



TESIS DOCTORAL

Evaluación de riesgos para la salud humana y riesgos ecológicos por la presencia de contaminantes elementales y radioactivos en suelos y plantas autóctonas alrededor de la zona industrial de Puchuncaví-Ventanas, Chile

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2017

Dedicated To My Parents,

Brother and Wife

ACKNOWLEDGMENTS

“We should feel empowered by where we came from and who we are, not hide it. It is important to acknowledge that everything we do affects our ancestors as much as they have affected us.”

– Lorin Morgan- Richards

After almost 5 years the time has come to write these words. When I look back and remember all the moments lived since that November 2011 that I stepped for the first time to "unknown" 5th plant of the Faculty of Chemistry I didn't have any idea about this new country, culture, and language. I knew there have been hard times that had to be faced, working many hours with new equipment in laboratory and without any results or even with weird results. I spent days and days for studying new topics and learning how to deal with chemical materials. But the best thing is that there have been many good things, very good moments shared in the Department, with breakfasts, meals or dinners, that compensate for everything else.

In the following lines I would like to thank the following people and institutions:

I owe my deepest gratitude to my supervisor Dr. Eduardo Pinilla Gill, without his continuous optimism concerning this work, enthusiasm, encouragement and support this study would never have been completed. I also express my warmest gratitude to my other supervisor Dr. Conrado Miró, who guided me into the world of radioactive elements and helped me more than enough to understand and digest this topic. I am deeply grateful to Dr. Francisco Cereceda as other supervisor, who worked too much as logistic and scientific core of this project during these years and give different pure scientific ideas to improve this thesis.

Thanks to Gobierno de España (Ministerio de Ciencia E Innovación, project CTQ2014-52309-P), Spanish Agency for International Cooperation to Development (AECID), the University of Extremadura, Junta de Extremadura, Air Quality Network of Extremadura, and Centro Nacional de Aceleradores (CNA, Seville).

I cannot thank enough the group of the Department of Analytical Chemistry, for being professional companions with whom I shared good memories. I have many good memories with them inside and outside of university. Julia, Carmen, María, and Elena, a piece of this thesis goes for you. In particular, I would like to thank Fernando and Charo that always shared their experience and knowledge with me.

My sincere thanks also go to Dr. Elena Chamizo Calvo who afforded me an opportunity to join their research team in CNA, and who gave access to the laboratory and research facilities. Without her precious support it would not be possible to conduct research into radioactive pollutants especially Pu and U.

To my friends inside and outside Spain, Francisco Llerena, Kike, Cristina, Emilio, Nielen, Ignacio, Bahram Aghajanzadeh, Mahmoud Hossein Nia, and Peyman. Special thanks should undoubtedly go to my friend Hassan who supported me for long time with his advice and kindness.

I thank and appreciate my mother and father amorously. For the first time in these 33 years, I am speechless! I can barely find the words to express all the wisdoms, loves and supports they've given me. They have been my first fan and for that I am eternally grateful. I hope this work will be a negligible thankful for their unbounded supports and kindness during these years I've moved to other countries. Also my brother who always supported me during these years and with his sense of humor I always forgot living far away from family how much is difficult.

Finally, my big and great thanks and love go to my wife, who has stood by me through all my travails, absences, fits of pique and impatience especially during last days of my work. She never stopped to show me her pure love even she was tired of her hectic and difficult life in another country.

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JUSTIFICATION AND AIMS

The present thesis is focused on optimization and experimental validation of strategies for human health and ecological risk assessment derived from exposition to environmental pollutants. It is a truly relevant subject from scientific, social and economic points of view, since these environmental assessment tools are essential for proper diagnosis of risks caused by anthropogenic emissions, and also to verify the effect of preventive and corrective measures. We have specifically study trace elements and radioisotopes, occurring in soils and native plants as the result of deposition of atmospheric emissions from different sources, especially of industrial origin. Most of the thesis is devoted to the investigation of environmental risks at the Puchuncaví area (Valparaíso Region, Chile), heavily impacted by industrial emissions from the beginning of the 1960s (industrial complex of Puchuncaví-Ventanas), but we have also studied the presence of uranium and plutonium radioisotopes in selected southern hemisphere locations. The work developed in this thesis has served to reinforce international scientific cooperation between the Universidad de Extremadura and Universidad Técnica Federico Santa María in Chile, supported by a research project funded by Agencia Española de Cooperación Internacional al Desarrollo (AECID).

The development of the general objective of the Thesis can be divided in the following specific aims:

1. To identify and quantify a set of environmental pollutants (trace elements and radioactive pollutants) in soils around the industrial area of Puchuncaví-Ventanas (V Region, Chile) during a long term campaign (2007-2011) to assess potential impact of industrial activities in the area by using modeling and chemometric tools.

2. Assessment of ecological risk at Puchuncaví soils by geoaccumulation index (Igeo), enrichment factor (EF), contamination factor (Cf), contamination degree (Cdeg), and integrated pollution index (IPI).
3. Assessment of human risk (non-carcinogenic and carcinogenic) at Puchuncaví, from deposition of elemental pollutants and radioactivity in soil samples.
4. Evaluation of selected native plant species growing at the Puchuncaví area for biomonitoring and phytoremediation respect to trace element pollution.
5. Investigation of the presence of some artificial radioactive pollutants in surface soils as a result of global fallout from nuclear weapons tests carried out in the 1950's and 1960's. These soil samples were collected from different sampling locations (South America and Africa) in the Southern Hemisphere.

ABSTRACT

As previously mentioned in the Justification of the thesis, our work has been focused on optimization and experimental validation of strategies for human health and ecological risk assessment derived from exposition to environmental pollutants, specifically trace elements and radioisotopes, occurring in soils and native plants as the result of deposition of atmospheric emissions from different sources, especially of industrial origin. Most of the thesis is devoted to the investigation of environmental risks at the Puchuncaví area (Valparaíso Region, Chile), heavily impacted by industrial emissions from the beginning of the 1960s (industrial complex of Puchuncaví-Ventanas), but we have also studied the presence of natural radiation and of uranium and plutonium radioisotopes in selected southern hemisphere locations.

After Introduction (chapter 1) and Materials and Methods (chapter 2) the results obtained in the thesis are summarized and discussed in chapter 3. This chapter contains the four scientific articles obtained from the work (3.1 to 3.4), then a general discussion in English (3.5) and the translation of the general discussion to Spanish (3.6).

The first article (presented in chapter 3.1), named ***“Long-term assessment of ecological risk from deposition of elemental pollutants in the vicinity of the industrial area of Puchuncaví-Ventanas, central Chile”***, published in the journal Science of the Total Environment, is an investigation about soil pollution by trace elements and compares ecological risk indexes related to industrial activities for the case study of Puchuncaví-Ventanas as a representative industrial zone. Selected elements (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba) were analyzed during a long-term period (yearly sampling campaigns during 2007–2011), at 5 sampling stations representing different degrees of impact. Geoaccumulation index (I_{geo}),

enrichment factor (EF), contamination factor (C_f), contamination degree (C_{deg}), and integrated pollution index (IPI) are critically discussed for quantitative ecological risk assessment.

The second article (presented in chapter 3.2), named ***“Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncaví-Ventanas industrial complex, Chile”***, published in the journal Environmental Pollution is a study about Puchuncaví as a model environment for evaluating the spatial gradient of human health risk, which is mainly caused by trace elemental pollutants in soil, but considering also radioactive risks. Soil elemental profiles and radioactivity in 121 samples from five selected locations representing different degrees of impact from the industrial sources.

The third article (chapter 3.3), ***“Trace elements levels in native plant species around the industrial site of Puchuncaví-Ventanas (central Chile): Evaluation of the phytoremediation potential”***, in preparation for the journal Environmental Science and Pollution Research, investigates uptake of selected trace elements (Cu, Sb, As, Pb, Cd, Zn, Cr, Mn, Ni, V, and Co) from soil and their accumulation in the biomass samples (leaves and flowers) of three selected native plants (namely *Oenothera Affinis*, OA; *Sphaeralcea Velutina*, SV and *Argemone Subfusiformis*, AS) around Puchuncaví-Ventanas. Trace elements measurements in the native plants of this area and the ability of transfer of these pollutants from soil to plant (transfer factor) have been assessed in order to identify the potential use of these plant species for phytoremediation.

In the fourth article (chapter 3.4), ***“Presence of fallout ^{236}U and plutonium isotopes, ^{239}Pu and ^{240}Pu , in soils from Southern Hemisphere”***, in preparation for the journal Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, we provided new data about concentration of ^{236}U , ^{239}Pu and ^{240}Pu in soil of the Southern Hemisphere (Chile in South-America, and Mozambique and South-Africa in Africa) due to the global fallout and also an important contribution of the so-called tropospheric fallout from

both the atmospheric nuclear tests performed in the French Polynesia and in Australia by France and United Kingdom, respectively.

RESUMEN

Como se ha comentado previamente en la justificación de la tesis, nuestro trabajo se ha centrado en la optimización y validación experimental de estrategias para la evaluación de riesgos ecológicos y riesgos para la salud humana, derivados de la exposición a contaminantes ambientales, específicamente elementos traza y radioisótopos, presentes en suelos y plantas autóctonas como resultado de la deposición de emisiones atmosféricas desde diferentes fuentes, especialmente desde fuentes industriales. La mayor parte de la tesis se dedica a la investigación de riesgos ambientales en la comuna de Puchuncaví (región de Valparaíso, Chile), fuertemente impactada por emisiones industriales desde los años sesenta del siglo XX, pero se ha estudiado también la presencia de isótopos de uranio y plutonio en lugares seleccionados del hemisferio sur.

Después de una Introducción (capítulo 1) y un capítulo de Materiales y Métodos (capítulo 2), se presentan y discuten en el capítulo 3 los principales resultados obtenidos. EL capítulo contiene los cuatro artículos científicos derivados de la tesis (3.1 a 3.4), seguidos de una discusión general en inglés (3.5) y en castellano (3.6).

El primer artículo (capítulo 3.1), denominado ***“Long-term assessment of ecological risk from deposition of elemental pollutants in the vicinity of the industrial area of Puchuncaví-Ventanas, central Chile”***, publicado en la revista Science of the Total Environment, es una investigación sobre la contaminación del suelo por elementos traza, y compara índices de riesgo ecológico relacionados con la actividad industrial para el caso de estudio de Puchuncaví-Ventanas, como zona industrial representativa. Se analizaron parámetros seleccionados (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba) durante un período prolongado (2007-

2011), en cinco puntos de muestreo representativos de diferentes grados de impacto. Se calcularon y discutieron el índice de geoacumulación (I_{geo}), el factor de enriquecimiento (EF), el factor de contaminación (C_f), el grado de contaminación (C_{deg}), y el índice de contaminación integrado (IPI), para una estimación cuantitativa del riesgo ecológico.

El segundo artículo (capítulo 3.2), titulado ***“Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncaví-Ventanas industrial complex, Chile”***, publicado en la revista Environmental Pollution, es un estudio sobre Puchuncaví como entorno modelo para evaluar el gradiente espacial del riesgo para la salud humana, causado fundamentalmente por elementos traza en el suelo, pero considerando también el riesgo radiactivo. Los perfiles de concentración de elementos traza y la radiactividad se estudiaron sobre 121 muestras de cinco localizaciones representando diferentes grados de impacto de las fuentes industriales.

El tercer artículo (capítulo 3.3) ***“Trace elements levels in native plant species around the industrial site of Puchuncaví-Ventanas (central Chile): Evaluation of the phytoremediation potential”***, en preparación para la revista Environmental Science and Pollution Research, investiga la absorción por vegetales autóctonos de elementos traza seleccionados (Cu, Sb, As, Pb, Cd, Zn, Cr, Mn, Ni, V, and Co) desde los suelos, y su acumulación en muestras de biomasa (hojas y flores) para el caso de tres especies nativas de la zona de Puchuncaví (*Oenothera Affinis*, *Sphaeralcea Velutina* y *Argemone Subfusiformis*). Se evalúa la capacidad de las plantas para transferir los contaminantes desde el suelo a la biomasa vegetal (factor de transferencia), para identificar el uso potencial de esas especies vegetales en fitorremediación.

En el cuarto artículo (capítulo 3.4) ***“Presence of fallout ^{236}U and plutonium isotopes, ^{239}Pu and ^{240}Pu , in soils from Southern Hemisphere”***, en preparación para la revista Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, se proporcionan nuevos datos sobre la concentración de ^{236}U , ^{239}Pu y ^{240}Pu en suelos del

hemisferio sur (Chile en Sudamérica, y Mozambique y Sudáfrica en África), debido a la deposición global y a la importante contribución de la denominada deposición troposférica debida a los ensayos nucleares realizados en la Polinesia Francesa y en Australia por Francia y por el Reino Unido, respectivamente.

CHAPTER 1.

INTRODUCTION

1. Introduction

1.1. Soil pollution

The pedosphere is the total superficial layer of Earth that consists of soil, and it is characterized by complex and dynamic interactive linkages with the lithosphere, the hydrosphere, the biosphere, and the atmosphere. Soil is a matrix composed of water, air, living organisms, and minerals, originated by decomposition of organic matter and weathering of bedrock. Geographical location, type of weathering processes, kind of bedrock, and climate can influence the physical and chemical properties of the soil. A typical soil profile is presented in Figure 1.1, showing the five basic horizons (O, A, E, B, and C) through a soil profile (Radojevic et al. 1999).

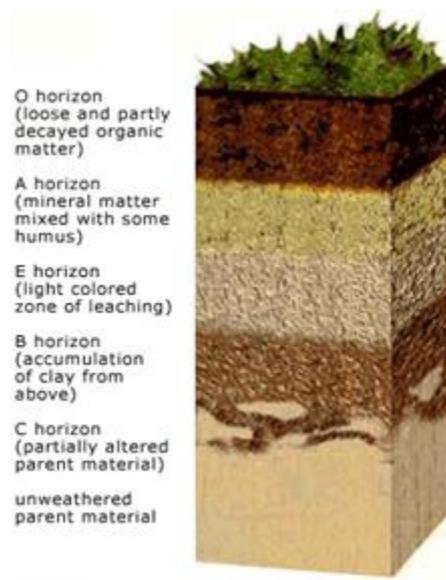


Fig.1.1. Schematic view of a soil profile and different layers definition

Around 149 million km² of global land on the world is covered by soil (Zabel et al. 2014). From this amount of soil just 93 million km² are biologically productive and it approximately includes 33% forest, 32% pastures and 11% crop land. Different interaction factors cause variation in soil depth and across landscapes. These different interaction factors include physical and

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chemical weathering, erosion and deposition, human and natural disturbances, combined with the effects of the different factors involved in soil formation, related with parent material, climate, living organisms (e.g., vegetation), topography and time. These factors can produce a very significant spatial diversity in soil, for example only in the United States 20,000 different kinds of soil have been identified and mapped (Moretto and Klacher 2015).

Recently, the impact of human in the biosphere is more significant than in the past. It is important to identify and assess the sphere of man's (anthroposphere) settlement and activity. Human's impact on the biosphere is intense and complex, and it has often led to un-repairable changes. Although geological and biological conversions of the Earth's biosphere have been very slow, changes induced by humans have become extremely quick in recent years. Natural balance of natural ecosystems that have been formed evolutionarily during a long period of time are now changed and disturbed by human activities in the short time (Kabata-Pendias 2011).

Soil contamination refers to a situation where chemical state deviates from the normal composition but does not have a detrimental effect on organisms, whereas soil pollution applies when an element or a chemical substance is detected in greater than natural (background) concentrations because of human activity and has a net detrimental effect on the environment and its components. Based on plant, animal, and human health perspective, soil is polluted when chemical substances begin to affect biological processes.

