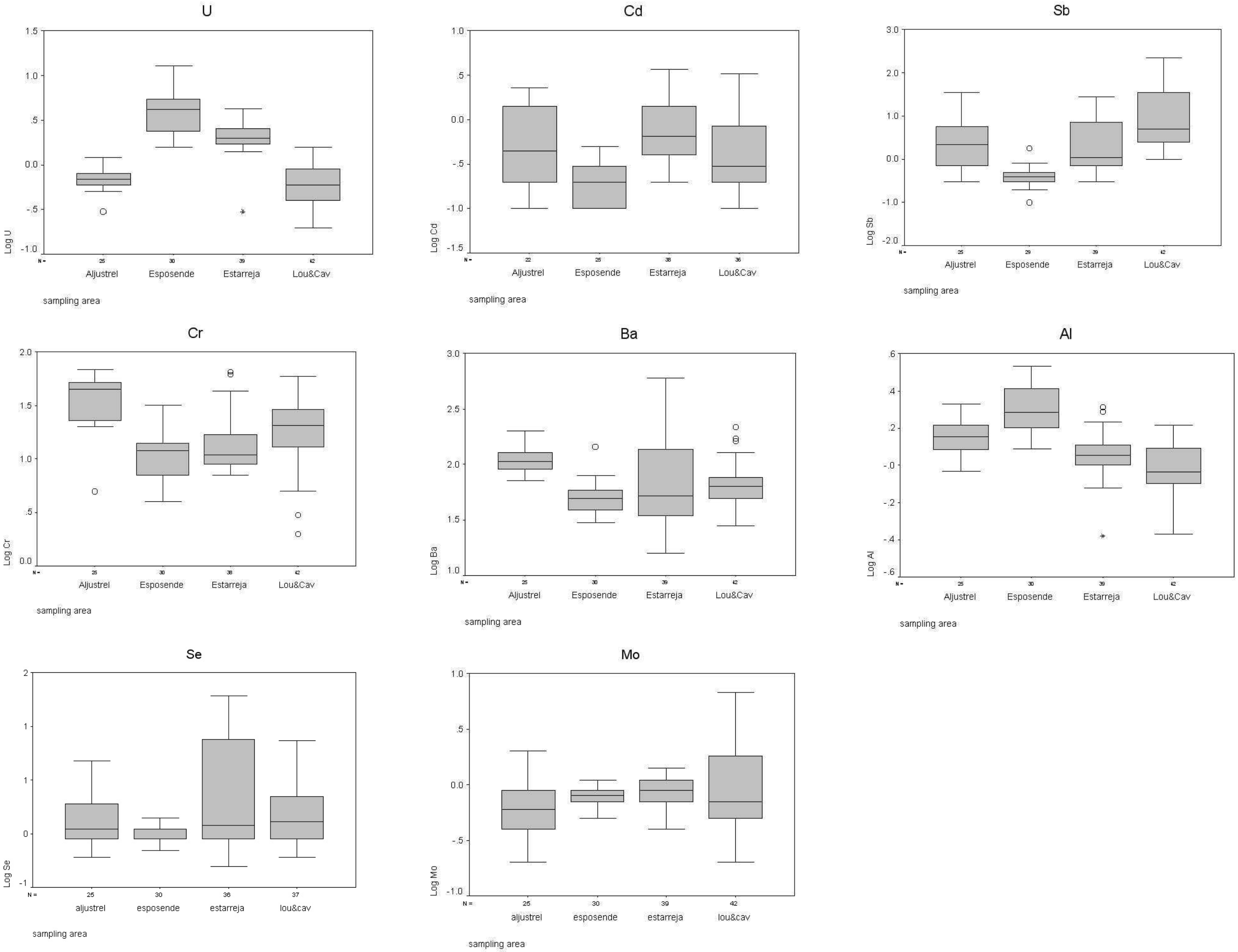


Figure 4 *(cont.)*

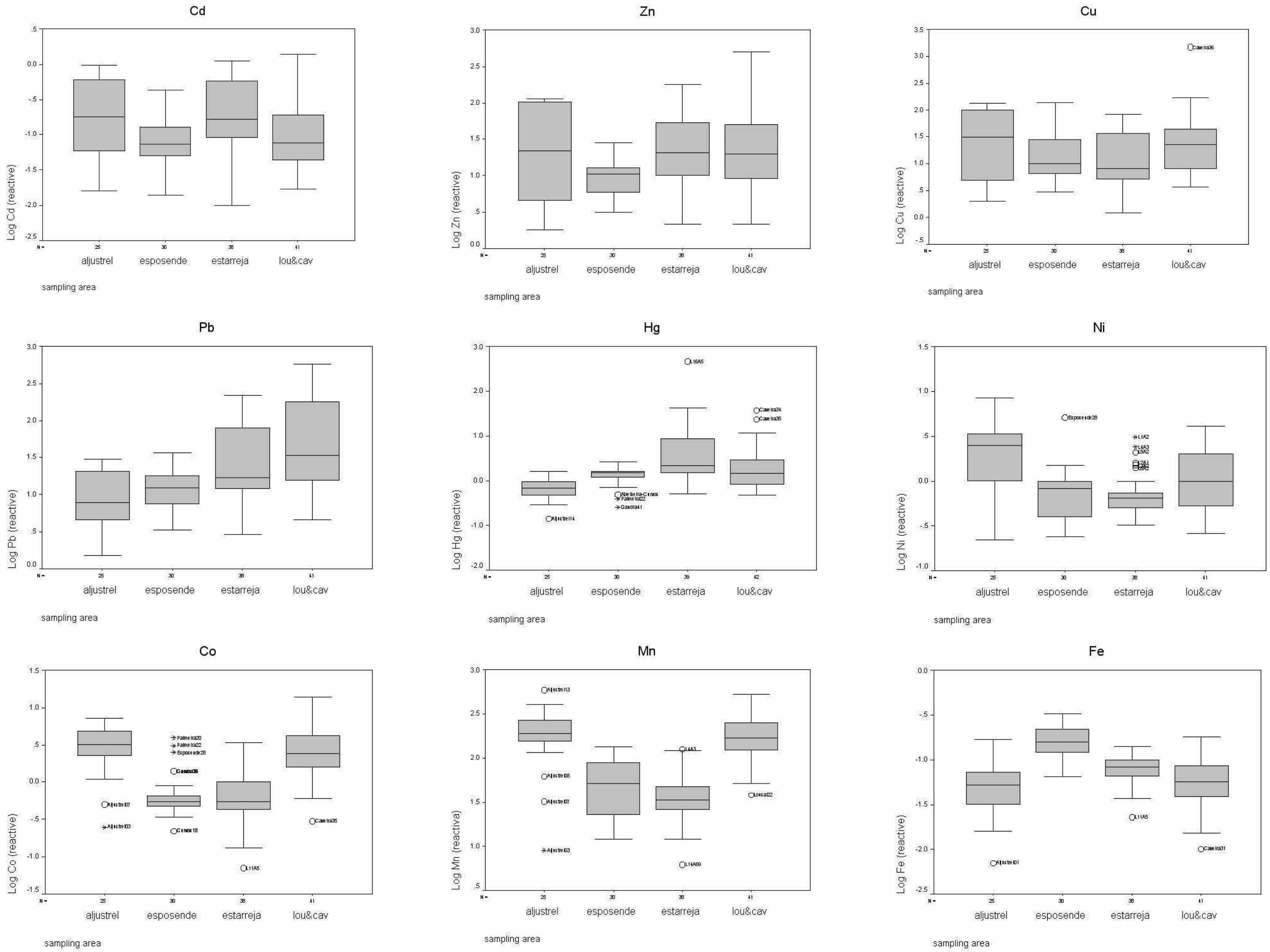


Figure 5: Boxplots of log transformed (Log_{10}) reactive concentrations of PTEs (µg kg^{-1}, for Hg and mg kg^{-1} for other elements) in soils from the four sampling areas