Pollution can rise in the environmental as the result of regular industrial activities or following an accident, as in the case of nuclear accidents or oil spills. Main important sources of soil pollution are industrial sources, agrochemical sources, urban sources, atmospheric sources, and incidental sources (Mirsal 2008):

- *Industrial sources*: industrial activities are important contributors to the soil pollution from the beginning of the industrial revolution. Mining and smelting, metallurgical, chemical and electronic industries are the most relevant types regarding environmental impact to soil.
- *Agrochemical sources*: main pollutants from agrochemical source are fertilizers, manure, pesticide, and fuel spillage from agricultural machinery.
- *Urban sources*: soil pollution by urban materials and processes is as old as urbanization history. In ancient days damage of the soil was limited by this source but modern urbanization has taken dimensions that are hardly controllable. Power generation emissions, soil pollution through transport activities, waste and sewage sludge, are important urban activities causing environmental impact in the soil.
- *Atmospheric sources*: wind-blown pollutants and acid deposits are important sources to raise pollutions in the soil.
- *Incidental sources*: warfare (explosives and poisonous gases) and industrial accidents have increased level of pollutants in the soil.

We will now discuss in some details the impact of the two classes of soil pollutants we have studied in this Ph D dissertation, namely trace elements and radioactive pollutants.

1.1.1. Elemental pollutants

Although trace elements are minor components of the solid soil phase, they play an important role in soil fertility. Knowledge of the association of trace elements with particular soil phases and their affinity to each soil constituent is the key to a better understanding of the principles governing their behavior in soils. The “normal concentrations” of trace elements in soils are of great interest as background values needed for any assessment of the degree of soil contamination. Present-day soils contain trace elements of various origins. Lithogenic elements are those that are directly inherited from the lithosphere (mother material).

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Pedogenic elements are of lithogenic origin also, but their concentration and distribution in soil layers and soil particles are changed due to pedogenic processes. Anthropogenic elements are all those deposited into soils as direct or indirect results of man's activities (Kabata-Pendias 2011).

The type and chemistry structure of the parent material affects the natural concentration of trace elements in soils (Mirsal 2008) but anthropogenic activities can also lead to soil deterioration. Introduction of different polluting substances, degradation of soil organic matter, and decreasing of the fecundity due to erosion and over use have been common problems since human started agriculture activities. E.g. in (Table 1.1), the average concentration of some heavy metals (mg kg^{-1}) in soils is shown. This table clearly shows that lead, cadmium, and mercury are enriched in the soil respect to mean crust values by anthropogenic activities. For example, the mean concentration of cadmium is six times higher than the mean concentration in the crust, whereas mercury and lead in soil are two times more concentrated than in the crust (Mirsal 2008).

Table.1.1. Elemental composition of the Earth's crust. All elements are in $\mu\text{g g}^{-1}$ (Mirsal 2008).

<i>Elements</i>	<i>Mean crust</i>	<i>Sandstone</i>	<i>Limestone</i>	<i>Soil</i>
<i>V</i>	160	20	45	108
<i>Cr</i>	100	35	11	84
<i>Ni</i>	80	9	7	34
<i>Zn</i>	75	30	20	60
<i>Cu</i>	50	30	5.1	26
<i>Co</i>	20	0.3	0.1	12
<i>Pb</i>	14	10	5.7	29
<i>Cd</i>	0.11	0.05	0.03	0.6
<i>Hg</i>	0.05	0.29	16	0.1

It is important to note that heavy metals may be harmful or even fatal for living organisms, but some of them are also, under certain conditions, essential for animals and plants, e.g. some of them are constituents of enzymes and other important organism's structures (Table 1.2). presents some essential and non-essential heavy metals for live organisms (Mirsal 2008).

Table1.2. Essential and non-essential heavy metals in animals and plants (Mirsal 2008).

	<i>Organisms</i>	<i>Heavy metals</i>
<i>Essential</i>	Animals	Co, Cr, Se
	Plants	B, Mo
	Animal&Plants	Cu, Mn, Fe, Zn
<i>Non-essential</i>	As, Cd, Hg, Pb, Pu, Sb, Ti, U	

However, human activities can extremely increase the level of heavy metals in the soil. Heavy metals constitute an ill-defined group of inorganic elemental pollutants, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (Merian et al. 2004). The heavy metals are contaminates in the soil because:

- 1) They become transferred from mines to random environmental locations where higher potentials of direct exposure occur.
- 2) They are mobilized by human cycle faster than relative nature pathways.
- 3) The concentrations of the metals in discarded products are relatively high compared to those in the receiving environment
- 4) The chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable (D'Amore et al. 2005).

We discuss now relevant properties of the most environmentally relevant trace elements related with soil pollution.

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-Lead

It is a bluish gray metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, PbSO₄), or oxygen (PbCO₃), and ranges from 10 to 30 mg kg⁻¹ in the earth's crust, and mean concentration of Pb for surface soils worldwide average is 32 mg kg⁻¹ (range from 10 to 67 mg kg⁻¹) (Kabata-Pendias 2011). Important anthropogenic industrial activities involving Pb are solders, alloys, cables or glass factories.

-Chromium

It is one of the less common elements and does not occur naturally in elemental form, but only in compounds (Smith 1995). The abundance of Cr in the Earth's upper crust averages 100 mg kg⁻¹ world soil average content of Cr in soils has been established as 59.5 mg kg⁻¹ (Kabata-Pendias 2011). An Important source of Cr pollution is considered to be from dyestuffs and leather tanning. Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content, and the amount of organic matter present. Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form (Smith 1995).

-Arsenic

Arsenic exhibits a fairly complex chemistry and can be present in several oxidation states (-III, 0, III, V) (Smith 1995). It occurs in a wide variety of minerals, mainly as As₂O₃, and can be recovered from processing of ores containing mostly Cu, Pb, Zn, Ag and Au. The average content of arsenic in the Earth's crust is estimated around 1.8 mg kg⁻¹ and the overall mean value of the total arsenic for different soil is estimated as 6.83 mg kg⁻¹. The background arsenic levels in top soils are generally low (Kabata-Pendias 2011). Main anthropogenic sources of arsenic are metal processing, chemical works based on S and P minerals, coal combustion, and geothermal power plants (Kabata-Pendias 2011).

-Zinc

Average Zn content in the Earth's crust is estimated at 70 mg kg^{-1} , and the same average level is for the worldwide soils but nowadays Zn concentrations are rising unnaturally by human activities. Most Zn is released during industrial activities such as mining, coal, waste combustion and steel processing.

-Cadmium

Cadmium is one of the three most poisonous heavy metals (Cd, Hg, and Pb) and is not known for any essential biological function (Campbell 2006). The average Cd content in the Earth's crust is 0.1 mg kg^{-1} and the world average soil Cd concentration is estimated as 0.41 mg kg^{-1} .

-Copper

Copper concentration in the Earth's crust ranges between 25 and 75 mg kg^{-1} and its average is 55 mg kg^{-1} . The general values for the average total Cu contents in soils of different groups all over the world range between 14 and 109 mg kg^{-1} (Kabata-Pendias 2011). Copper is the third most used metal in the world and its production and consumption are growing. Main anthropogenic sources of copper in the environment are copper mining activities, metal and electrical manufacturing, agricultural and domestic use of pesticides and fungicides, leather processing, and automotive brake pads. Natural sources of copper pollution are volcanic eruptions, windblown dust, and forest fires. Major sources of pollution (mainly non ferric metal smelters) present halos in which Cu concentrations in surface soils decrease with distance, which is especially pronounced in a downwind direction (Kabata-Pendias 2011).

-Mercury

Mercury is usually recovered as a byproduct of ore processing (Smith 1995). Major emissions of Hg are found in chlor-alkali production (being replaced by novel technologies due to environmental concern), vinyl chloride monomer production, artisanal gold mining, and

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batteries, control instruments, electrical lightings production, and Hg production. The average content of Hg in soils of different groups all over the world range between 0.58 and 1.8 mg kg⁻¹ and the worldwide mean content is estimated as 1.1 mg kg⁻¹. The background levels of Hg in soils are not easy to estimate due to the widespread Hg pollution and global atmospheric transport and deposition (Kabata-Pendias 2011).

-Nickel

The mean Ni abundance has been estimated around 20 mg kg⁻¹, whereas in the ultramafic rocks Ni ranges from 1400 to 2000 mg kg⁻¹ (Kabata-Pendias 2011). Nickel exists in the environment only at very low levels. Amount of Ni in the soils is strongly dependent on its contents in parent material. However, the concentration of Ni in surface soils shows the additional impact of both soil-forming processes and anthropogenic. Regarding biological activity, it is essential in small doses, but it is dangerous when the maximum tolerable amounts are exceeded. The most relevant sources of nickel contamination in soils come from steel and other metal products, metal plating industries, combustion of fossil fuels, and nickel mining and electroplating (Khodadoust et al. 2004).

-Vanadium

Vanadium is present in the earth's crust with average concentration 150 mg kg⁻¹. Highest concentration of V are reported in soils of mafic rock and the average vanadium content of soils worldwide has been calculated to vary from 18 to 115 mg kg⁻¹. Industrial activities like certain minerals ores, burning of coal and oil have increased the deposition of vanadium in soils (Merian et al. 2004).

-Manganese

Manganese levels in the earth crust vary from 411 to 550 mg kg⁻¹ and its highest levels occur in loamy and calcareous soils (Kabata-Pendias 2011). The main sources of anthropogenic

environmental pollution by manganese are factories of alloys, steels, and iron and also the production and use of fertilizers, fungicides, organomanganese fuel additives, and dry- cell batteries (Mirsal 2008).

-Strontium

Strontium is a relatively common element in the Earth's crust and its contents range is between 260 and 370 mg kg⁻¹. Main source of Sr pollution are coal combustions and sulfur mining (Kabata-Pendias 2011).

-Antimony

Antimony is found at very low levels throughout the environment, soil usually contains very low concentrations of antimony (less than 1 mg kg⁻¹). However, higher concentrations have been detected at hazardous waste sites and at antimony-processing sites (Burton 2002). The average content of Sb in the Earth's crust is estimated as 0.2 mg kg⁻¹ and mean background Sb levels of soils of various countries range from 0.25 to 1.04, and average is 0.67 mg kg⁻¹ (Kabata-Pendias 2011).

-Potassium

Potassium constitutes about 2.4% of the Earth's crust and is the seventh most abundant element. Potassium estimation in Earth's crust have been made with representative values within a wide range from about 0.1% for limestone to more than 4% for granites. Important uses of potassium are related to agricultural industry as K₂O form, electrolytic production, drilling fluids in oil industry, aluminum smelting, metal plating, and applications in the food and pharmaceutical industries (Merian et al. 2004).

-Barium

Barium is a common and quite ubiquitous element; its mean content in the Earth's crust

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amounts to 400 mg kg^{-1} , and ranges from 250 to 584 mg kg^{-1} in the upper continental crust and average range for soils on the world scale is from 362 to 580 mg kg^{-1} (Kabata-Pendias 2011).

-Cobalt

Cobalt is a comparatively rare element, ranking 32nd in abundance in the earth's crust (Merian et al. 2004). It is highly concentrated in mafic rocks, up to 200 mg kg^{-1} , when compared to its content in acid igneous rocks, $1\text{-}15 \text{ mg kg}^{-1}$, and to its abundance in the upper continental crust $10\text{-}12 \text{ mg kg}^{-1}$. It is also likely to concentrate in black shales. The Co content of soils is inherited mainly from parent materials. The worldwide mean value of Co in surface soils is calculated as 10 mg kg^{-1} . Usually, higher levels of Co are in heavy loamy soils (Cambisols) and, sometimes, in organic soil (Histosols) (Kabata-Pendias 2011). Soils near ore deposits, phosphate rocks, or ore smelting facilities, and soils contaminated by airport traffic, highway traffic, or other industrial pollution may contain high concentrations of cobalt. Small amounts of cobalt may be released into the atmosphere from coal-fired power plants and incinerators, vehicular exhaust, industrial activities relating to the mining and processing of cobalt containing ores, and the production and use of cobalt alloys and chemicals (Agency of Toxic Substances and Diseases 2004).

About international and national level regulations about potentially toxic trace elements levels in soils, we now comment the most outstanding rules in force.

- *Basel Convention, 1989*: The overarching objective of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal is to protect human health and the environment against the adverse effects of hazardous wastes. The main objects of this convention are; 1) the reduction of hazardous waste generation and the promotion of environmentally sound management of hazardous wastes, wherever the place of disposal, 2) the restriction of trans boundary movements of hazardous wastes except where it is perceived to be in accordance with the principles of environmentally sound management, and 3) a

regulatory system applying to cases where trans boundary movements are permissible (“Basel Convention Home Page,” n.d.).

- *Rotterdam Convention, 1998*: The text of the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade was adopted on 10 September 1998 by a Conference of Plenipotentiaries in Rotterdam, the Netherlands. The Convention entered into force on 24 February 2004. Main objects of this convention are; 1) to promote shared responsibility and cooperative efforts among parties in the international trade of certain hazardous chemicals in order to protect human health and the environment from potential harm, 2) to contribute to the environmentally sound use of those hazardous chemicals, by facilitating information exchange about their characteristics, by providing for a national decision-making process on their import and export and by disseminating these decisions to parties (“Rotterdam Convention Home Page,” n.d.).

Several countries, including the USA, Japan, Canada, Australia, Brazil and several developing countries have established soil protection policies which include legislation, guidance, documents, monitoring systems, identification of risk areas, inventories, remediation programs and funding mechanisms for contaminated sites for which no responsible party can be found. The European Union launched a thematic strategy for soil protection in 2006 (European Commission 2006).

1.1.2. Radioactive pollutants

Radioactive substances are of priority concern in any list of pollutants of public interest. In fact, any environment, even pristine areas, is radioactive due to the emissions from natural materials. Environmental radioactivity and nuclear processes has been essential in the origin and evolution of the elements, the Universe, the Earth, and probably life.

Naturally occurring radionuclides of terrestrial origin (also called primordial radionuclides) are present in various degrees in all media in the environment, including the human body itself.

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Only those radionuclides with half-lives comparable to the age of the earth, and their decay products, exist in significant quantities in these materials. Irradiation of the human body from external sources is mainly by gamma radiation from radionuclides in the ^{238}U and ^{232}Th series and from ^{40}K . These radionuclides are also present in the body and irradiate the various organs with alpha and beta particles, as well as gamma rays. Some other terrestrial radionuclides, including those of the ^{235}U series, ^{87}Rb , ^{138}La , ^{147}Sm , and ^{176}Lu , exist in nature but at such low levels that their contributions to the dose in humans are small (United Nations Scientific Committee on the Effects of Atomic Radiation 2000). The main natural contributors to external exposure from gamma rays are ^{238}U , ^{226}Ra , ^{232}Th series and ^{40}K (Bhangare et al. 2014). Terrestrial radiation was divided to external and internal exposures by (United Nations Scientific Committee on the Effects of Atomic Radiation 2000).

External exposures outdoors arise from terrestrial radionuclides present at trace levels in all soils. The specific levels are related to the types of rock from which the soils originate. Higher radiation levels are associated with igneous rocks, such as granite, and lower levels with sedimentary rocks. There are exceptions, however, as some shales and phosphate rocks have relatively high content of radionuclides. There have been many surveys to determine the background levels of radionuclides in soils, which can in turn be related to the absorbed dose rates in air. The latter can easily be measured directly, and these results provide an even more extensive evaluation of the background exposure levels in different countries. All of these spectrometric measurements indicate that the three components of the external radiation field, namely from the gamma-emitting radionuclides in the ^{238}U and ^{232}Th series and ^{40}K , make approximately equal contributions to the externally incident gamma radiation dose to individuals in typical situations both outdoors and indoors.

The natural radioactive emissions from soils have anxiously increased during the last 200 years when industrial revolution has started. Anthropogenic activities such as cement production

and metal treatment industries increase the release of radionuclides into the atmosphere and soil (Mirsal 2008).

Soil pollution by artificially produced radioactivity occurs through handling, transporting, testing, and the use of nuclear materials in warfare and industry. The important sources of artificial radioactivity are nuclear power stations, atomic tests, belligerent activities, and major nuclear accidents. In addition, the contributions of atomic nuclear weapon testing and nuclear center accidents like Chernobyl are two other reasons for making polluted the nature. As reported by Hardy et al. amount of radio activity passing to the environment from weapon tests is about 1.2×10^{16} Bq of $^{239,240}\text{Pu}$ and 2.8×10^{14} Bq of ^{238}Pu respectively (Hardy et al. 1973). In other report, quantity of ^{137}Cs 9.5×10^{17} Bq was also added (United Nations Scientific Committee on the Effects of Atomic Radiation 2000). Nuclear tests nearly doubled atmospheric concentration of radioactive ^{14}C in the Northern Hemisphere before they started to decrease after the 1963 Treaty of partial ban of nuclear tests.

As a consequence of the radioactive contamination of the atmosphere due to the nuclear tests, or accidents, etc. occurs the so-called "fallout" radioactive or deposition: radioactive products in the atmosphere are deposited either dry or wet on the surface of the earth. Fallout can be global or local. After an atmospheric nuclear explosion or nuclear accidents at power plants, the fission products, the non fissioned material, and other residual material vaporized by the heat produced in the explosion start condensation generating different classes of particles. Larger "hot" particles up to some millimeters are responsible of local fall-out (usually within 24 hours from the explosion), whereas particles in the micrometer range or below that can reach the stratosphere and globally transported before deposition as long term global fallout. The dangerous effects of these particles were underestimated at the beginning of nuclear development but then their harmful effects were clearly demonstrated. Radiobiological risks of fallout are especially dangerous in the long term, due to the accumulation of

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radioisotopes (as ^{90}Sr , ^{137}Cs and radioplutoniums) in the body as a consequence of ingestion of contaminated food.

We discuss now relevant properties of the most environmentally relevant radioactive pollutants related with soil pollution.

- Radium (Ra)

Radium was discovered by Marie Skłodowska Curie, a Polish chemist, and Pierre Curie, a French chemist, in 1898. The pure element is almost colorless, but it combines with atmospheric oxygen making a black layer on its surface. All isotopes of radium are highly radioactive with half-lives much shorter than the age of the Earth. Radium is generally strongly retained by soils (unless otherwise noted, below radium refers to ^{226}Ra , the most concerned isotope). As mentioned by Austrian soil studies in cropped and plowed for 9 years low migration velocities (0.4 per year) were observed (Atwood 2010). Radium still occurs in the environment, as the isotopes ^{223}Ra , ^{224}Ra , ^{226}Ra , and ^{228}Ra are part of the decay chains of natural thorium and uranium isotopes. Radium's most stable and long lived isotope is ^{226}Ra , it has a half-life of about 1600 years. It decays into ^{222}Rn , a radioactive gas, through alpha decay. Radium had been used to make self-luminous paints for watches, aircraft instrument dials and other instrumentation, but has largely been replaced by cobalt-60, because it is less dangerous radioactive. A mixture of radium and beryllium will emit neutrons and is used as a neutron source. Lots of treatments that were used in the early 1900s, these days are not used anymore because of dangerous effects of radium some examples of these effects are anemia, cancer, and genetic mutations (Harvie 1999).

- Thorium (Th)

Thorium was discovered 150 years ago by Jons Jakob Berzelius, and chemistry of the element was subsequently developed by Gerhard Carl Schmidt and Marie Curie. Thorium is a radioactive element that occurs naturally in low concentrations (about 10 parts per million) in

the earth's crust (Atwood 2010). It is about three times as abundant as uranium and about as abundant as lead or molybdenum. Thorium in its pure form is a silvery-white heavy metal that is about as dense as lead. ^{232}Th is common isotope in the nature although there are some other isotopes in small amounts. The half-life of ^{232}Th and ^{230}Th as most concern isotopes are very long (14 billion and 77,000 years respectively). Thorium exists in earth's crust in small quantity. The chief commercial source is monazite sands in the United States Brazil, India, Australia, and South Africa. The main use of thorium is for preparation of the Welsbach mantle for portable gas lanterns, for coating tungsten wire for components of electronic equipment, for adding in ceramic ceramic items such as crucibles for increasing their heat resistance, to refractive glass to allow for smaller and more accurate camera lenses, for welding rods and electric bulb filaments to improve product performance, and finally as a fuel in nuclear reactors. Thorium naturally exists in soil, rocks, surface water, groundwater, plants, and animals at low concentrations. The mobility of thorium in soil is very low, in most soils thorium will remain strongly sorbed and the mobility is very slow. The contaminations of groundwater through the transport of thorium from soil to groundwater will not occur in most soils (Atwood 2010).

- Uranium (U)

Uranium is element found in the upper layers of the Earth's crust. Uranium exists only as unstable, radioactive isotopes which undergo a long chain of radioactive decays to end up finally as stable isotopes of lead. Uranium is widely distributed throughout the earth with deposit in many countries being of economic value. The Earth's crust contains around 2.4 mg kg^{-1} uranium (Atwood 2010). Range of uranium is from 1 to 10 mg kg^{-1} in sandstone, shale or limestone, while granite contains up to 15 mg kg^{-1} uranium (Merian et al. 2004). As mentioned by UNSCEAR the most natural uranium use in energy production industry in fission reactors and nuclear research reactors (United Nations Scientific Committee on the Effects of Atomic

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Radiation 2000). Natural uranium is formed by three isotopes: ^{238}U , ^{235}U and ^{234}U . 99,284 % of a given mass is ^{238}U , 0,711% is ^{235}U , and 0,0085% is ^{234}U . ^{238}U to ^{235}U is constant in the earth crust with particular exceptions.

Uranium decays emitting an alpha particle very slowly. The half-life of uranium-238 is approximately 4.47 billion years and uranium-235 is 704 million years. Many contemporary uses of uranium make use of its unique nuclear property. Uranium-235 is distinguished by being the only element found in nature that is a fissile isotope. Uranium-238 is fissionable by fast neutrons, and is also fertile material (which can be transmuted into a nuclear reactor in plutonium-239 which is fissile). While uranium-238 possesses a small probability of spontaneous fission or when bombarded by fast neutrons, uranium-235 is more likely to be fissioned by being bombarded by thermal neutrons, which is why the reaction is primarily responsible for the generation of heat in a nuclear reactor, and is the main source of fissile material for nuclear weapons. Both uses are possible because of the uranium's ability to sustain a nuclear chain reaction.

^{235}U is used as fuel in nuclear power plants and in some nuclear weapon designs. To produce fuel, natural uranium is separated into two portions. The fuel portion has more ^{235}U than normal, called enriched uranium, while the surplus portion has less ^{235}U than normal and it is called depleted uranium. Natural, enriched or depleted uranium is chemically identical.

Another less studied (due to analytical complexities) but interesting isotope of U is ^{236}U . This isotope is produced by the $^{235}\text{U} (n, \gamma) ^{236}\text{U}$ and $^{238}\text{U} (n, 3n) ^{236}\text{U}$ reactions in nuclear explosions and also via thermal neutron capture on either anthropogenically in nuclear power plants or in nature. Some thermonuclear devices used natural uranium tampers, other depleted uranium and, and others enriched uranium. Its half-life is $2,342 \times 10^7$ years.

-Plutonium (Pu)

Plutonium is an element with silvery bright color, after oxidation the color will change to dull gray, yellow, or olive green with atomic number 94, melting point 639.4 °C, and boiling point 3228 °C in period 7 of periodic table. It was discovered by Seaborg and his research team in 1940 in California University. Pu is a hazardous radiological element and it must be handled with specialized equipment, a few milligrams in kilogram are mortal for animals.

Among 18 different isotopes of Pu, there are five common isotopes; ^{238}Pu , ^{239}Pu , ^{240}Pu , ^{241}Pu , and ^{242}Pu and all of them are fissionable. Something is different between isotopes are their half-life.

^{239}Pu , an alpha-emitter with a half-life of 24,110 years, was used as the fuel of most of these devices, due to its fissile nature and its relatively easy production mechanism (neutron activation of the naturally-occurring ^{238}U). Therefore, this is the most important actinide present in the nuclear debris. The second most abundant one is ^{240}Pu (alpha-emitter, $T_{1/2} = 6,564$ years), produced by neutron activation on ^{239}Pu . As both radionuclides are closely related, the study of the $^{240}\text{Pu}/^{239}\text{Pu}$ atomic ratio offers very valuable information on the plutonium source, ranging from more than 0.4 for reactor-grade plutonium and high-yield nuclear tests (thermonuclear tests) to 0.01 for weapon-grade plutonium. The Southern Hemisphere houses about 20% of the global inventory of these radionuclides (United Nations Scientific Committee on the Effects of Atomic Radiation 2000) (Valković 2000).

-Radioactive potassium

There are several radionuclides of potassium with 28 known isotopes (Atwood 2010). However just there are three natural isotopes (^{39}K , ^{40}K and ^{41}K). ^{40}K is the only radionuclide of naturally occurring potassium (natural abundance: 0.012%) and because of its extremely long half-life of 1.28×10^9 years.

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Potassium-40 undergoes decay to stable ^{40}Ca 89% of the time emitting a 1.314 MeV β -particle in the process. The remaining 11% undergoes decay by positron emission, emitting a characteristic photon with a energy of 1.460 MeV, highly useful for identification and quantification of ^{40}K by γ -spectrometry.

About legal regulations on radiological protection, Spanish legislation is very demanding. For more details see the address: <https://www.csn.es/normativa-del-csn/normativa-espanola>.

Consejo de Seguridad Nuclear (CSN) has particular relevance. Among its functions, the CSN proposes to the Government the necessary regulations in the matter of nuclear safety and radiation protection, as well as the revisions of the same as it deems convenient. In addition, it adapts the national legislation to the international, especially which derived from the directives of the European Union. Likewise, it elaborates and approves the Technical Instructions, Circulars and Guides related to nuclear and radioactive installations and other activities related to the subjects of its competence. Instructions are technical standards on nuclear safety and radiation protection mandatory for affected subjects. Instructions are communicated to the Congress of Deputies (Congreso de los Diputados) before its approval by the Governing Council and subsequent publication in the Official State Gazette (Boletín Oficial del Estado, BOE).

The primary international activity in the field of multilateral relations is the participation of the CSN in the governing bodies, advisory committees and technical working groups of the International Atomic Energy Agency (IAEA) and the European Union.

At the international level within the European Union (EU), its Member States cooperate through Treaties, which are binding agreements signed by States. Among these treaties is Euratom which establishes the basic normative framework for the creation and safe development of a common nuclear industry and nuclear market.

Of particular importance is the International Atomic Energy Agency (IAEA) within the United Nations system. It was established in 1957 in Vienna (Austria) as a specialized governmental agency, with a mission to serve as a forum for scientific and technical cooperation, and to promote the contribution of nuclear energy to peace, health and prosperity in the world. To promote technological and physical safety, the IAEA develops standards of a supportive nature and promotes the achievement and maintenance of these high levels of safety and security in the peaceful uses of nuclear energy in all its Member States.

1.2. Monitoring and assessment of soil pollution

Very diverse soil pollution monitoring methods have been developed and they can be roughly classified in three different categories:

- a) Methods used as screening test to estimate overall soil quality respect to pollution impact.
- b) Standard methods required by local, regional or federal regulations for soil pollution assessment in compliance of official regulations.
- c) Research oriented methods for monitoring the progress of pollutants transfer and evolution, clean-up of the soil from the pollutants, and also for monitoring of biological, physical and chemical processes in the environment.

The complex nature of the soil matrix, and the often strong binding of pollutants with soil particles are the main difficulty in soil pollution monitoring, making it a harder analytical challenge compared to ambient water or ambient air monitoring in the context of environmental analysis. It is also relevant the difficulties to access representative samples in a solid three dimensional matrix where the pollutants tend to migrate downward and become less accessible. The most desirable techniques for soil analysis normally should be nonobtrusive, inexpensive, and easy to carry out using the field samplings instrument (Aelion 2004).

1.2.1. Soil analysis

1.2.1.1. Elemental pollutants

The determination of elemental pollutants in soil samples, within the frame of pollution assessment campaigns, implies the design of a proper sampling scheme, a variety of sample pretreatment protocols (notably homogenization and a range of extraction / digestion strategies) and final measurement of analyte concentrations. The following techniques are generally used for the determination of trace elements in single soil fractions (Hlavay et al. 2004): electrochemical techniques, atomic absorption spectrometry (GF-AAS, F-AAS), inductively coupled plasma atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectrometry (ICP-MS), neutron activation analysis (NAA), spark source mass spectrometry (SSMS), ultraviolet spectrometry (UV-Spec), X-ray fluorescence spectrometry (XRF), and atomic fluorescence spectrometry (coupled with hydride generation). More direct methods for the determination of trace elements on soil and sediment fractions are used to allow a direct assessment of the different phases in soils and the determination of trace elements bound to these particular phases. Investigation of the relevance of the results with respect to bioavailability is still under discussion.

Detailed descriptions of analytical strategies for trace element assessment in soil pollution studies are compiled in specific treaties on the subject, e.g. (Carter and Gregorich 2008) (Pansu and Gautheyrou 2006) , and reviews, e.g. (Hlavay et al. 2004) (Wei and Yang 2010).

A large number of experimental studies have demonstrated the use of the aforementioned strategies for soil pollution assessment, so we'll comment here only some recent examples. E.g. Guillén et al (Guillén et al. 2011) (Guillén et al. 2012) monitored the Cu, Zn, Cd, Ni, Pb, Cr, and As in polluted soil in the city of Huelva (Spain). Reagents were used in this research work are acetic acid, hydroxylamine hydrochloride, hydrogen peroxide, and ammonium acetate. Chemical analyses of soil samples were carried out by inductively coupled plasma mass

spectrometry (ICP-MS). Nygård et al studied the distribution of 32 Elements in Organic Surface Soils (Li, Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ge, As, Rb, Sr, Y, Zr, Mo, Ag, Cd, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Hf, Tl, Pb, and Bi) by ICP-MS too (Nygård et al. 2012). Nadal et al investigated metal pollution of soils and vegetation in an area with petrochemical industry (Nadal et al. 2004). Yaylali-Abanuz described heavy metal contamination of surface soil around Gebze industrial area, Turkey (Yaylali-Abanuz 2011). In a similar studied carried out also in Turkey, Yay et al identified 6 enriched elements as a consequence of anthropogenic activities (Al, Ca, Cr, Cu, Fe, Mg, Mn, Zn, Na, K, Pb, Ni and Cd) in urbanized soil of Ankara (Yay et al. 2008). Reagents in this research work were HNO_3 and HF. Major, minor and trace elements were analyzed by atomic absorption spectrometry (AAS). Other research groups used similar approaches, like Díaz-Somoano et al for the study of the impact of a semi-industrial coke processing plant in the surrounding surface soil (Díaz-Somoano et al. 2012).

Gowd et al measured selected trace elements (Ba, Cr, Cu, Pb, Sr, V, and Zn) in and around Jajmau (Kanpur) and Unnao industrial area in India. X-ray fluorescence spectrometry was used for analyzing the HMs in the soil samples (Srinivasa Gowd et al. 2010).

Nanos and Rodríguez Martín published a multiscale analysis of heavy metal contents in soils, particularly about the spatial variability of seven heavy metals (Cd, Cr, Ni, Pb, Zn, Cu and Hg) in the agricultural soils of the Duero river basin in Spain by optical emission spectrometry (IPC) with an ICP-AES plasma spectrometer (Nanos and Rodríguez Martín 2012). In the same study, mercury in soil extracts was determined by cold vapour atomic absorption spectrometry (CVAAS) in a flow-injection system. In this research just was used acid HCl for dissolution.

Fernández-Caliani published a risk-based assessment of multimetallic (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, V and Zn) soil pollution in the industrialized peri-urban area of Huelva, (Spain) by inductively coupled plasma-optical emission spectrometry (ICP-OES) after a multi-acid digestion by HClO_4 , HNO_3 , HCl, and HF (Fernández-Caliani 2012).

1.2.1.2. Radioactive pollutants

There is a wide variety of analytical protocols and techniques for analyzing the radioactive elements in environmental matrices such as air, water, sediment, atmospheric aerosols, biological samples (urine, feces, and human milk), and soil. Proper selection of analytical strategies depends on the type of radionuclides selected as analytes, and the type of radiation they emit. In Chile, there are not nuclear facilities of industrial level as nuclear power plants or nuclear fuel processing plants, so the optimum strategy for investigating radioactivity in soils is studying natural isotopes present in the soil matrix (gamma emitters) and radionuclides deposited through global fallout as radioplutoniums (^{239}Pu y ^{240}Pu) and ^{236}U . The appropriate analytical protocols will be described in detail in the Materials and Methods section.