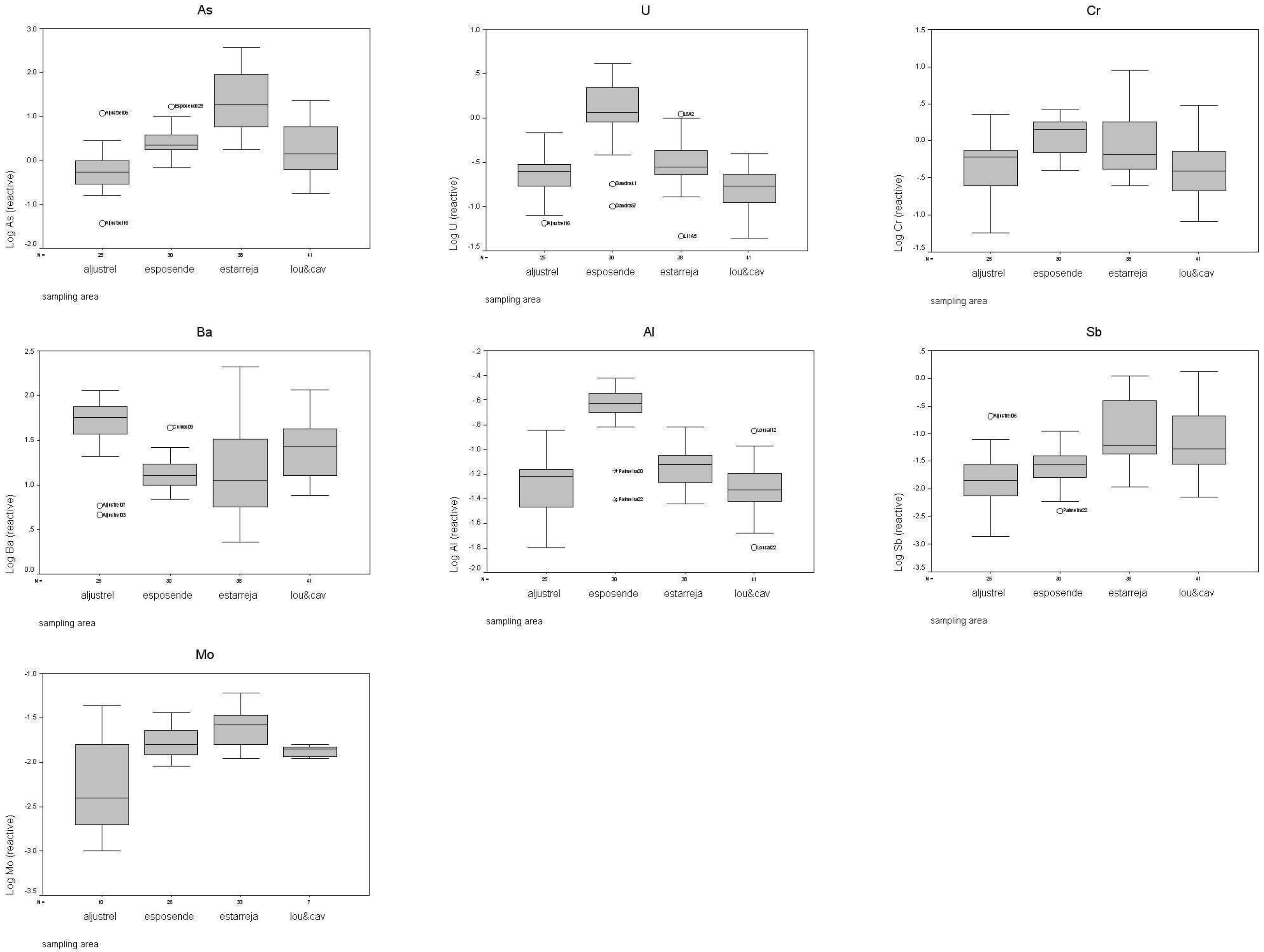


Figure 5 *(cont.)*

Annex III

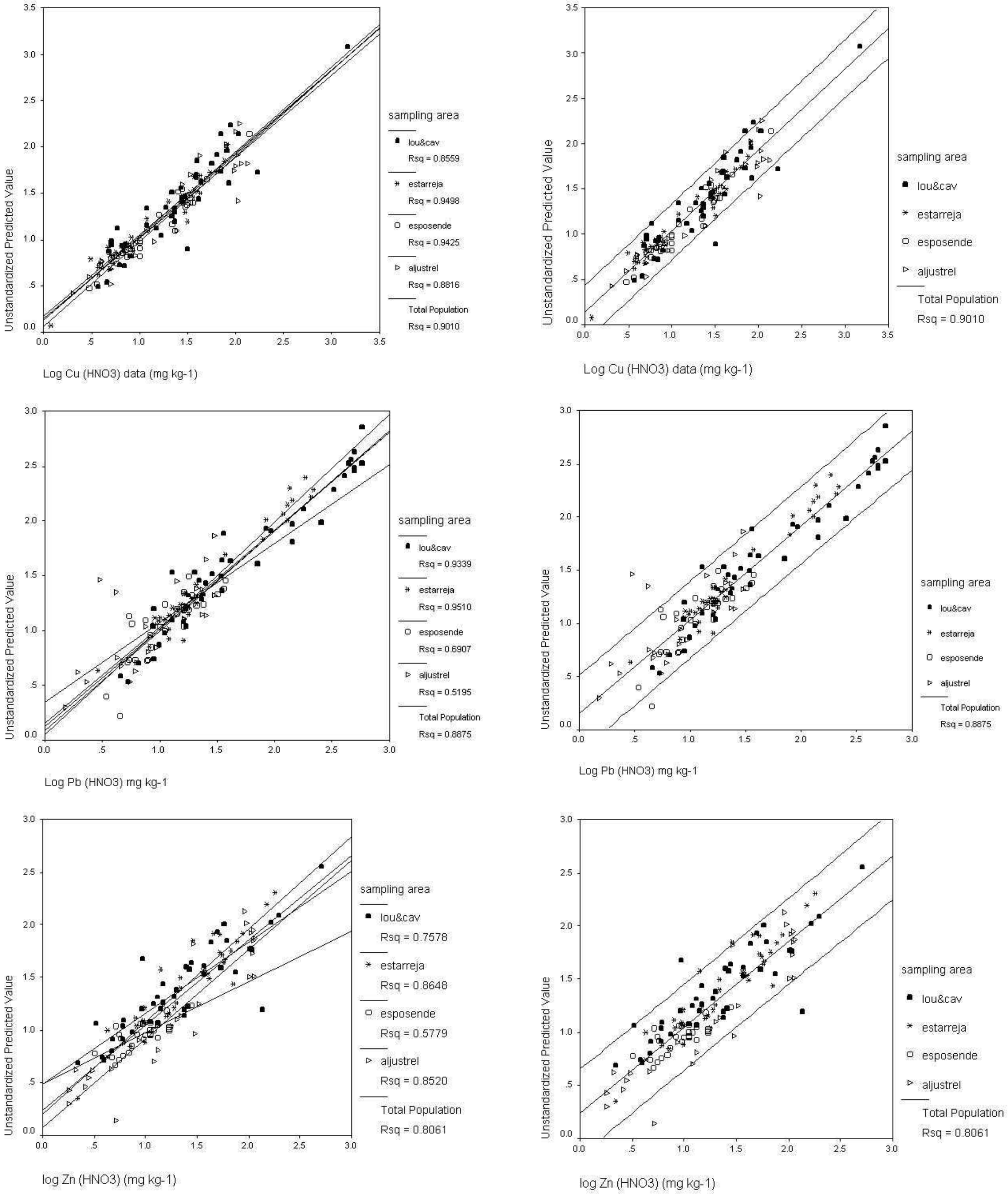


Figure 1: HNO_3-extractable concentrations predicted by the linear multiple regression models against measured data. Model performance by sampling area is also shown.

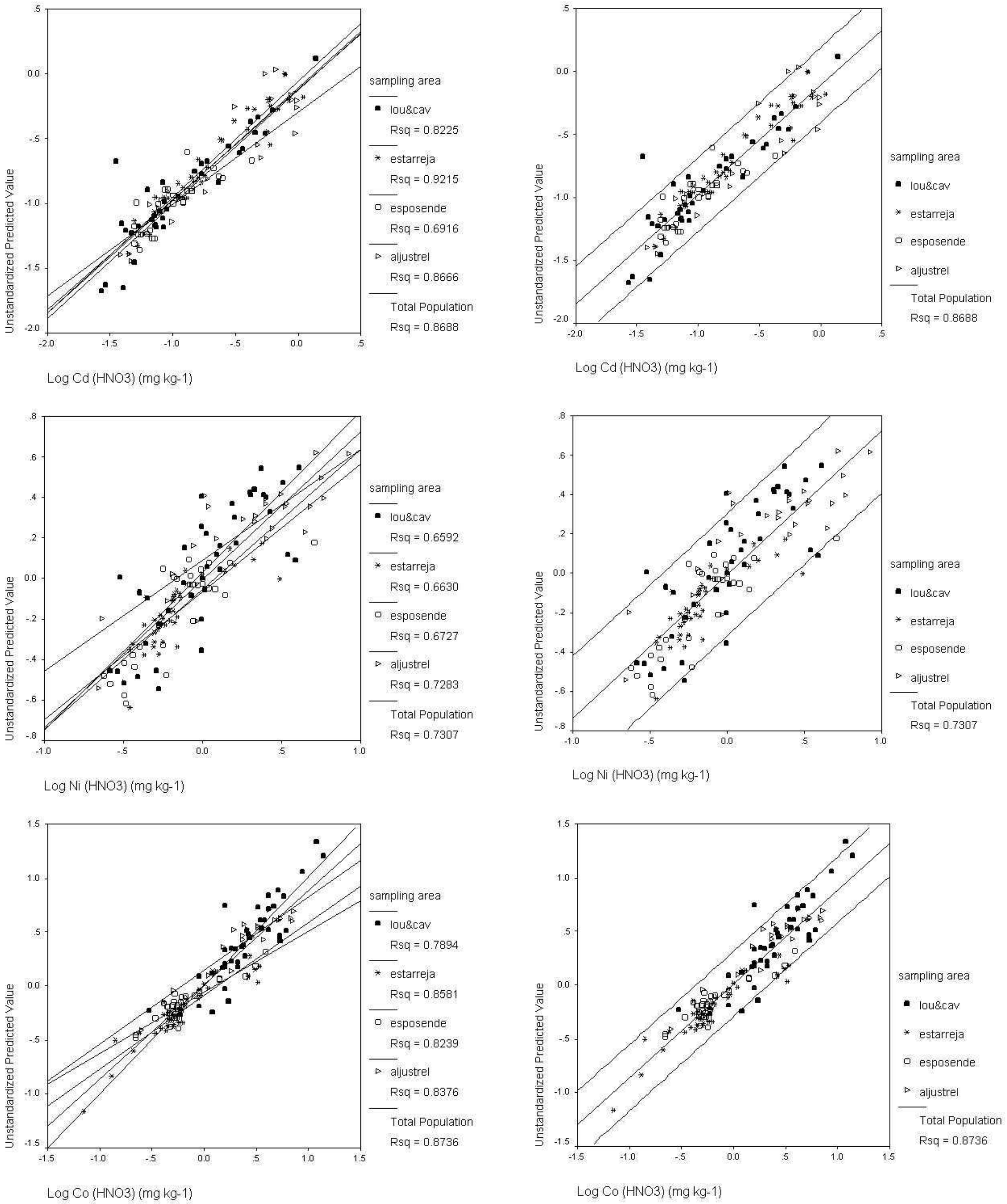


Figure 1 *(cont.)*

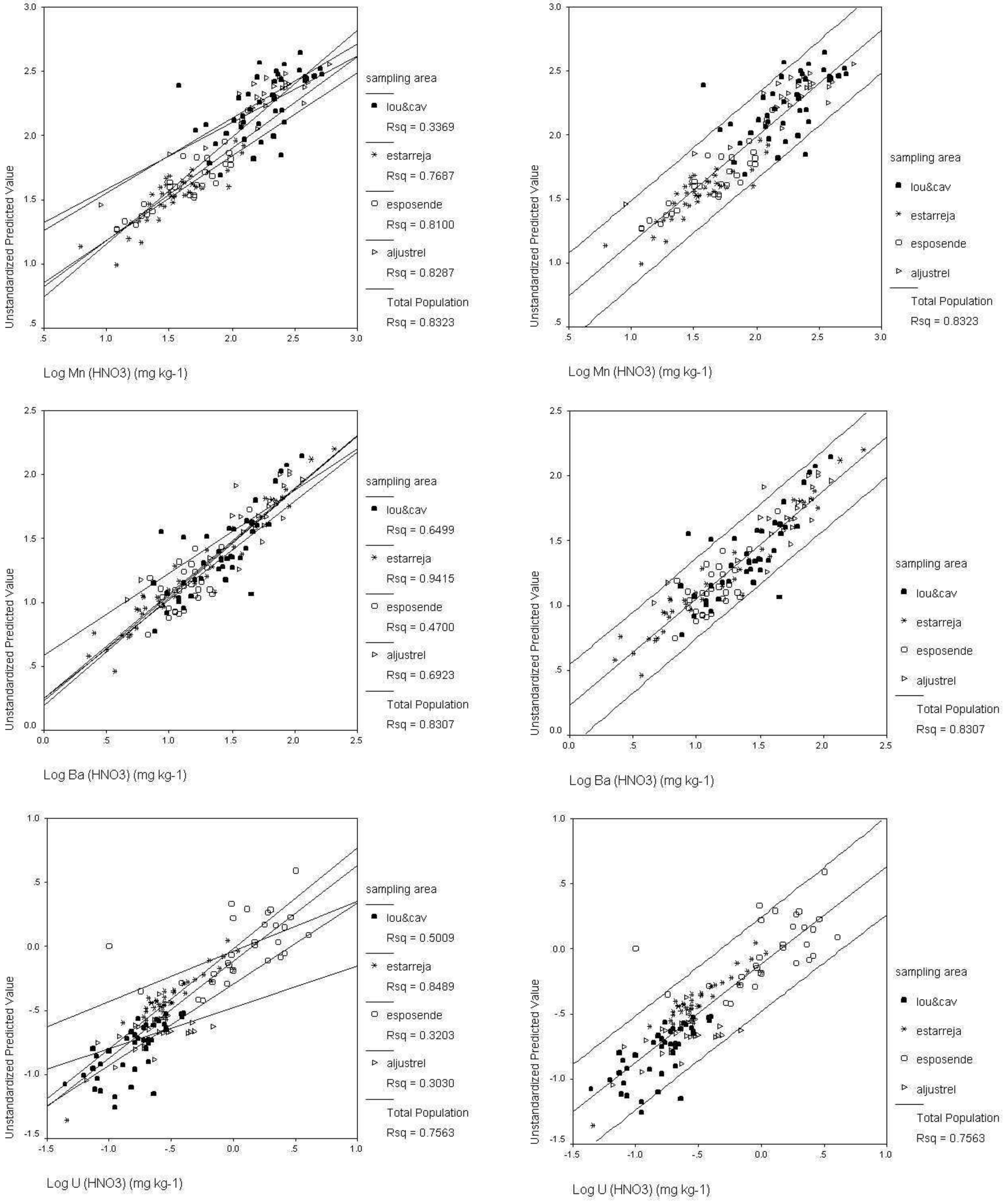


Figure 1 *(cont.)*

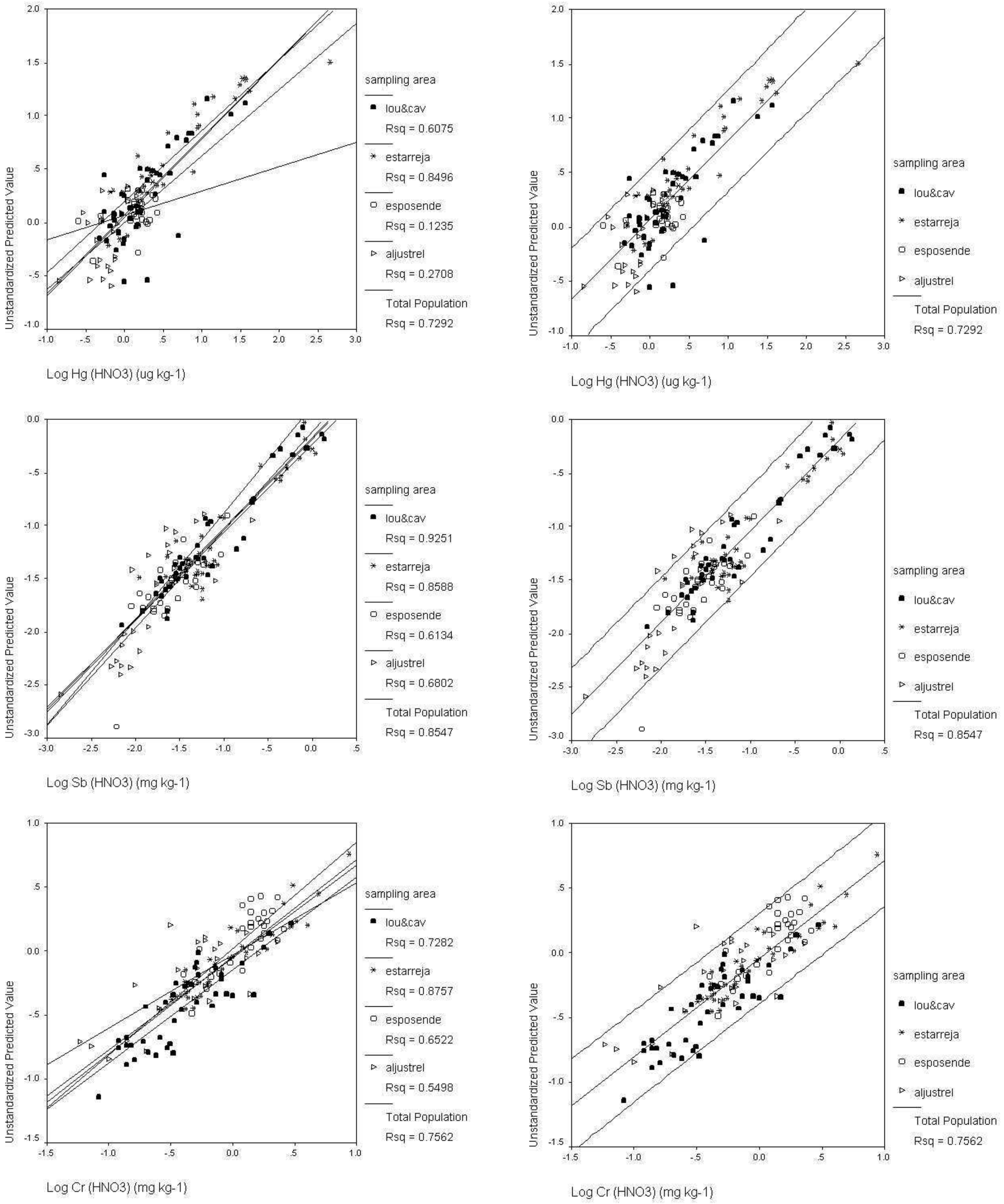