1.2.2. Biomonitoring of trace elements pollution with higher plants

Biomonitoring, in the general sense, may be defined as the use of organisms and biomaterials to obtain information on certain characteristics of the biosphere. With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wolterbeek 2002).

To grow and complete the life cycle plants need accumulate macronutrients (such as N, P, K, S, Ca, and Mg) and micronutrients (such as Fe, Zn, Mn, Ni, Cu, and Mo). The uptake mechanism in plants is selective plants preferentially acquiring some ions over others. Ion uptake selectivity depends upon the structure and properties of membrane transporters, these characteristics allow transporters to recognize bind and mediate the trans-membrane transport of specific ions. The concentration of elements in plants and the effect of these elements on the metabolism of plants has been extensively studied and the basic information on many topics is available in monographs on plant physiology or plant nutrition. The metabolic fate and role of

each element in the plants can be characterized in relation to some basic processes like: 1) Uptake (absorption), and transport within a plant, 2) Enzymatic processes, 3) Concentrations and forms of occurrence, 4) Deficiency and toxicity, and 5) Ion competition and interaction. The reaction of plants to chemical stresses that are caused by both shortage and excesses of elements in the soil cannot be defined exactly because plants have developed during their evolution, ontogeny, and phylogeny several biochemical mechanisms that have resulted in adaptation to and tolerance of new or chemically imbalanced environments. Therefore, investigation should do for a particular plant-soil system. Plants have different tendencies in the uptake of elements from the soil. Three different uptake characteristics are recognized; accumulation, indication, and exclusion. Differences largely depend on specific ability of plants and huge differences in element uptake between plant species and between genotypes of a species (Kabata-Pendias 2011). There are different tendencies to the elements accumulation by vegetables. This tendency in order is (1) legumes with low tendency, (2) root vegetables with moderate tendency, and (3) leafy vegetables with high tendency (Kabata-Pendias 2011).

The role of trace elements (TEs) in plants has been studied for long time and it is a basic topic for many studies. The role of each element in plant can be characterized in relation to some basic process as mentioned by Kabata-Pendias (Kabata-Pendias 2011); 1) uptake (absorption), and transportation in a plant 2) enzymatic processes 3) concentrations and forms of occurrence 4) deficiency and toxicity and 4) ion competition and interaction. Trace elements concentrations in plants are highly associated with the chemical composition of growth media. Reaction of plants to trace elements in soils depends on different factors, In addition some general values as transfer factor (TF) expressed by plant / soil. Important source of TEs for plants are the soil or solution nutrient in general their growth media. In addition, plants can absorb TEs from aerial parts. Binding of TEs to the soil is one of most important factors for absorption them by plants. It means in general plants easily absorb the kind of TEs that dissolve in the soil solutions in either ionic or chelated and complexed forms. Root is most

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important pathway to absorb TEs. There some factors can affect the uptake of TEs by plants like, the plant specific ability, soil factors, pH, water regime, clay content, cation exchangeable capacity, soil organic matter, nutrient balance, other TEs concentration, and climate condition. There are different processes involved in mechanisms of plant adaptation to the TEs excess in growth media (Kabata-Pendias 2011), such as: Complexing and chelating of ions outside plant cells (mainly roots), binding of ions to cell walls, selective uptake of ions, immobilization in various organs as immobile compounds, including minerals, restructured influx through the plasma membrane, release by leaching from foliage, extraction from leaf tips in forms of salts, Volatilization of toxic compounds, efflux of element excess from roots, and removal of elements with falling leaves or needles.

Plants have used different mechanisms for tolerating high concentration of metals in soil. Some plants have tolerated by preventing the uptake of toxic metals uptake into root cells. These plants have little potential for metal extraction. Second group of plants have evolved specific mechanisms for detoxifying high metal levels accumulated in the cells. Third group of plants called indicators, extension of metal concentration in these plants reflect the metal accumulate in the rhizospheric soil. These types of plants normally have been used for mine prospecting to find new ore bodies (Raskin et al. 1994). Some type of plants, that can accumulate TEs above certain concentrations in their leaves are called hyperaccumulators. Hyperaccumulators should contain concentrations of trace metals in leaves higher than the values presented in (Table 1.3) (Greger 1999):

Table1.3. Minimum level of trace elements in hyperaccumulators species

<i>Element</i>	<i>Concentration in leaves (mg kg⁻¹)</i>
<i>Cd</i>	>100
<i>Co, Cu, Ni, Pb</i>	>1000
<i>Mn, Zn</i>	>10,000

Soil quality can be monitored by measuring the concentration of the pollutants directly in the soil or in deposition, by constructing models depicting the spread of pollutants or by using biomonitors. Direct measurements provide important information about the level of pollutants, but they are expensive and there is a risk of contamination when determining low concentrations. The models provide information about extensive area and they can be used to produce predictions of future evolution of soil quality. Bioindication and biomonitoring have proven to be excellent and cheap ways to observe these impacts of external factors. Biomonitoring refers to process which in that using living organisms or part of them can obtain quantitative information on environmental quality (Atabadi et al. 2010).

The term biomonitor is used to refer to an organism, or a part of it, that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations (Markert et al. 1997). Figure 1.2 depicts different exposure ways of contaminants in plants. There are two mechanisms for transportation of metals from soil to plant root; 1) convection or mass flow and 2) diffusion. Due to convection, soluble metal ions move from soil solids to root surface. From the rhizosphere, water is absorbed by roots to replace water transpired by leaves. Water uptake from rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. Some ions are absorbed by roots faster than the rate of supply via mass flow. In addition, plants have specialized mechanisms to

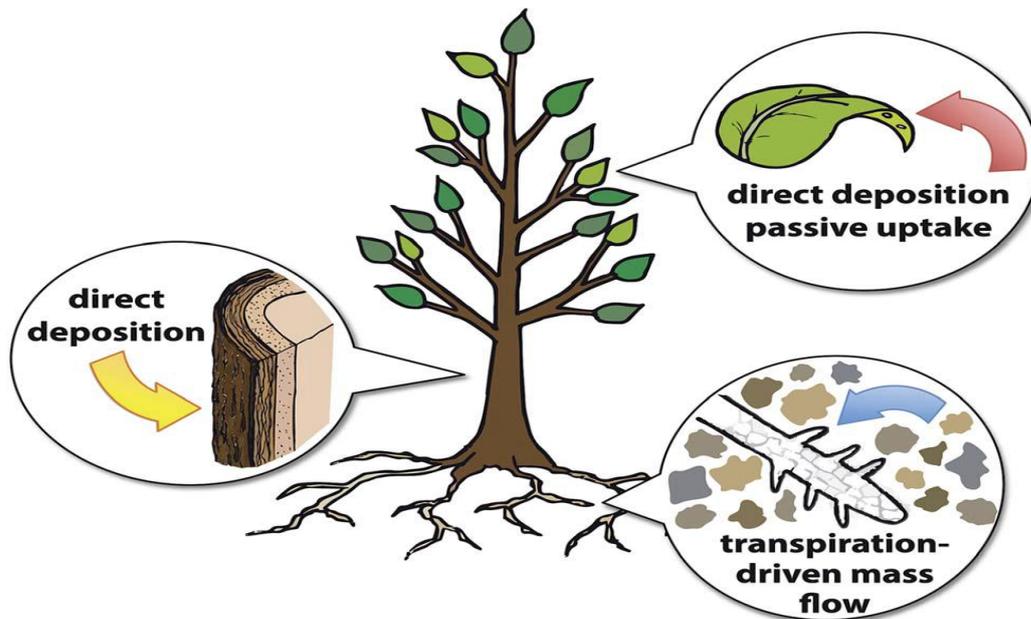


Fig.1.2. Mechanisms of concentration of environmental contaminants which plants can accumulate (Lin et al. 2015).

increase the concentration of metal ions in soil solution. For example, at low ion supply, plants may alter the chemical environment of the rhizosphere to stimulate desorption of ions from soil solids into solution. Such a mechanism is rhizosphere acidification due to H^+ extrusion from roots (Crowley et al. 1991).

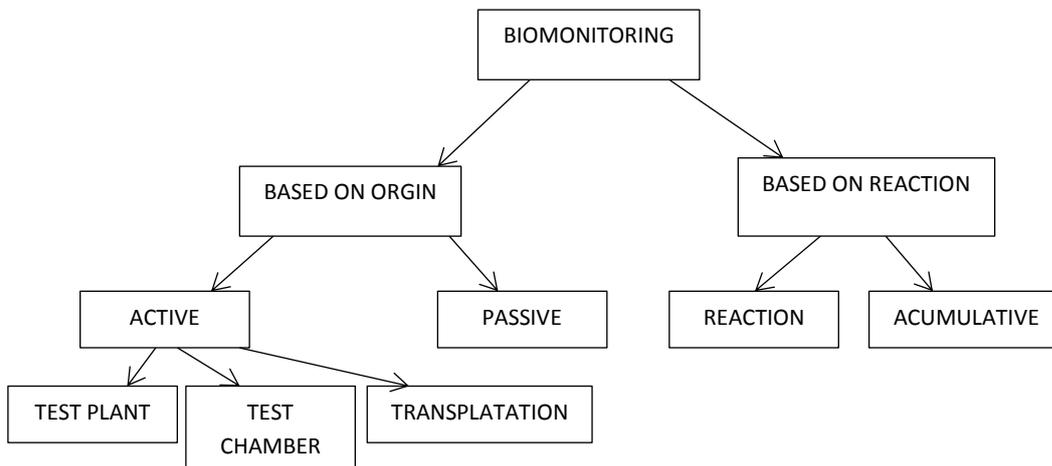


Fig.1.3. Schematic figure of heavy metal biomonitoring using different materials and sampling techniques.

Biomonitoring methods can be divided into several types (Figure 1.3). Active biomonitoring includes the exposure of well-defined species under controlled conditions and can be divided into transplantation, test plant, and test chamber methods. In the transplantation procedure organisms (mosses and lichens) are translated from unpolluted areas to the polluted site under consideration. The exposure time thus is well defined, but the change in uptake efficiency due to climatic change is usually applied to testing either synergistic or single species effects of pollutants on sensitive biomonitors. Transplant techniques appear to be useful, in particular at relatively high pollutant levels. One distinct advantage, compared to the use of indigenous species, is that of well-defined exposure time, but the reproducibility of this technique appears not to be very satisfactory for parameters such as air concentration and deposition rate (Čeburnis and Steinnes 2000). Passive biomonitoring refers to the observation or chemical analysis of native plants. In other words, in passive methods use the biological material taken from the site of growth (Markert et al. 2003).

Both lower and higher plants are used for biomonitoring. Lower plants are resistant to many substances which are highly toxic for other plants species. They are able to survive in such diverse and often extreme environment; these sedentary organisms possess an equally diverse set of physiological adaptations (Fernandez et al. 2006).

The use of higher plants, especially different outside parts (leaves and barks), for monitoring purposes is becoming more and more widespread. The main advantages are greater availability of the biological material, simplicity of species identification, sampling and treatment, harmless sampling and ubiquity of some genera, which makes it possible to cover large areas. Higher plants also exhibit greater tolerance to environmental changes which is especially important for monitoring areas with elevated anthropogenic influence (Berlizov et al. 2007).

1.2.3. Risk assessment

There are many different definition of the term environmental risk, but based on EPA definition risk is the chance of harmful effects to human health or to ecological systems resulting from exposure to an environmental stressor (stressor is any physical, chemical, or biological entity that can induce an adverse response). Stressors may adversely affect specific natural resources or entire ecosystems, including plants and animals, as well as the environment with which they interact (US EPA, ORD 2016a). Risk assessment has been used to characterize the nature and magnitude of health risks to humans and ecological receptors from chemical contaminants and other stressors that may be present in the environment (US EPA, ORD 2016b).

Based on EPA definition, the risk assessment process usually begins by collecting measurements that characterize the nature and extent of chemical contamination in the environment, as well as information needed to predict how the contaminants will behave in the future. The risk assessor evaluates the frequency and value of human and ecological exposures that may occur as a consequence of contact with the contaminated medium, both now and in the future. This evaluation of exposure is then combined with information on the inherent toxicity of the chemical (that is, the expected response to a given level of exposure) to predict the probability, nature, and magnitude of the adverse health affect that may occur. In real life, information is usually limited on one or more of these key data needed for risk assessment calculations. This means that risk assessors often have to make estimates and use judgment when performing risk calculations, and consequently all risk estimates are uncertain to some degree. For this reason, a key part of all proper risk assessments protocols is a fair and open presentation of the uncertainties in the calculations and a characterization of how reliable (or how unreliable) the resulting risk estimates really are.

In this thesis we have applied estimations for ecological and human risk derived from exposure to the soils in the surroundings of the Puchuncaví-Ventanas industrial area, so we'll describe briefly the fundamentals of both types of environmental risk assessment.

Ecological risk assessment is the process for evaluating how likely it is that the environment may be impacted as a result of exposure to one or more environmental stressors such as chemicals, land change, disease, invasive species and climate change.

Ecological risk assessments can help identify environmental problems, establish priorities, and provide a scientific basis for regulatory actions. The process can identify existing risks or forecast the risks of stressors not yet present in the environment. However, while ecological risk assessments can play an important role in identifying and resolving environmental problems, risk assessments are not a solution for addressing all environmental problems, nor are they always a prerequisite for environmental management. Many environmental matters such as the protection of habitats and endangered species are compelling enough that there may not be enough time or data to do a risk assessment. In such cases, professional judgment and the mandates of a particular statute will be the driving forces in making decisions.

Before setting up an environmental risk assessment it is important to set out problem formulation. Problem formulation is the problem being addressed and the boundaries within which any decisions on environmental risk are to be made. It can typically define the risk of what, to whom (or which part of the environment), where (location) and when (in time) (EHSC 2008).

Base on EHSC definition environmental risk assessment can be thought of as containing the following key stages: 1) hazard identification 2) identification of the consequences 3) estimation of the magnitude of the consequences 4) estimation of the probability of the consequences 5) evaluating the significance of a risk.

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There are a wide range of uses of environmental risk assessment. Although the specific methodology and the responsibility for carrying out the assessment may vary, the core principles and the key stages of the process are same in each case. There are different ranges of legislation that include the principles of environmental risk assessment in relation to chemicals. In 1998 European Environment Agency has published the list of these legislations but the area is changing rapidly and providing a complete list is almost impractical. Some examples of the use of environmental risk assessment are given below (EHSC 2008):

- Assessing the impacts of chemicals used at existing sites (for example for the Control of Major Accident Hazards (COMAH) Regulations (1999), Environmental Permitting Regulations (2010) and other similar legislation).
- Assessing the impacts of products generated by individual companies/sites due to their use or transport etc.
- Assessing potential impacts of new developments, new sites or new processes as part of the planning procedure (for example in relation to the Town and Country Planning Regulations (2011)). This is often known as Environmental Impact Assessment or EIA.
- Assessing the impacts of products, processes or services over their life cycle (life cycle assessment or LCA).
- Consideration of risks to the environment in a company's environmental management system (EMS) or eco-management and audit scheme (EMAS). Such schemes are based on continual environmental improvement in which risk assessment plays an important part.
- Registration, evaluation, authorization and restriction of chemicals regulation. Environmental risk assessment is a key component of determining the safe use of chemicals under this legislation.

In recent year general people has more concern about harmful chemicals and substances in the nature matrices like air, soil, and drinking water. Health risk assessment is a scientific tool designed to help people and government answer the basic questions like (WHO 2010):

- 1) What chemical exposures pose the greatest risks? Can the risks be ranked to allow a country to spend its resources in the most efficient way?
- 2) What are the risks of drinking this water? Should drinking-water be provided from a different, safer source?
- 3) Is this chemical spill dangerous? What is the appropriate emergency response?
- 4) Is it “safe” to build homes on this old hazardous waste site? Should we clean up this contaminated soil?
- 5) What, if any, limits on chemical exposure should be established in occupational settings, in consumer products, in environmental media and in food?
- 6) Should limits be set for chemical emissions from industrial, agricultural or other human activities?