Figure 1 *(cont.)*

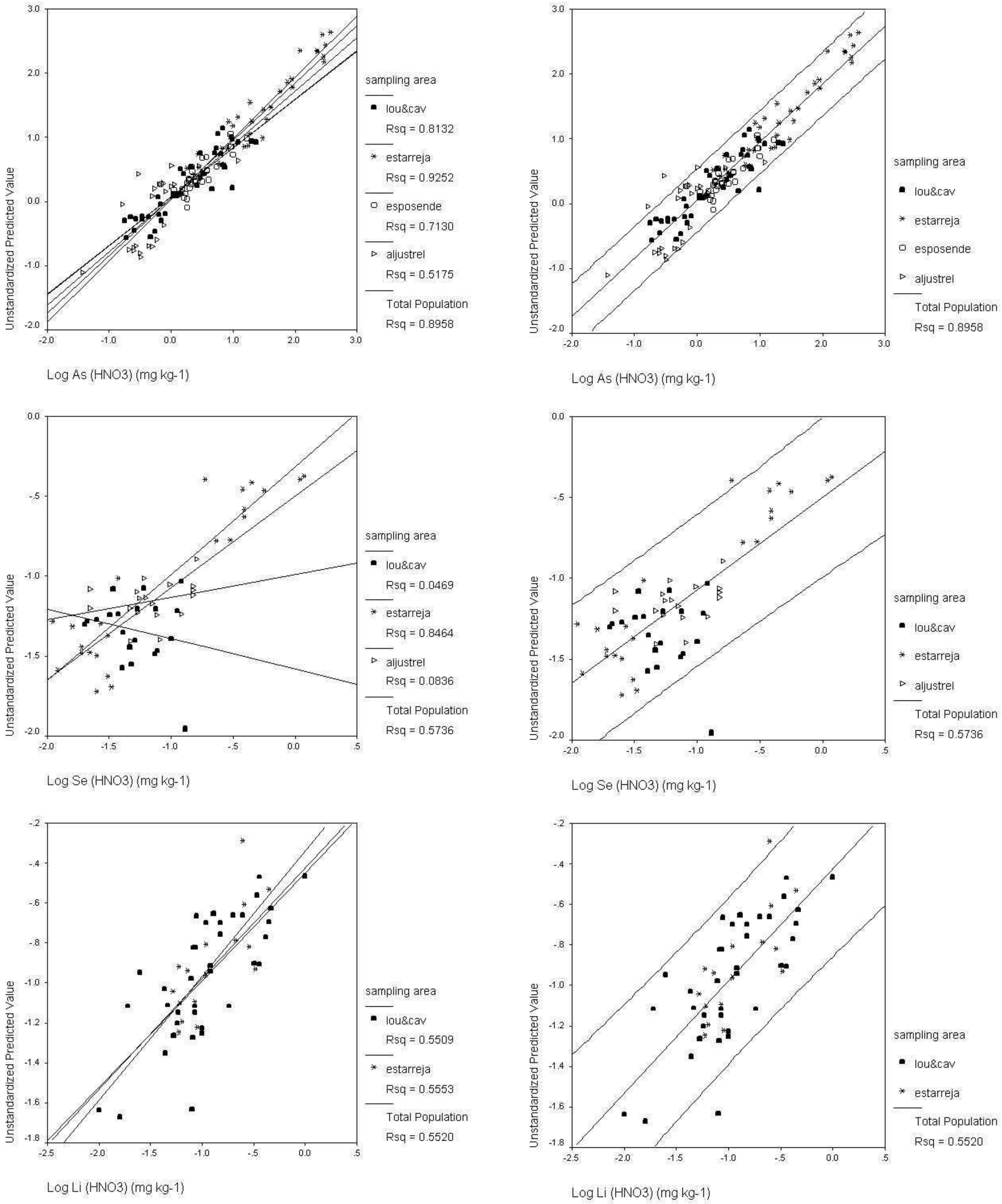


Figure 1 *(cont.)*

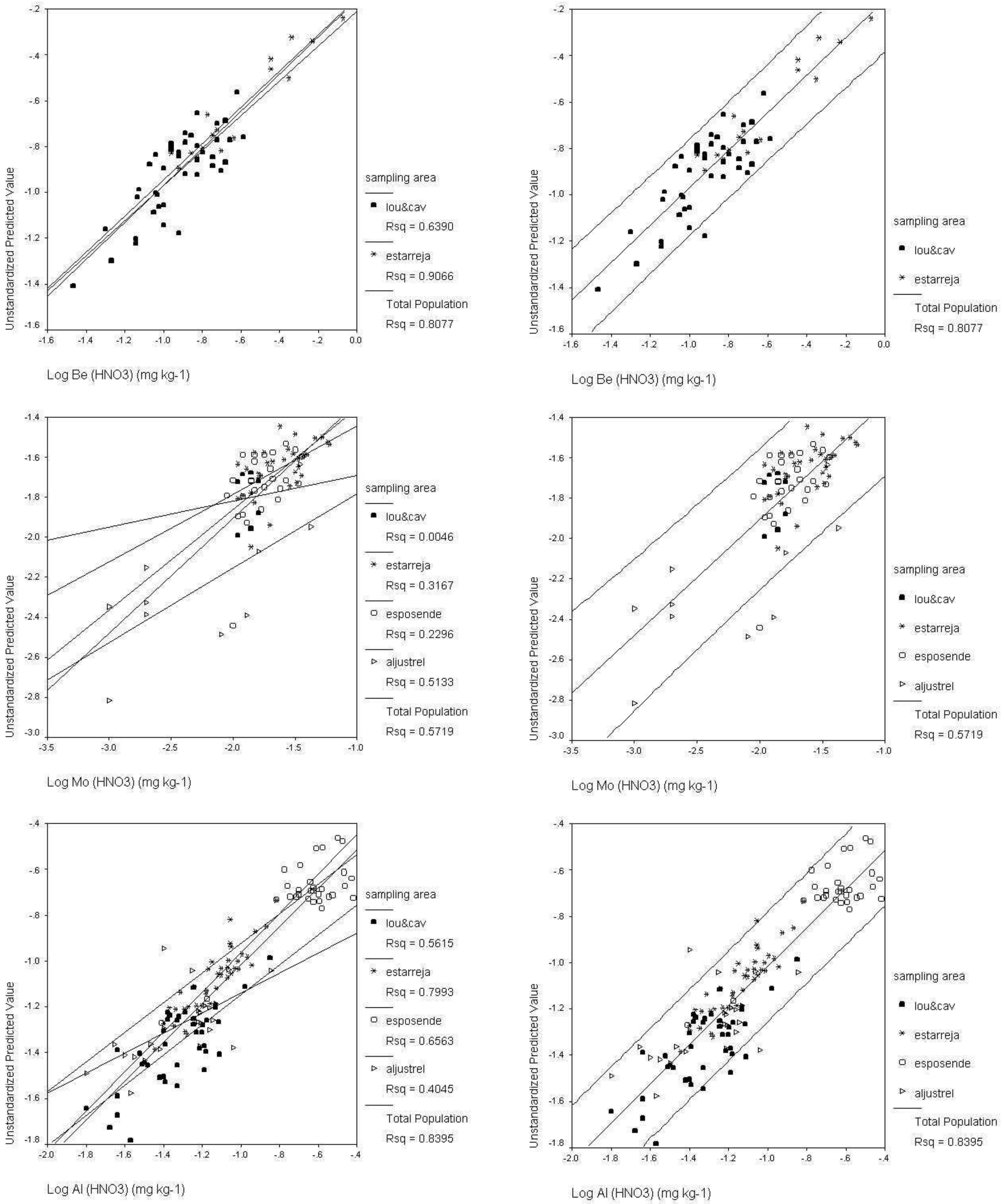


Figure 1 *(cont.)*

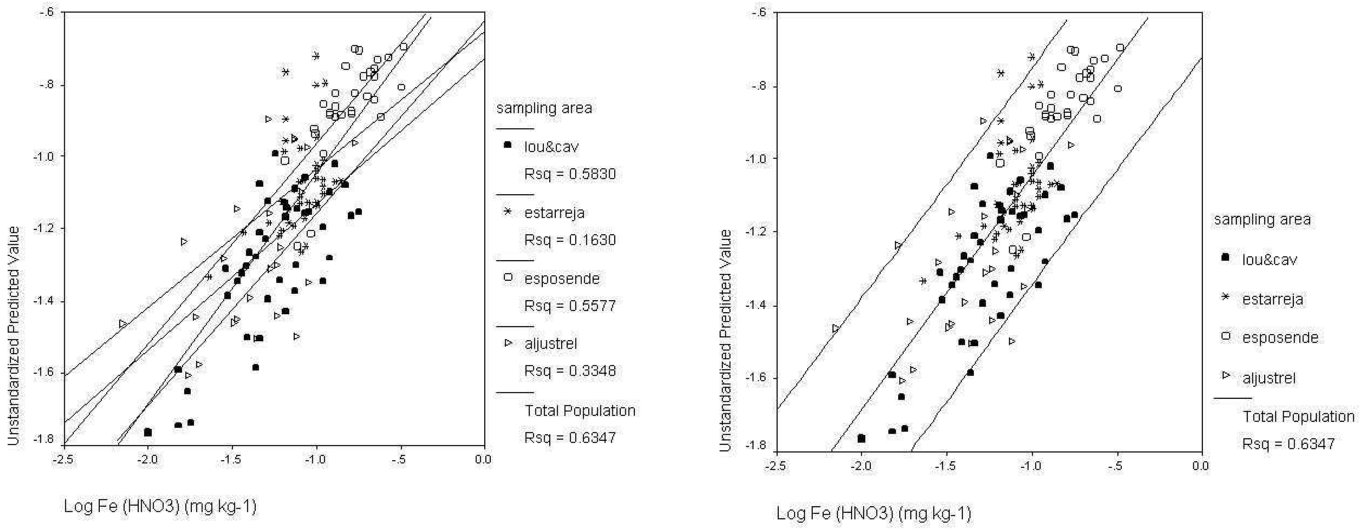


Figure 1 *(cont.)*

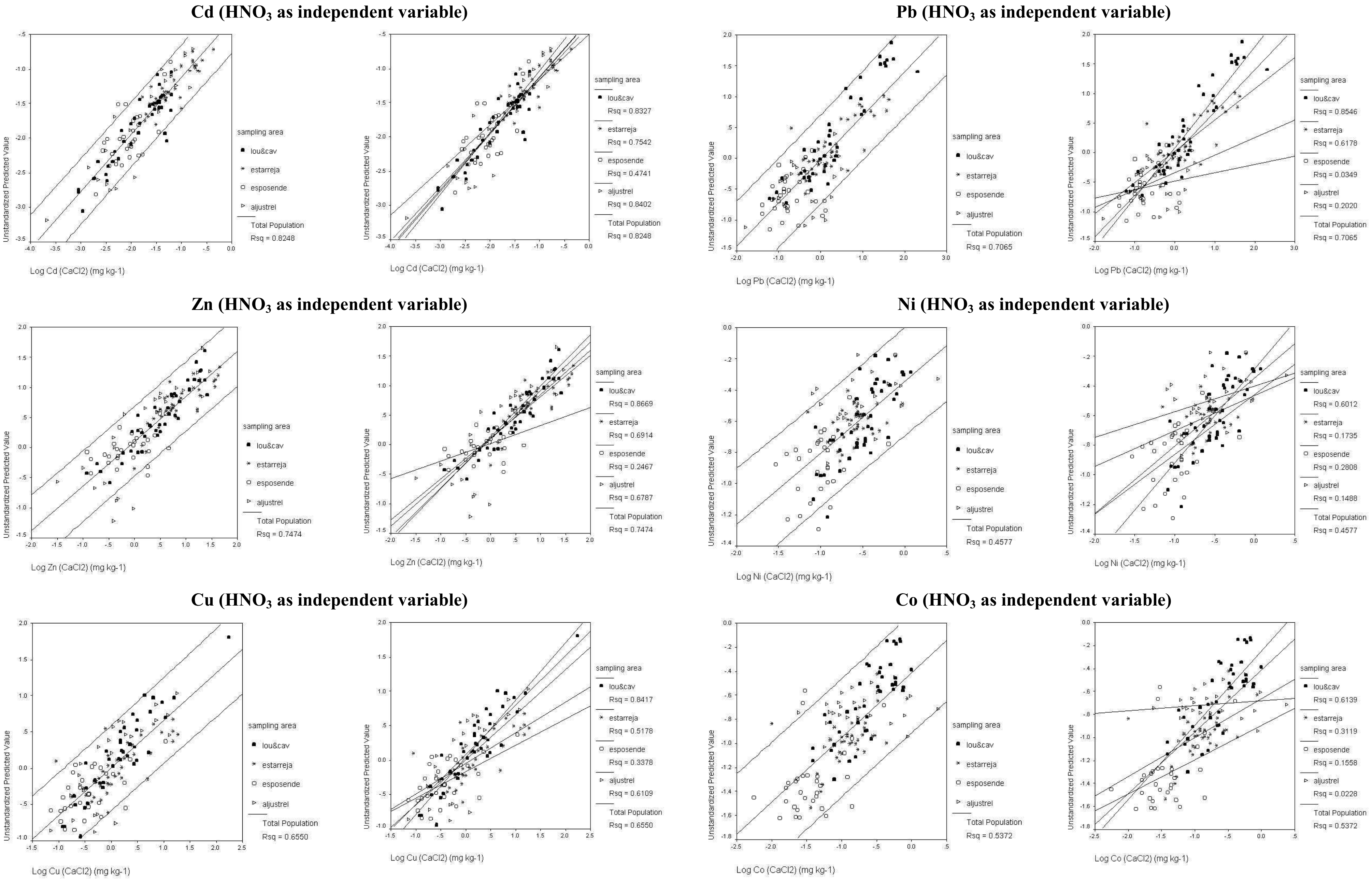
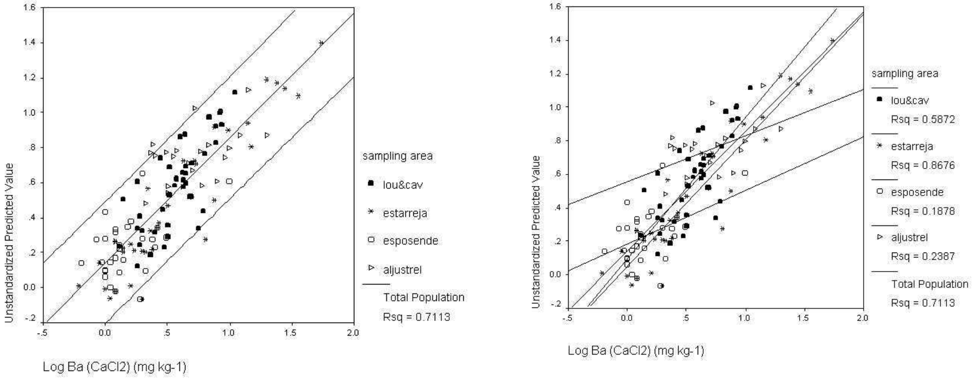
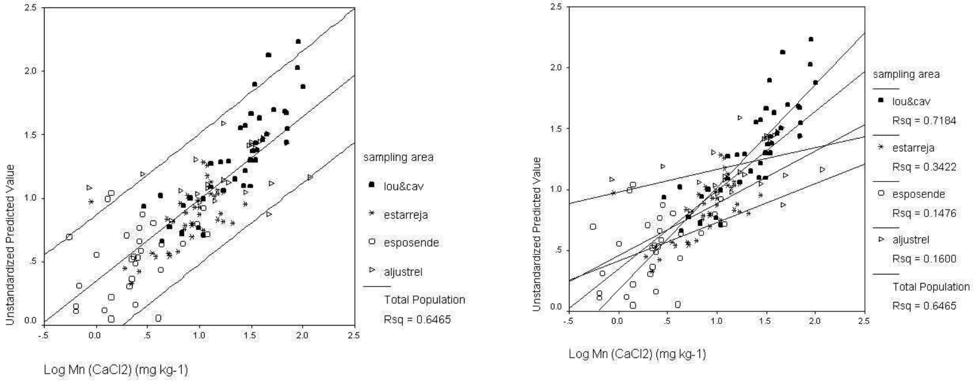