As defined by EPA human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media. Human health risk assessment of chemicals refers to methods and techniques that apply to the evaluation of hazards, exposure and harm posed by chemicals, which in some cases may differ from approaches used to assess risks associated with biological and physical agents. In general, as defined by EPA human health risk assessment includes 4 basic steps (US EPA, ORD 2016b):

- 1) Hazard identification: Examines whether a stressor has the potential to cause harm to humans and / or ecological systems, and if so, under what circumstances,
- 2) Dose-Response assessment: Examines the numerical relationship between exposure and effects,

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3) Exposure assessment: Examines what is known about the frequency, timing, and levels of contact with a stressor,

4) Risk characterization: Examines how well the data support conclusions about the nature and extent of the risk from exposure to environmental stressors.

Human health risk assessments of chemicals can be performed to evaluate past, current and even future exposures to any chemical found in air, soil, water, food, consumer products or other materials. Normally risk assessment is limited by lack of complete information. In general, term of risk is depends on following: 1) Quantity of chemicals present in an environment matrix like air, soil, and water, 2) The amount of contact (exposure) a person has with the pollutant in the medium, and 3) The toxicity of the chemical.

1.3. Study area at Puchuncaví-Ventanas

1.3.1. General characteristics

The Puchuncaví-Ventanas industrial area is located in a district belonging to the V Region of Chile, Valparaiso Province (Figure 1.4). The area is located in the Chilean mainland coast (34° 45' S, 71° 29' W), limiting north with the municipality of Zapallar, south with the municipalities of Quillota and Quintero, west to Nogales and La Cruz and west by the Pacific Ocean (Bay of Quintero) and 58 km North from Valparaíso (Regional Capital Region V), 45 km North from

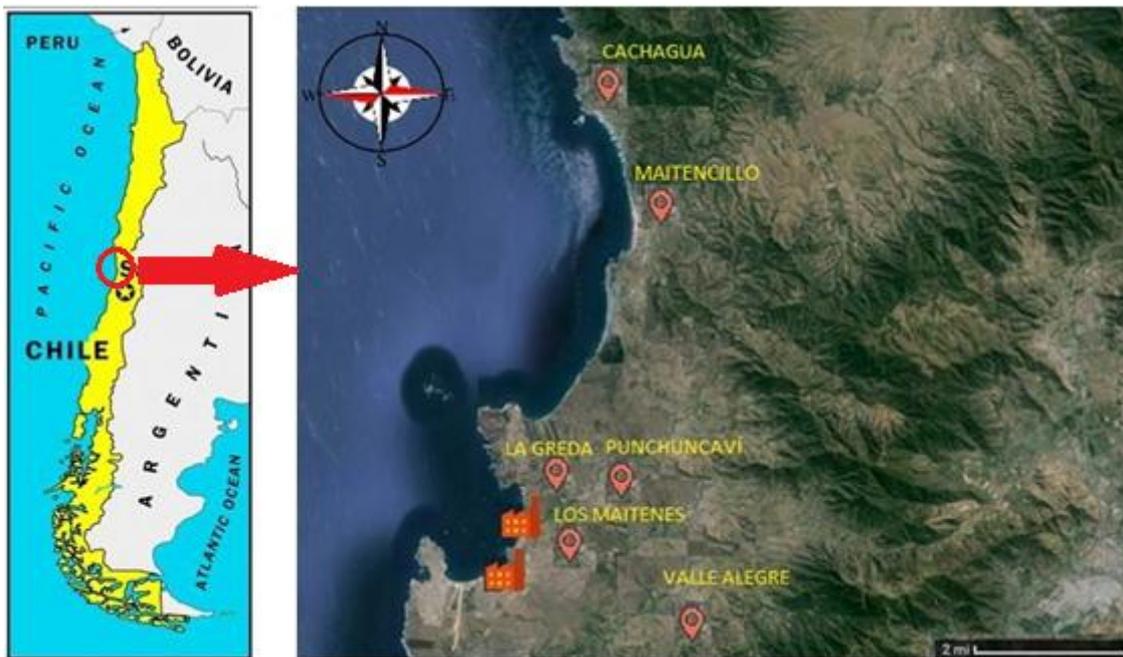


Fig.1.4. Puchuncaví-Ventanas industrial area and soil sampling points.

Viña del Mar and 160 km North-West from Santiago de Chile. It comprises an area of 301 km², with a population of 13,000. The main communication ways are the F-30E Road, Highway 5 North (via Catapilco), and Nogales-Puchuncaví way. The climate of the study area is temperate warm with winter rains, prolonged dry season (7-8 months) and high cloudiness, with the sea due to its proximity modeling the thermal contrasts. The rain has a monthly average of 3.6 mm during the autumn months, 31.1 mm during the winter months and 10 mm in the spring. It is a relatively dry region with an average annual rainfall of 370 mm which is concentrated in the months of May to August.

As mentioned by Neaman et al. (Neaman et al. 2009) soil structure in the topsoil (0-30 cm) in Puchuncaví-Ventanas is sandy loam, it included clay (13–18%), sand (65–74%) and silt (13–17%). In addition, a coarse texture that implies low nutrient availability and limited water holding capacity (Neaman et al. 2009). In general, prevalent SW winds in the zone are expected to transfer atmospheric pollutants inland.

Puchuncaví region has transferred quickly from a mainly rural area to be a region with a significant degree of urbanization, in relation to its provision of services and the presence of

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industrial sites. Since the 1950s, the sector of Ventana, located in the Bay of Quintero belonging to the region Puchuncaví, has undergone a huge capital investment. A side effect of this industrial activity has been the worsening of environmental quality conditions (Cantin 2003). The historical background referred to the area distinguish the two periods that marked the development and growth of the Industrial Complex of Ventana into the municipality. On the other hand, the regional interest in having a development and the devaluation of the traditional economy Puchuncaví led to the construction of the Industrial Complex without adequate consideration of the environmental aspect, carrying out the project without incorporating in its design components fundamental for reducing emissions into the environment (Rueda Holgado, 2015).

The increasing installation of new industrial projects on the coast of the V Region and particularly in the communes of Puchuncaví, Ventanas and Quintero, the installation of a terminal for liquefied natural gas in Quintero and a new thermal power plant and the expansion of the smelter Ventanas, are responsible for a number of negative effects on the development of agriculture and tourism, as well as a potential risk to human health (González, Sergio; Ite 1992).

Today, Quintero from the peninsula to the tip of Ventanas is developed in many important private bay and port facilities. This is a wide bay with great depth and allowing the reception of vessels over 16 meters underwater, thereby facilitating the operation with large ships. Moreover, its location in central Chile, its good communications, and willingness to grow both in area of land and sea, make this area a very attractive place for large industrial investments. Quintero Bay currently has four ports:

1. Ventanas: a multipurpose terminal for loading and unloading solid bulk and liquid bulk, general cargo, oil, copper and gold concentrate, and for disembarkation cement and asphalt.

2. Terminal Oxiquim: large hub port with loading and unloading of liquid bulk such as fuel and chemicals.
3. The R.P.C.: port has three terminals: the L.P.G., for landing liquefied gas, Multicrudo for landing the oil and Monoboya for crude oil.
4. The port center of Muelle Asimar: characterized by being the only dock capable sector support higher weights.

1.3.2. Pollution sources

As previously mentioned, Puchuncaví-Ventanas is one of the main industrial areas of Chile, comprising a wide range of industrial factories and activities with potential risks to human and environmental health. We describe now the most important facilities in the Industrial Complex (Fig 1.5)(Rueda Holgado, 2015):



Fig.1.5. industrial factories in Puchuncaví-Ventanas

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1. AES GENER S.A. – CENTRAL VENTANAS

Ventanas thermoelectric plant (Fig 1.6) has two generating units coal-fired power with a total electrical output of 338 MW. Each unit is connected to an electrostatic precipitator three fields which retains suspended in the exhaust flow of exhaust ducts, reducing emissions of particulate matter into the atmosphere. Coal reception is via Puerto Ventanas SA, who leases its facilities to AES GENER for receiving coal through bulk cargo ships and transport to the



Fig.1.6. AES GENER S.A.

storage area is covered by conveyor belt. The company sells part of the received coal to external customers, in addition to dispatch of the fuel to the Laguna Verde plant.

2. CODELCO DIVISIÓN VENTANAS

It is a copper smelter and refinery (Fig. 1.7) that produces copper cathodes, gold bullion and silver shot. Production of company was 1,792,000 metric tons of electrolytic copper in 2013.

Also, for the processing of anode sludge refinery plant in noble metals, there were 150,952 kg of silver and 5,649 kg of gold; and the acid plant produced 351,537 tonnes of sulfuric acid.



Fig.1.7. CODELCO DIVISIÓN VENTANAS

3. COMMERCIAL CATAMUTÚN

It is a company divided into two areas: coal division and division steam. The coal division is engaged in the import and distribution of thermal coal. Ships can be supplied from 40 to 55,000 tons at rates of 8 to 15,000 tonnes per day.

4. ENAP TERMINAL QUINTERO

In the Quintero terminal of the National Oil Company, ENAP, crude oils that are imported and transported by marine vessels in the Bay of Quintero are unloaded and transported by submarine to ponds that are installed in the terminal pipelines are stored, subsequently sent to the refinery Concon.

5. HARBOUR OF VENTANAS S.A.

The loading and unloading of copper concentrate involve manipulating it, not only through conveyor belts but also for crane operation, exposed to the wind. The possibility of falling overboard and consequent sediment contamination is evident, which makes it advisable to install a protection system to prevent fugitive dust.

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6. MELÓN CEMENT

This company has within the port areas of Port of Ventanas with a dome cellar for storing clinker-bauxite, compounds used for the manufacture of cement.

7. OXIQUIM S.A.

This company has two establishments in the Ventanas area, a maritime terminal that discharges liquid granules (methanol, styrene, ethyl acetate, etc.) through discharge ducts installed in its quay and an establishment of grinding of granular substances, Located in the northern sector of the bay of Quintero, immediately in the south of the Port of Ventanas.

8. COPEC

The company COPEC has in operation in the area of Ventanas a plant of lubricants, inside which are carried out operations of heating of fluids.

9. PACSA

The fuel and asphalt terminal, PACSA, was installed in 1999.

10. GASMAR S.A.

Gas terminal that regularly provides services to the butane and propane volumes with which ENAP supplies by sea the demands of its customers in central Chile. Under this modality, it offers ship unloading and loading services, product storage and its dispatch to the SONACOL pipeline.

11. NEW THERMOELECTRIC POWER PLANT

Corresponds to the construction and operation of a monoblock thermoelectric generating unit designed to consume solid fuels, coal and / or petroleum coke, by means of a circulating fluidized bed technology boiler.

12. TERMINAL OF LIQUID NATURAL GAS, LNG

It is the reception, storage and regasification terminal for Liquefied Natural Gas (LNG), which operates in Quintero Bay and supplies natural gas to central Chile.

Some environmental impact assessment studies have already been approved, including the following companies: Lafarge, Petcoque Macovensa collection, cement mill, new thermoelectric plant Ventanas III, expansion of the Ventanas smelter, thermoelectric plant Endesa and thermoelectric plant Campiche.

1.3.3. Previous pollution assessment studies

1.3.3.1. Soils

González and Bergqvist determined heavy metals contents and their spatial distribution patterns in soil within the Quintero and Puchuncaví municipalities (Valparaíso Province, V region) (González and Bergqvist, 1986). The same research group reported changes of similar metal contents concentration in soil samples from same area after 8 years to evaluate changes (González and Ite 1992). De Gregori et al carried out the redox speciation of selenium present at ultratrace levels (De Gregori et al. 2002). The same group conducted a work aimed to the monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile including the Puchuncaví-Ventanas industrial area (De Gregori et al. 2003). Ginocchio et al. reported Cu, Zn, Pb and Cd concentrations in different soil layers around the area (Ginocchio et al. 2004). Copper mobility in soil around the copper smelter was investigated by (Neaman et al. 2009). The same author reported results about the effectiveness of lime and compost of in situ immobilization of trace elements in soil by using earthworms as bioindicators of toxicity (Neaman et al. 2012). Parra et al. studied trace elements distribution in particle size fractions for contaminated soil in this industrial area (Parra et al. 2014). Bernalte et al. monitored mercury in soils affected by industrial emissions in the area (Bernalte et al. 2015).

1.3.3.2. Native plants

Ginocchio investigated the effects of the copper smelter plant on local vegetation and grassland in the industrial Puchuncaví Valley (Ginocchio 2000). De Gregory et al. monitored copper and selenium concentrations in rainwater, soils and *alfalfa* samples at various sites from three different zones of Valparaiso region (De Gregori et al., 2000). González et al. determined copper concentrations in 22 different native species near the Ventanas smelter in order to compare copper concentration levels (González et al. 2008a). Meier et al. studied some LMWOA (Low Molecular Weight Organic Acids) root exudation pattern and Cu accumulation capability in shoot and root tissues of two native Chilean metallophytes (*Oenothera affinis* and *Imperata condensata*) and agricultural plants (*Lupinus albus* and *Helianthus annuus*) (Meier et al. 2010a). Muena et al. evaluated the effects of liming and nitrogen fertilization on its cotyledon emergence, survival, biomass production and copper accumulation (Muena et al. 2010a). González et al. evaluated the effects of the biodegradable chelate of methylglycinediacetic acid (MGDA) on copper extraction by *Oenothera picensis* species (González et al. 2011).

1.3.3.3. Analysis of radionuclides

Studies on radioactivity in soils in South America are much scarcer than those in other areas of the planet, especially in the northern hemisphere. However, we can mention Malanca et al. in Brazil (Malanca et al. 1996), Sajó-Bohus et al. in Venezuela (Sajó-Bohus et al. 1999), Ayub et al. in Argentina (Ayub et al. 2008) and O. Moure in Uruguay (Odino Moure et al. 2010). These authors report values of natural radionuclides such as ^{226}Ra , ^{232}Th and ^{40}K in soils.

To date, the distribution of plutonium isotopes in the Northern Hemisphere is well understood. However, the published information on the Southern Hemisphere is very scarce. The most important data base is due to the Department of Energy of United States (DOE), which in 1973 started to study the presence of $^{239,240}\text{Pu}$ in undisturbed soils from different

areas of Africa and South America within a global scale project aimed at determining the worldwide $^{239,240}\text{Pu}$ inventory (Hardy et al. 1973). Later on, the same samples were re-evaluated for $^{240}\text{Pu}/^{239}\text{Pu}$ atomic ratio determination purposes and for ^{237}Np studies (Kelley et al. 1999).

In addition, a much more recent work was developed by the CNA of Seville, and the University of Extremadura. In this last work new data on the impact of nuclear tests to South America through the study of ^{239}Pu and ^{240}Pu in soils from different areas of Chile was provided (Chamizo et al. 2011). On the other hand, and to best knowledge of the author, the present work is the first study on ^{236}U concentrations and $^{236}\text{U}/^{239}\text{Pu}$ atom ratios in the Southern America.

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CHAPTER 2.

MATERIALS and METHODS

2. Materials and methods

2.1. Materials

2.1.1. Trace elements in soil and plants

2.1.1.1. Equipments and reagents

Equipments

➤ *Planetary milling*

Dried soil and plant sub-samples were mechanically homogenized in a Planetary Mono Ball Mill model PULVERISETTE 6 (FRITSCH) (Fig.2.1). Technical data:

- Grinding bowls (80, 250, 500 ml) and grinding balls (0.1 - 40 mm).
- Rotational speed of main disk is between 100-650 rpm.

➤ *Microwave*

A MARS 5 (CEM Corporation, Matthews, NC, U.S.A.) microwave was used for digestion of the plant samples (Fig.2.2). The characteristics of this equipment are:

- Power between 0 and 1200 W.
- Internal cavity coated of fluoropolymer.
- RTP-300 plus temperature sensor.
- ESP-1500 plus pressure sensor.
- Ventilation of the cavity with air extraction.
- PerfectCircle™ rotor with capacity for 15 vessels.