Figure 2: $CaCl_2$-extractable concentrations predicted by the linear multiple regression models against measured data. Model performance by sampling area is also shown.

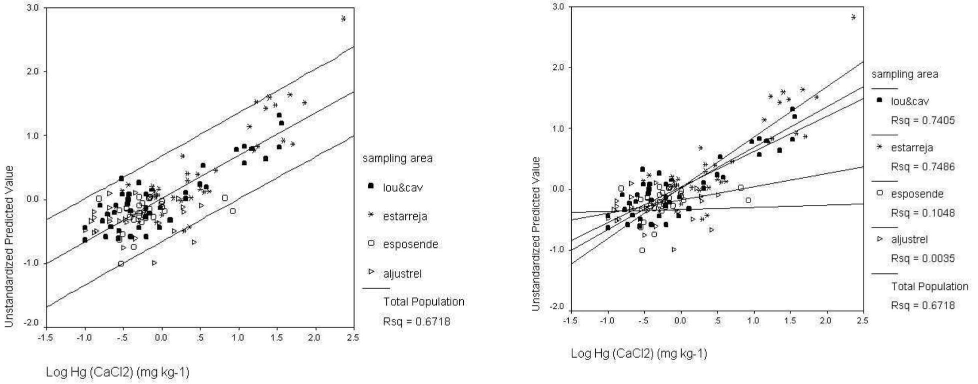
Ba (HNO_3 as independent variable)



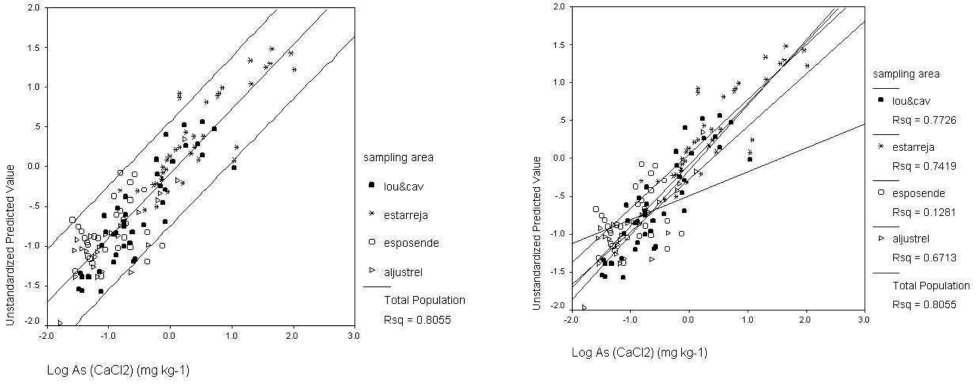
Mn (HNO_3 as independent variable)



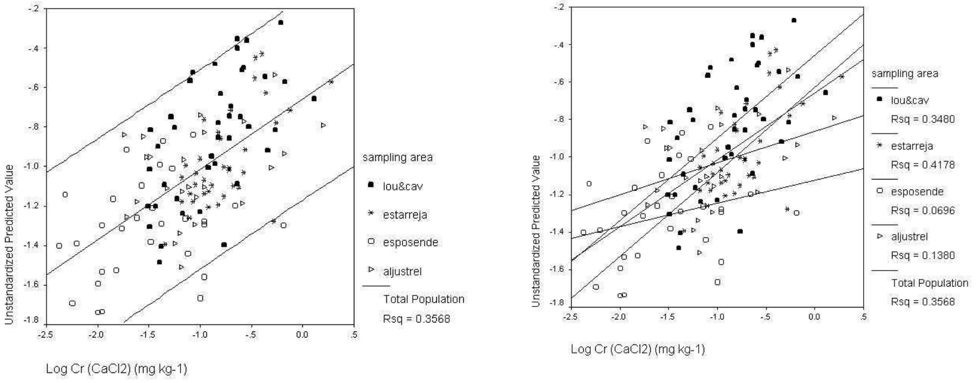
Hg (HNO_3 as independent variable)



As (HNO_3 as independent variable)



Cr (HNO_3 as independent variable)



U (HNO_3 as independent variable)

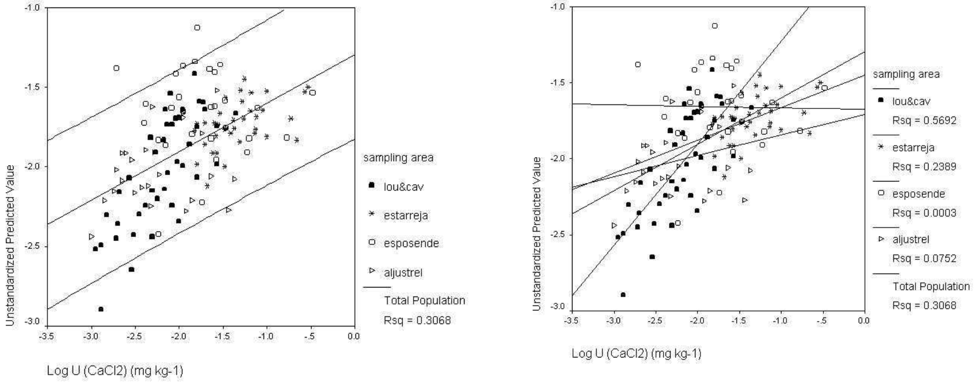
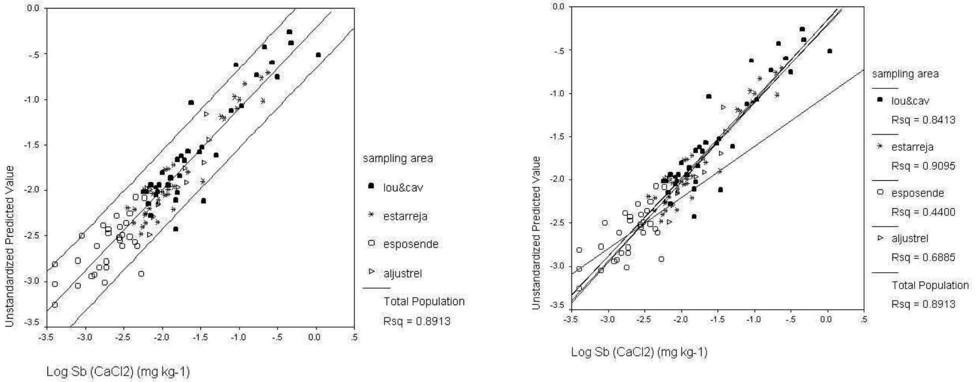
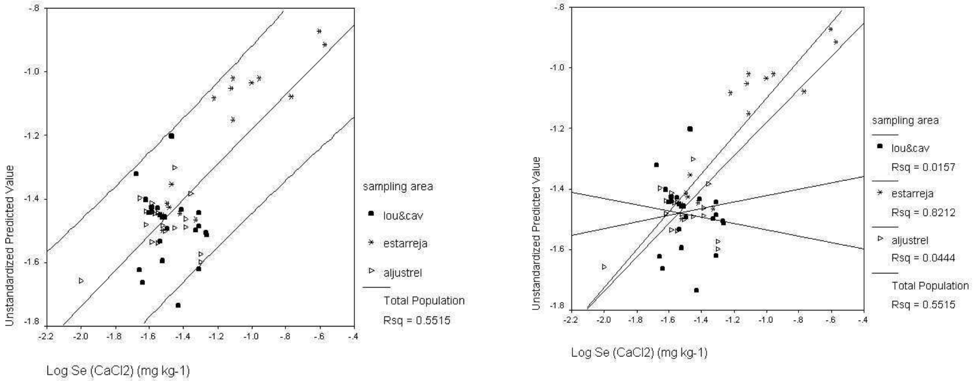


Figure 2 *(cont.)*

Sb (HNO_3 as independent variable)



Se (HNO_3 as independent variable)



Be (HNO_3 as independent variable)

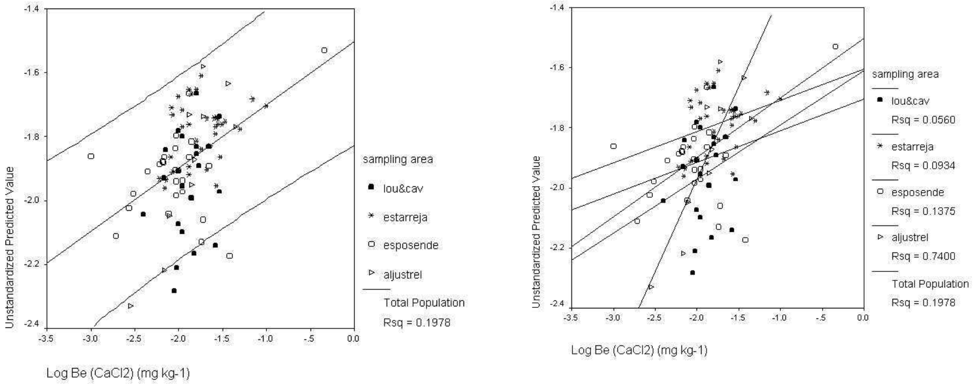


Figure 2 *(cont.)*