Fig.2.1. Planetary milling machine



Fig.2.2. Microwave MARS 5

Materials and methods

➤ *Inductively coupled plasma mass*

spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometer (ICP-MS) model ELAN 9000 (PerkinElmer, Waltham, Massachusetts, USA) was used for determining complete profiles of elements in samples of soil and plants. The main components



Fig.2.3. ICP-MS PerkinElmer ELAN 9000

of this equipment are (Fig.2.3):

- Auto sampler model ASX-520, which works under laminar flow hood Telstar Mini H.
- Automatic peristaltic pump.
- Cross-Flow nebulizer resistant to HF and blockage due to particles or suspended solids.
- Scott chamber, as spray chamber to separate and discard the drops size exceeding 10 microns generated during nebulization, so that only small drops that remain in suspension in the gas flow are carried into the plasma.
- Quartz torch and RF coil for plasma generation.
- Cones (sampling and skimmer) made of nickel.
- Lenses responsible for leading the generated ions to the mass spectrometer.
- Quadrupole mass spectrometer made of gold-coated ceramic material.
- SimulScan detector, which works in two stages and has a similar operation as photomultipliers, transforming the count produced by ions into measurable electrical signals.
- Vacuum system consisted of two pumps (turbo molecular and mechanical), models DS602 and DS302 (Varian, USA).
- Polyscience cooling system (PerkinElmer, Waltham, Massachusetts, USA).
- Elan® software version 3.4 that controls all system components to carry out the analysis in an almost completely automatic manner.
- Air Liquide manual reset gas center, CLSA-1 model, which provides a continuous supply of gas

to a circle by automatic transition from argon source in use, nearly exhausted, to the standby source.

Other equipments

- Polypropylene Fritsch 2 mm stainless steel mesh.
- Microbalance Mettler Delta Range® Toledo model AX205 with an accuracy of 10 mg.
- Teflon digestion vessels (Savillex, USA).
- Nitric acid distiller quartz, provided by Kürner (Rosenheim, Germany).
- Ultrapure water generator system, model Wasselab Ultramatic (Navarra Water Treatment s.l., Pamplona, Spain).
- Pipettes Socorex, model ACURA 535 and ACURA 825 (Lausana, Switzerland).
- Digging tool and containers for collecting soil samples, all made of polypropylene materials.

Main reagents

- HNO₃ suprapure distilled from PA-ACS-ISO nitric acid (Panreac, Barcelona, Spain).
 - HF Panreac Hiper Plus, (Barcelona, Spain).
 - HClO₄ suprapure 70% degree (Merck, Darmstadt, Germany).
 - HCl 35% degree Hiper Plus (Panreac, Barcelona, Spain).
 - In (III) standard solution, quality ICP (Scharlab, Spain): 1 g L⁻¹ in nitric acid 5%.
 - Y (III) standard solution, quality ICP (Scharlab, Spain): 1 g L⁻¹ in nitric acid 5%.
 - Ge (II) standard solution, quality ICP (Scharlab, Spain): 1 g L⁻¹ in nitric acid 5%.
 - Montana Soil Standard Reference Material ® 2710a Material from the U.S. National Institute of Standards and Technology (NIST).
 - Tomato Leaves Standard Reference Material ® 1573a from the U.S. National Institute of Standards and Technology (NIST).
 - Rye Grass Standard Reference Material ® 281 from the European Community Bureau of Reference (BCR).

2.1.2. Radioactive pollutants in soil

2.1.2.1. Equipments and Reagents

Equipments

- *Hot plate*
- *TEVA[®] and UTEVA[®] cartridges (Fig.2.4).*
- *Vacuum box (Fig.2.5).*
- *Press machine (Fig.2.6).*



Fig.2.4. TEVA and UTEVA cartridges

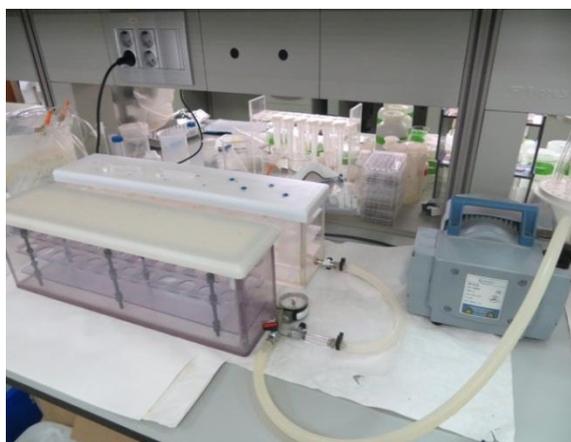


Fig.2.5. Vacuum box



Fig.2.6. Press machine

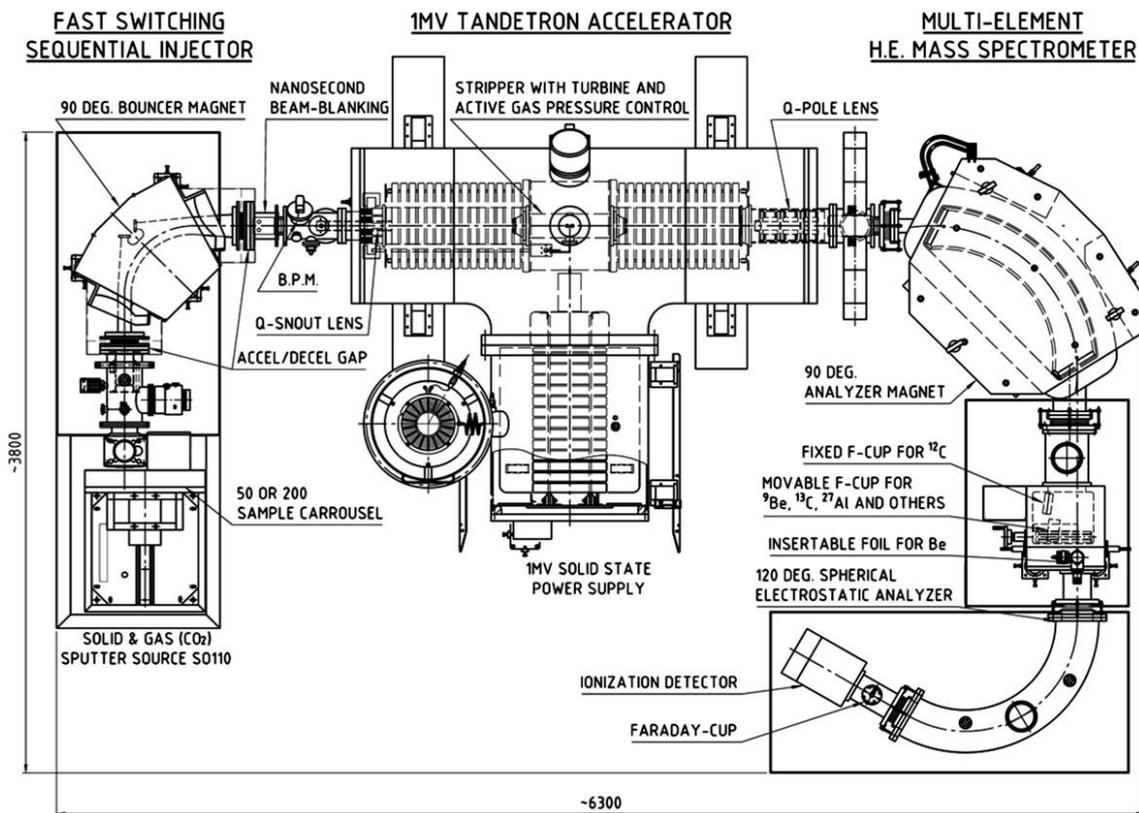


Fig.2.7. Main components of the 1 MV AMS system at the CNA

➤ Accelerator mass spectrometry (AMS)

1 MV Accelerator mass spectrometry (AMS) system at Centro Nacional de Aceleradores de Sevilla (CNA) was used for analyzing ^{236}U and $^{239,240}\text{Pu}$ in samples of soil. The main structure components of this equipment show in Fig.2.7.

Compared to other compact AMS facilities, the HVEE 1 MV system at CNA stands out due to its original and versatile ion-optical design. Thanks to the Q-Snout lens placed at the entrance of the low-energy acceleration tube, a beam waist can be formed in the middle of the stripper channel keeping the extraction energy from the ion source constant, usually at 35 keV; after the acceleration process, the focusing properties of the ion beam can be adjusted for any selected charge state to allow an optimized beam transport thanks to an electrostatic quadrupole triplet lens placed at the exit of the accelerator (Fig.2.7).

Plutonium isotopes ($^{239,240,242}\text{Pu}$) are injected into the accelerator as $^x\text{Pu}^{16}\text{O}^-$ at 35 keV from a cathode containing plutonium oxide dispersed in an iron oxide matrix mixed with aluminum

Materials and methods

powder. After acceleration processes the Pu^{3+} ions arrive at the detector with energy of 2.7 MeV, and are counted from the total energy signal provided by the first anode of the gas ionization detector. To achieve this, the counting gas (isobutene) pressure is increased to 6 mbar in order to stop all the ions under the first anode. Plutonium counts are almost completely resolved from the ones associated with molecular fragments with the same M/q ratio in 2^+ charge state when a 75 nm thick $8 \times 8 \text{ mm}^2$ silicon nitride window is used (Chamizo et al. 2008b) (Chamizo et al. 2008a).

Uranium isotopes are measured using a similar technique for plutonium isotopes. Uranium isotopes are extracted from the Cs- sputter ion source as UO^- ions (e.g. $^{236}\text{U}^{16}\text{O}^-$). The sample material consists of uranium oxide dispersed in an iron oxide matrix, which is mixed with niobium at a Fe:Nb mass ratio of 1:3. After extraction, the negative ions are mass-analyzed by the low-energy magnet (LEM); accelerated to the high-voltage terminal of the accelerator operating at about 670 kV where the molecular ions are dissociated and the uranium stripped to U^{3+} ; reaccelerated and, finally, mass, charge and energy analyzed by the high-energy spectrometer. Ar gas is used as a stripper. The high-energy spectrometer consists of a 90° magnet (high-energy magnet, HEM) followed by a 120° electrostatic analyzer (ESA), both with radii of 85 cm. The ESA is followed by a gas ionization chamber (GIC) and the total energy signal from this is used to count the minor uranium isotopes at about 2.7 MeV energy. The samples are spiked with ^{233}U ($T_{1/2} = 1.59 \times 10^5 \text{ y}$) when the uranium concentration in the sample has to be determined. The major isotopes (e.g. $^{238}\text{U}^{3+}$) are detected as beam currents in the movable off-axis Faraday cup (FC3) placed at the image position of the HEM. The uranium isotopes are injected and analyzed sequentially using the so-called Slow Sequential Injection Analysis System (SSI) software. More details can be found in (Chamizo et al. 2015).

➤ **Gamma spectrometry**

The equipment used in gamma spectroscopy (Fig.2.8) includes an energy-sensitive radiation detector, electronics to process detector signals produced by the detector, such as a pulse sorter (i.e., multichannel analyzer), and associated amplifiers and data acquisition data readout devices to generate, display, and store the spectrum. Other components, such as rate meters and peak position stabilizers, may also be included.

We have used a high-purity germanium detector. Gamma spectroscopy detectors are passive materials that wait for a gamma interaction to occur in the detector volume. The most important interaction mechanisms are the photoelectric effect, the Compton Effect, and pair creation pair production. The photoelectric effect is preferred, as it absorbs all of the energy of the incident gamma ray. Full energy absorption is also possible when a series of these interaction mechanisms take place within the detector volume. When a gamma ray undergoes a Compton interaction or pair production, and a portion of the energy escapes from the detector volume without being absorbed, the background rate in the spectrum is increased by one count. This count will appear in a channel below the channel that corresponds to the full energy of the gamma ray.

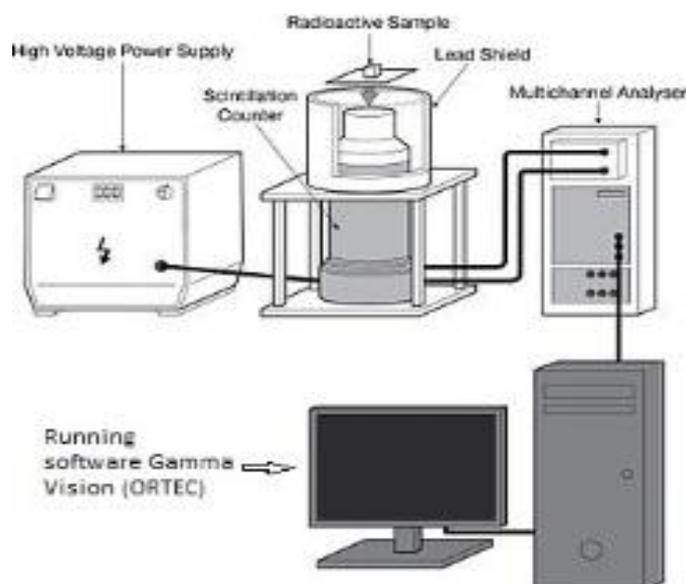


Fig.2.8. Schematic view of gamma spectrometry equipment.

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The voltage pulse produced by the detector is shaped by a PREAMPLIFIER. The PREAMPLIFIER takes the very small voltage signal produced by the detector, reshapes it into a Gaussian shape. Then the signal is amplified by the AMPLIFIER. Next the analog-to-digital converter (ADC) converts that signal into a digital signal. Next the multichannel analyzer (MCA) classifies the receiving signals creating a spectrum that represents counts versus channels (or energy). The number of channels can be changed in most modern gamma spectroscopy systems by modifying software or hardware settings. The number of channels is typically a power of two; common values include 512, 1024, 2048, 4096, 8192, or 16384 channels. The choice of number of channels depends on the resolution of the system and the energy range being studied; in our case was 4096 channels.

The multichannel analyzer output is sent to a computer, which stores, displays, and analyzes the data. A variety of software packages are available from several manufacturers, and generally include spectrum analysis tools such as energy calibration, peak area and net area calculation, and resolution calculation.

Gamma rays detected in a spectroscopic system produce peaks in the spectrum. These peaks can also be called lines by analogy to optical spectroscopy. The width of the peaks is determined by the resolution of the detector, a very important characteristic of gamma spectroscopic detectors, and high resolution enables the spectroscopist to separate two gamma lines that are close to each other. Gamma spectroscopy systems are designed and adjusted to produce symmetrical peaks of the best possible resolution. The peak shape is usually a Gaussian distribution. In most spectra the horizontal position of the peak is determined by the gamma ray's energy, and the area of the peak is determined by the intensity of the gamma ray and the efficiency of the detector.

The most common figure used to express detector resolution is full width at half maximum (FWHM). This is the width of the gamma ray peak at half of the highest point on the peak distribution. Resolution figures are given with reference to specified gamma ray energies.

Not all gamma rays emitted by the source that pass through the detector will produce a count in the system. The probability that an emitted gamma ray will interact with the detector and produce a count is the efficiency of the detector. High-efficiency detectors produce spectra in less time than low-efficiency detectors. In general, larger detectors have higher efficiency than smaller detectors, although the shielding properties of the detector material are also important factors. Detector efficiency is measured by comparing a spectrum from a source of known activity to the count rates in each peak to the count rates expected from the known intensities of each gamma ray.

The determination of gamma-emitting radioisotopes in samples of soil was carried out using a gamma spectrometry system of Laboratorio de Radiactividad Ambiental de la Universidad de Extremadura (LARUEX). Radioactivity measurements were carried out with a Ge (HP) semiconductor detector, 45% relative efficiency at 1.33 MeV, coupled to a 4096-channel analyser (Fig.2.9).



Fig.2.9. Equipment of high resolution gamma spectrometry

Main reagents

- ^{242}Pu was obtained from a standard solution supplied by National Physical Laboratories (NPL, England).
- ^{233}U was obtained from a standard solution provided by International Atomic Energy Agency Environment Laboratories in Monaco (IAEA-EL).
- HNO_3 Ultra-pure 8 M, (HPS, England).
- HCl Ultra-pure 9 M (HPS, England).
- H_2O_2 with PA purity (HPS, England).
- NaNO_2 with PA purity (HPS, England).
- $\text{Fe}(\text{OH})_3$ with high purity standards (HPS, England).
- NH_3 Suprapur.
- Fe^{3+} (pure iron) standard solution (HPS, England).
- Niobium powder 98% (ALDRICH, Germany).
- IAEA-410, reference material (sediment from Bikini atoll) for ^{236}U and $^{239,240}\text{Pu}$ analysis
- Cocktail QCY-48 supplied by Amersham for calibrate the detector efficiency

- IAEA-6-SOIL reference material for gamma spectrometry analysis.

2.2. Methods

2.2.1. Trace elements in soil and plant samples

2.2.1.1. Sampling and determination of trace elements in soil

Soil samples were analyzed for monitoring trace elements in this research carried out during five monitoring campaigns in winter 2007, 2008, 2009, 2010 and 2011. Main characteristic and location of the sampling areas within and around the Puchuncaví-Ventanas industrial complex were mentioned in (Table 2.1).

Table 2.1. Characteristics of sampling areas (Geographical coordinates, distance, and pH)

<i>Station</i>	<i>Latitude - Longitude</i>	<i>Distance From Industrial area (km)</i>	<i>pH</i>
La Greda(LG)	32° 44' 57" S - 71° 28' 30" W	1.69-NE	6.8 - 8.3
Los Maitenes (LM)	32° 45' 41" S - 71° 27' 18" W	2.39-E	4.4 - 5.9
Puchuncaví village (PU)	32° 43' 17" S - 71° 24' 43" W	8-NE	6.4 - 6.7
Valle Alegre (VA)	32° 48' 30" S - 71° 26' 10" W	6.72-SE	6 - 6.3
Maitencillo (MA)	32° 36' 5" S - 71° 25' 56" W	18-N	6.8 - 8.3
Cachagua (CH)	32° 34' 52" S - 71°26' 55" W	20.3-N	6.8- 8.3

These activities are in the frame of a large scale and long term ongoing environmental monitoring program in the area. Five samples were taken at randomly selected points around each designed sampling zone for each sampling campaign. To take soil samples preparing the storage containers of samples, such as sampling tools, buckets, shovels, sifters was necessary and utensils collection which are inert material (polypropylene) not to interfere in subsequent analyzes. The preparation consisted of a bath of 24 hours in 10% nitric acid, then rinsing with abundant water, then washing with distilled water and finally finished with acetone wash. The soil sampling was done after a week. Each place had their sampling tools to avoid cross contamination. The sampling protocol is as follows:

- Selection and demarcation of a square meter of soil (Fig.2.10).



Fig2.10. Measuring 1 m² of soil

- Clear sampling surface area such as removal leaves, roots, and seeds (Fig.2.11).



Fig.2.11. Cleaning the surface of soil

- Drilling of the sampling area with a depth of 5 cm (Fig.2.12).



Fig.2.12. Drilling the soil

- Homogenization of soil in the area demarcated for sampling.



Fig.2.13. Gathering about 3 kg soil

- Collection approximately 3 kg sample in a bucket previously treated and identified. The bucket is covered with plastic film to avoid any interference (Fig.2.13).
- Soil samples were finally transferred to the respective laboratory for treatment and subsequent analysis.

Materials and methods

In the laboratory, soil samples were appropriately dried in stove by heating at 50 °C for 72 h and then sieved through a polypropylene 2 mm mesh. Soil subsamples were mechanically homogenized in a planetary mill at 500 r.p.m for 15 min and manually sieved to 0.2 mm using a stainless steel mesh. The samples were then stored in the fridge (4 °C) until analysis.

50 mg of soil and reference material samples were placed in Teflon digestion vessels (Savillex, USA). 2.5 mL HNO₃ and 5 mL HF were added and the mix was left to react for some minutes. The vessels were then closed and heated to 90 °C in the stove for 8 h. After cooling, the vessels were opened, 2.5 mL HClO₄ was added and the solution was evaporated to dryness on a plate at 200 °C. 1 mL HNO₃ was then added and the solution was again evaporated to dryness. The samples were finally taken with 2.5 mL HNO₃ and water was added to a total volume of 50 ml. 50 µL of a 10 mg L⁻¹ In (III) and Y (III) solution was used as internal standards. Montana Soil Reference material (NIST code 2710a) was used to assess the accuracy of the experimental results.

After digesting process, the concentrations of heavy metals in the soil samples were determined by a standard ICP-MS protocol for As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba by equipment PerkinElmer ELAN 9000 (Waltham, Massachusetts, USA). Equipment conditions and the measurement parameters used in the analytical determination were 1000 W RF power, carrier gas flow of 1 L min⁻¹; voltage lenses 7.25 V, wash time between samples of 35 s to obtain three replicates of each sample.

2.2.1.2. Assessment of potential ecological risk

➤ *Geoaccumulation index (I_{geo}):*

A common approach to estimating the enrichment of metal concentrations above background or baseline concentrations is to calculate the geoaccumulation index (I_{geo}) (Loska et al. 2004).

The index is built on the background level of natural fluctuations including very low

anthropogenic input. I_{geo} has been widely applied to several trace elements in Europe (Yaylali-Abanuz 2011). This index is expressed in Eq. (1):

$$I_{geo} = \log_2 (C_n / 1.5 B_n) \quad (1)$$

Where C_n is the measured concentration of element “n” in the soil sample, B_n is the geochemical background value of element n in the upper Earth's crust (Wedepohl 1995), and the factor 1.5 is included to consider the natural fluctuation of the concentration of a given substance. I_{geo} values are categorized in seven groups as follows: Practically uncontaminated ($I_{geo} \leq 0$); uncontaminated to moderately contaminated ($0 < I_{geo} < 1$); moderately contaminated ($1 < I_{geo} < 2$); moderately to heavily contaminated ($2 < I_{geo} < 3$); heavily contaminated ($3 < I_{geo} < 4$); heavily to extremely contaminated ($4 < I_{geo} < 5$) and extremely contaminated ($I_{geo} < 5$).

➤ *Enrichment factor (EF):*

A common approach to estimating the anthropogenic impact on natural matrices is to calculate a normalized enrichment factor (EF) for metal concentrations above uncontaminated background levels. It is a convenient tool for plotting geochemical trends across large geographic areas, which may have substantial variations in the soil. The EF method normalizes the measured heavy metal content with respect to a sample reference metal such as Fe, Sc, Ti, Al, Ca, or Mn (Abraham and Parker 2007). Mn was selected as a reference element in this study. The value of the enrichment factor was calculated according to Eq. (2):

$$EF = [C_n (\text{sample}) / C_{ref} (\text{sample})] / [B_n (\text{background}) / B_{ref} (\text{background})] \quad (2)$$

Where C_n (sample) is the concentration of element n in the sample, C_{ref} (sample) is the concentration of the reference element (Mn) in the sample, B_n (background) is the concentration of element n in the upper Earth crust (Wedepohl 1995), and B_{ref} (background) is the concentration of the reference element in the Earth crust.

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Enrichment factor values are grouped in five categories: Deficiency to minimal enrichment ($EF < 2$); Moderate enrichment ($2 < EF < 5$); Significant enrichment ($5 < EF < 20$); Very high enrichment ($20 < EF < 40$) and extremely high enrichment ($EF > 40$) (Sutherland 2000).

➤ *Contamination factor (C_f), contamination degree (C_{deg}), and integrated pollution index (IPI):*

The assessment of soil contamination was also carried out using the contamination factor and degree. As mentioned by Håkanson (Hakanson 1980), C_f is ratio between the concentration of selected pollutants (PCBs, Hg, Cd, As, Cu, Cr, Zn, and Pb) in sediment samples and reference preindustrial concentration values. It has been later adapted to the study of soil pollution by using upper crust elemental concentrations (Wedepohl 1995) as reference values. In our case, PCBs were not measured and for this reason we've replaced this parameter by Sb because its lethal dose (LD_{50}) is in same range PCBs. So they both can have similar eco toxic effect. C_f is expressed by Eq. (3):

$$C_f = C_n (\text{sample}) / C_n (\text{background}) \quad (3)$$

C_f was divided into four categories: Low contamination ($C_f < 1$); Moderate contamination ($1 \leq C_f < 3$); Considerable contamination ($3 \leq C_f < 6$) and very high contamination ($C_f > 6$) (Hakanson 1980).

The sum of contamination factors for all elements examined represents the contamination degree (C_{deg}) of the environment. Eq (4) presented equation for calculating C_{deg} :

$$C_{deg} = \sum C_f \quad (4)$$

C_{deg} is divided into four groups as C_f : Low contamination ($C_{deg} < 8$); Moderate contamination ($8 \leq C_{deg} < 16$); Considerable contamination ($16 \leq C_{deg} < 32$) and very high contamination ($C_{deg} > 32$).

The integrated pollution index (IPI) is calculated as the average of the contamination factors calculated for each sample (Yaylali-Abanuz 2011). IPI results are divided in four categories Low: pollution level ($IPI \leq 1$); Moderate pollution level ($1 < IPI \leq 2$); High pollution level ($2 < IPI \leq 5$) and extremely high pollution level ($IPI > 5$).

2.2.1.3. Assessment of potential human risk

➤ *Exposure doses:*

Residents living in Punchuncavi Valley are potential receptors of pollutions. Due to the difference on their age and pollutions contact, we divided the subjects into three groups: children, adults, and shepherd / Farmer. Generally, exposure of soil pollutions can occur via three main paths: (1) ingestion of soil due to their deposition (2) dermal absorption of trace elements in soil adhered to exposed skin, and (3) direct inhalation of soil through mouth and nose. Exposure doses for ingestion, dermal, and inhalation pathways were calculated by Eq (5), (6), and (7) respectively. Ingestion absorption:

$$ADI_{ing} = [(C_s \times IngR \times EF \times ED) / (BW \times AT)] \times 10^{-6} \quad (5)$$

Where ADI_{ing} is the average daily intake of the element (i) from the soil ($mg\ kg^{-1}$ of body weight-day); C_s is the concentration of the element in soil ($mg\ kg^{-1}$); $IngR$ is the ingestion rate of soil for adults ($100\ mg\ day^{-1}$), for shepherd or farmer adults ($330\ mg\ day^{-1}$), and for children ($200\ mg\ day^{-1}$); EF is the exposure frequency ($350\ days\ year^{-1}$); ED is the exposure duration to non-carcinogenic pollutants for adults (30 years) and for children (6 years), and for carcinogenic for adults (24 years) and for children (6 years); BW is the average body weight of the exposed person for adults (70 kg) and for children (15 kg); and AT is the time period over which the dose is averaged for non-carcinogenic for adults (10,950 days) and for children (2,190 days) (Ordóñez et al. 2011). About C_s values, we averaged the concentration of the

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element in the samples collected at each sampling location during the yearly 2007-2011 campaigns. Dermal absorption:

$$ADI_{der} = [(C_s \times SA \times SL \times ABS \times EF \times ED) / (BW \times AT)] \times 10^{-6} \quad (6)$$

Where ADI_{der} is the average daily intake of elements from dermal absorption (mg kg^{-1} of body weight-day), C_s is the concentration of the element in soil (mg kg^{-1}), SA is the skin surface area available for contact, for adults (5700 cm^2), for shepherd or farmer adults (3300 cm^2), and for children (2800 cm^2), SL is soil to skin adherence factor for adults ($0.07 \text{ mg cm}^{-2} \text{ day}^{-1}$), for shepherd or farmer adults ($0.3 \text{ mg cm}^{-2} \text{ day}^{-1}$) and for children ($0.2 \text{ mg cm}^{-2} \text{ day}^{-1}$), ABS is dermal absorption factor, 0.03 for As and 0.001 for other elements, EF , ED , BW and AT as detailed in the previous equation for ingestion (Ordóñez et al. 2011). Inhalation absorption:

$$ADI_{inh} = (C_s \times inhR \times EF \times ED) / (PEF \times BW \times AT) \quad (7)$$

Where ADI_{inh} is the average daily intake of the element (i) from the soil (mg kg^{-1} of body weight-day), C_s is the concentration of the element in soil (mg kg^{-1}), $InhR$ is the inhalation rate of soil for adults (20 mg day^{-1}) and for children (7.6 mg day^{-1}). PEF is particle emission factor (1.36×10^9). EF , ED , BW , and AT as in the previous paragraph for ingestion.

➤ *Non-carcinogenic risk assessment*

Non-carcinogenic risk is usually evaluated by the hazard quotient (HQ) (unitless). HQ was calculated as the exposure value divided by the reference dose RfD (mg kg^{-1} of body weight day^{-1}), which is the toxicity threshold value of each chemical. RfD values were taken from (Ordóñez et al. 2011), based on the US Risk Assessment System (Eq 8).

$$HQ = ADI / RfD \quad (8)$$

$HQ \leq 1$ means adverse health effects are unlikely. $HQ > 1$ indicates probable adverse health effects. $HQ > 10$ indicates high chronic risk (Meza-Montenegro et al. 2012).

The hazard index (HI) is calculated for the total chronic hazard for each exposure pathway. The HI estimates the risk of mixing contaminants by summing individual HQ. HI was calculated by Eq. (9):

$$HI = \sum HQ_i \quad (9)$$

HI is divided into two groups; $HI < 1$ suggests that adverse health effects are unlikely even for sensitive populations, whereas the potential for chronic effects may be a concern when $HI > 1$. The last parameter that is presented in this study for non-carcinogenic risk is cumulative HI. It is calculated by the sum of the individual HI values for each of the main exposure pathways Eq. (10):

$$\text{Cumulative HI} = \sum HI \quad (10)$$

If cumulative $HI \leq 1$ means the exposed population is unlikely to experience obvious non-carcinogenic effects. In contrast, cumulative $HI > 1$ implies that there is a chance that non-carcinogenic effects may occur, with a probability that tends to increase as HI increases. Cumulative HI can exceed the target hazard level due to either, one or more pollutant with an HQ exceeding the target hazard level, or the sum of several pollutants specific HQ_s that are each less than the target hazard level. The total HI might therefore overestimate the potential for non-carcinogenic health effects.

➤ *Carcinogenic risk assessment*

Carcinogenic risk was evaluated by Eq. (11) and the detailed calculating process was followed by EPA guidelines (Ordóñez et al. 2011). The estimated value was the incremental probability of an individual developing any type of cancer over a lifetime due to carcinogenic exposure. The key risk assessment parameter derived from the carcinogen risk assessment process is the

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slope factor (SF). The slope factor is a toxicity value that quantitatively defines the relationship between dose and response.

$$\text{Risk} = \text{ADI} \times \text{SF} \quad (11)$$

Where risk is the unitless, ADI has been previously defined, and SF is the carcinogenicity slope factor ($\text{mg kg}^{-1} \text{ day}^{-1}$). Results of carcinogenic risk are divided into four groups, risk $> 1.0 \times 10^{-4}$ are viewed as unacceptable, $1.0 \times 10^{-4} < \text{risk} < 1.0 \times 10^{-6}$ is considered an acceptable range depending on exposure conditions, and risk $< 1.0 \times 10^{-6}$ is considered to pose not significant health effects. Furthermore, total risk (risk_{tot}) was calculated as the sum of the risks pathways we have considered.

2.2.1.4. Sampling and determination of trace elements in plant

Plant samples are gathered during one day journey (on November 8, 2012). The gathered samples are: *Oenothera Affinis* (OA), it is a native biannual herb and hemicryptophyte that grows up to 0.5-0.8 m, drought-resistant, easy to spread and produces a large biomass (Fig.2.14). *Sphaeralcea Velutina* (SV), it is an endemic small, round and dense shrub (up to 0.7m tall), nanophanerophyte plant (Fig.2.15). *Argemone Subfusiformis Ownbey* (*Papaveraceae*) (AS). With the common name Chilean Prickly Poppy is a native annual herb and therophyte. It has erected and prickly stems and leaves and reaches up to 0.7 - 1.2 m high (Fig.2.16). Prospection and species identification were conducted in November 2012. In each of the sampling sites, aerial biomasses of the selected species were collected. For the identification of plant species, the work of Navas Bustamante (Navas Bustamante 1979), Marticorena & Quezada (Marticorena and Quezada 1985), and Matthei et al. (Matthei J. et al. 1995) were used. For each identified species two individuals were sampled, located no more than 3 m away from each. Both individuals were considered the same sample and representative of the same soil.



Fig.2.14. *Oenothera Affinis* in La Greda sampling area



Fig.2.15. *Sphaeralcea Velutina*



Fig.2.16. *Argemone Subfusiform* is in Los Maitenes sampling area

Samples were transported to the laboratory in plastic bags (Fig.2.17). The sampled plants were washed twice with tap water to remove soil particles and then twice with distilled water. The plant material were dried for 48 h at 40 °C and finally manually triturated and kept in polyethylene bags until its analysis (Poschenrieder et al. 2001).



Fig.2.17. Gathering *Sphaerakcea Velutina* and keeping in plastic bag

Plant sub-samples were mechanically homogenized in a planetary mill and manually sieved through 0.2 mm using a stainless steel mesh. According to digestion methodology described by Wiseman et al (Wiseman et al. 2013), 150 mg of real sample (flower and leaf) and 300 mg of reference material were weighed and placed into Teflon vessels. Afterwards, samples were pre-digested overnight with 4.5 ml HNO₃ and 1.5 ml HCL (9 ml HNO₃ and 3 ml HCL for reference material samples). Pre-digested samples filtered 2 or 3 times then they moved to the Teflon microwave vessels and digested in microwave at 160 ° C (600 w, ramp time 15 min, and 15 minutes holding time).

The samples moved again to the Teflon vessels and they were evaporated on heater at 95 °C until near dryness. 0.5 ml of concentrated HF was added to the real samples (1 ml for reference material samples) to remove the silicate. Samples were then heated until they almost dried. 1 ml of concentrated HNO₃ was added to the real samples (2 ml for reference material samples) then they were evaporated until near dryness. This last step was repeated twice. The real samples were finally taken with 0.5 mL HNO₃ and water to a total volume of 10 ml and reference materials diluted with 2.5 mL HNO₃ and water to a total volume of 50 ml. 50 µL of a 10 mg L⁻¹ In (III) and Ge (II) solution was used as internal standards. The digested samples were analysed by PerkinElmer ELAN 9000 ICP-MS equipment (RF power 1,000 W, Ar

plasma flow rate 1 L min⁻¹, washing time 35 s). Each sample was assayed in triplicate. Each sample had three replications. In addition, blank samples were analysed and there weren't significant concentration of studied elements.

2.2.1.5. Soil to plant transfer factor

The transfer factor (TF) is used to evaluate the transfer efficiency of heavy metals from the soil to the plant, which is defined as the ratio of the heavy metal concentration in the plant to that in the soil. Therefore, the TFs of heavy metals from the soil to external parts of plants were calculated as Eq. (12) (Cheng et al. 2015) (Kassaye et al. 2012b):

$$TF = C_{\text{plant}} / C_{\text{soil}} \quad (12)$$

Where TF (based on dry weight) is the transfer factor of the heavy metal from the soil to plant, C_{plant} is element concentration in plant (mg kg⁻¹), and C_{soil} is element concentration in soil (mg kg⁻¹). The results will be categorised based on their TF to low (less than 0.09), medium (0.1-0.3), and high (1-2) levels. In addition, TF < 1 indicate low uptake by plants compared to the soil reservoir and TF > 1 indicate high uptake by plants (Kassaye et al. 2012b).

These values are needed for many assessment models to predict the concentration of an element for a given plant species at an anticipated contamination level in the soil (Chojnacka et al. 2005).

2.2.2. Radioactive pollutants in the soil

2.2.2.1. Sampling

The samples were collected in undisturbed areas, in point away from trees and buildings, at the least inclination possible, no changes have occurred by human intervention and animal to the road in a radius of 200 meters. Soil samples were collected to a depth of 5 cm from different locations in Southern Hemisphere (Table 2.2).

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Table 2.2. Locations and technics were used for radioactive pollutants analysis

<i>ID</i>	<i>Site</i>	<i>Country</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Gamma spectrometry</i>	<i>AMS</i>
1	La Greda	Chile	32° 43' S	71° 24' W	Yes	Yes
2	Los Maitenes	Chile	32° 46' S	71° 27' W	Yes	Yes
3	Puchuncaví	Chile	32° 45' S	71° 28' W	Yes	Yes
4	Valle Alegre	Chile	32° 48' S	71° 26' W	Yes	Yes
5	Maitencillo	Chile	32° 36' S	71° 25' W	Yes	No
6	Santa Bárbara	Chile	42° 51' S	72° 47' W	No	Yes
7	Durban	South-Africa	29° 52' S	30° 57' E	No	Yes
8	Maputo	Mozambique	25° 57' S	32° 17' E	No	Yes

The procedure for sampling surface soil was performed according to the Spanish normative (2006), inspired by (CEEM, 2001), (ASTM,1995), and (AFNOR 1999). A sample was composed of 3 sub-samples. To accommodate non-negligible variability soil a square of 2x2 m was established. Three sub-samples are collected in the center square of 2 m side and two of its vertices. Next the extraneous matter (stones, leaves, seeds or roots) was eliminated. To sample a square steel structure, 50x50 cm length, with edges on one of the sharp edges and height corresponding to the depth of five centimeters is used. The distance between two sampling points taken at different times was about 300 m approximately.

Approximately, 3 kg of subsample were extracted with a plastic spade placed into a conditioned plastic container. The containers were immediately closed and transferred to the lab.

2.2.2.2. Accelerator mass spectrometry (AMS) methodology for analyzing the ^{236}U and $^{239,240}\text{Pu}$

These analysis were carried out in the Centro Nacional de Aceleradores (CNA) of Sevilla. For each sample, three subsamples were collected and homogenized. About 7 g soils were selected aliquoted from each site; spikes were added (about 2 pg ^{242}Pu and ^{233}U) and thoroughly homogenized with a few ml of diluted HCl. These artificially added are not present in the samples of interest, and are used to estimate the $^{239,240}\text{Pu}$ and ^{236}U concentrations, respectively, in the original soil samples, using the so-called isotope dilution method. ^{242}Pu was obtained from a standard solution (NPL, England); ^{233}U was obtained from a standard solution (IAEA-EL). The samples were then calcined at 600 °C in a muffle furnace for about 6 h to destroy organic material, and the ashed sample material was transferred to Teflon® vessels and leached with 8 M HNO_3 , 9 M HCL and H_2O_2 for about 5 h at 100 °C in a hot plate (Fig.2.18) at atmospheric pressure. Ultra-pure HNO_3 and HCl were used, to avoid ^{238}U contamination.

The resulting supernatant containing the anthropogenic uranium and plutonium were separated by centrifugation (Fig.2.19), filtered through a 1.2 μm syringe membrane, evaporated to dryness, and dissolved in about 10 ml of 0.5M HNO_3 . A $\text{Fe}(\text{OH})_3$ co-precipitation, using the iron present in the sample, was then applied to preconcentrate the actinides and remove the major matrix components.



Fig.2.18. Drying leached

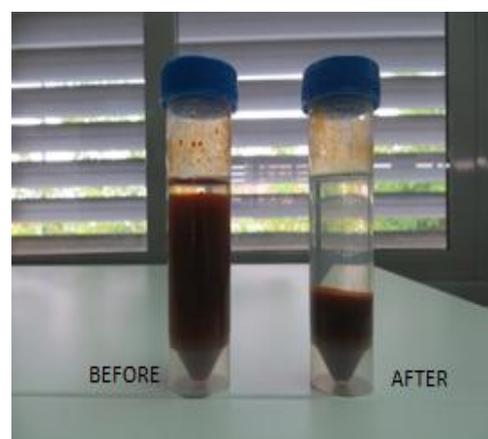


Fig.2.19. Samples after centrifuging

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The resulting precipitate was then dissolved in 15 ml of 8M HNO₃, and the chemical separation of U and Pu fractions were performed using UTEVA and TEVA resins in a vacuum box (Fig.2.20).



Fig.2.20. Using UTEVA and TEVA resins in a vacuum box

1 mg of Fe³⁺ was added to the solutions to coprecipitated the uranium or plutonium fractions with Fe(OH)₃. Those precipitates were transferred to quartz crucibles, dried, baked at 600 °C for 1 h to convert the uranium or the plutonium to the oxide form, mixed with about 3 mg of Nb powder and pressed into appropriated aluminum cathodes.

2.2.2.3. Gamma spectrometry methodology for analysing the ²²⁶Ra, ²³²Th and ⁴⁰K

Soil samples were dried at 80 °C for 48 h, sieved through a 2 mm mesh, weighed and hermetically sealed in radon-impermeable plastic containers. About 150 g of sample material were used for measurements of each soil sample. Before measurements, the containers were kept sealed for 4 weeks, in order to reach equilibrium of the ²³⁸U and ²³²Th series and their respective progeny. Spectra were recorded during 48 h. The reliability of the spectrometer was checked by measuring the activity of an IAEA reference material, IAEA-6-SOIL. The ²³²Th activity was determined by means of the γ -emissions of ²²⁸Ac (911 keV) and ²⁰⁸Tl (583.01 keV) and that of ²²⁶Ra by means of those from ²¹⁴Bi (609.3 and 1764.5 keV) and ²¹⁴Pb (351.9 keV) assuming that both radioactive families are left in secular equilibrium. Lastly, we determined the ⁴⁰K by

using the 1460.7 keV γ -emissions. The specific activities (A) for ^{226}Ra , ^{232}Th and ^{40}K were calculated by Eq. (12):

$$A = N / (t \times P \times \epsilon) \quad (12)$$

Where N is the net counts, t is the data collection time, P is the emission probability and ϵ is the efficiency of the detector for the corresponding peak. The error was calculated from the combined uncertainty in the efficiency of the detector and the uncertainty in the net count rate. In the case of using several gamma-ray peaks for the calculations of the activity (i.e. ^{226}Ra and ^{232}Th), uncertainty in the yield is also included. The analysis of gamma spectrum obtained was performed using the software Gamma Vision (ORTEC). The detector efficiency was calibrated using certified mixed gamma standards QCY-48, supplied by Amersham, for the energy range 60-1900 keV. Spectra were recorded during 48 h.

2.2.2.4. Assessment of potential human risk

The human beings are exposed mainly to natural sources of radioactivity. More than 80% of the radiation dose received by mankind is due to natural radiation sources (Tufail 2012). Since soil minerals can be used in industries and building constructions, the γ -ray radiation hazards due to the specified radionuclides are assessed by several different indices. The term “radium equivalent activity” (Ra_{eq}) was introduced for the activity of a radionuclide, which would have the same therapeutic effect as 1 mg of ^{226}Ra . It represents the specific activities of ^{226}Ra , ^{232}Th and ^{40}K by a single quantity, which takes into account the radiation hazards associated with them. Ra_{eq} is a weighted sum of activities of the above three natural radionuclides based on the estimation that 370 Bq kg^{-1} of ^{226}Ra , 259 Bq kg^{-1} of ^{232}Th or 4810 Bq kg^{-1} of ^{40}K produce the same gamma ray dose. It presented by Eq. (13):

$$Ra_{eq} = A_{Ra} + 1.43A_{Th} + 0.077A_K \quad (13)$$

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Where A_{Ra} , A_{Th} , and A_K ($Bq \cdot kg^{-1}$) are the activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K , respectively. In general, the maximum value of Ra_{eq} in building materials must be less than 370 $Bq \cdot kg^{-1}$ for safe use (Beretka and Matthew 1985).

In addition, Beretka and Mathew defined two other indices that represent the external and internal radiation hazards. The external hazard index is obtained from Ra_{eq} expression through the supposition that its maximum value allowed (equal to unity) corresponds to the upper limit of Ra_{eq} ($370 Bq \cdot kg^{-1}$).

This index value must be less than unity in order to keep the radiation hazard insignificant; i.e. the radiation exposure due to the radioactivity from construction materials is limited to 1.0 $mSv \cdot y^{-1}$. Then, the external hazard index can be defined as Eq. (14):

$$H_{ex} = \frac{A_{Ra}}{370} + \frac{A_{Th}}{259} + \frac{A_K}{4810} \leq 1 \quad (14)$$

Dose rate calculation (D) is important to estimate the dose of radiation received for a man. Conversion factors to transform specific activities A_{Ra} , A_{Th} , and A_K , in absorbed dose rate at 1 m above the ground (in $nGy \cdot h^{-1}$ by $Bq \cdot kg^{-1}$) are calculated by Monte Carlo method and the values are (United Nations Scientific Committee on the Effects of Atomic Radiation 2000) Eq. (15):

$$D (nGy \cdot h^{-1}) = 0.0417A_K + 0.462A_{Ra} + 0.604A_{Th} \quad (15)$$

In natural environmental radioactivity situations, the absorbed dose can be converted into annual effective dose E using equation (16). The conversion factor from absorbed dose to effective dose, (Q) is $0.7 Sv \cdot Gy^{-1}$ for environmental exposure to gamma-rays of moderate energy and T is one year, i.e., 8760 h, as proposed in (United Nations Scientific Committee on the Effects of Atomic Radiation 2000).

$$E (mSv \cdot y^{-1}) = T \times Q \times D \times 10^{-6} \quad (16)$$

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CHAPTER 3.

RESULTS and DISCUSSION

3. Results and discussion

3.1. Article 1

Science of the Total Environment 527–528 (2015) 335–343

<http://www.sciencedirect.com/science/article/pii/S0048969715300681>

Long-term assessment of ecological risk from deposition of elemental pollutants in the vicinity of the industrial area of Puchuncaví-Ventanas, central Chile

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Abstract

The present work investigates soil pollution by elemental contaminants and compares ecological risk indexes related to industrial activities, for the case study of Puchuncaví-Ventanas, a relevant industrial zone located in central Chile. Selected elements (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba) were analysed during a long-term period (yearly sampling campaigns during 2007-2011), at 5 sampling stations representing different degrees of impact. PCA and cluster analysis allowed identifying a copper smelter and a coal fired power

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plant complex as major pollution sources. Geoaccumulation index (I_{geo}), enrichment factor (EF), contamination factor (C_f), contamination degree (C_{deg}), and integrated pollution index (IPI) are critically discussed for quantitative ecological risk assessment. I_{geo} , EF and C_f indexes are producing comparable environmental information, showing a moderate to high pollution risks in the area that demands further monitoring and adoption of prevention and remediation measures.

Capsule: Long term assessment of elemental pollution around an industrial area. New insight on ecological risk indexes for trace element pollution in soils, by critical comparison among them.

Key words: Soil, metal, ecological risk assessment, industrial pollution, pollution indexes

1. Introduction

Among the most significant soil pollutants, trace elements are relevant due to both acute and long-time toxic effects in the soils and related environmental media (Yaylah-Abanuz, 2011), since soil pollutants can enter the human body by ingestion, dermal contact, and inhalation, aside to the food chain pathway starting by plant uptake (Meza-Montenegro et al., 2012). The potential ecological risk associated to soil contamination is a very controversial issue in recent years (Meza-Montenegro et al., 2012), so ecological risk assessment aims to provide information to measure and predict soil pollutant threats for humans and the environmental health (Fairbrother et al., 2007; Wei and Linsheng Yang, 2010). Different indexes have been proposed to predict the environmental quality of soil and sediments (Caeiro et al., 2005).

Punchuncaví-Ventanas, one of the main industrial areas of Chile, comprises a wide range of industrial factories and activities implying potential risks to human and environmental health. Most environmentally relevant activities in this area are the CODELCO División Ventanas copper refinery and the AES Gener coal-fired power plants complex (three operating units) facilities. For example, these activities are responsible of 68.1% (copper refinery) and 30.7%

(coal-fired power plants) of SO₂ emissions in the area, according to local authority's reports (UNTEC, 2012). Aside to these main sources, there is a wide set of less impacting industrial activities operating in the area, briefly described as follows. Puerto Ventanas is a provider of dock and port facilities. Cementos Melón is a producer of national construction materials. It has three activity areas: cement, concrete aggregate, and mortars. Catamutún Import, sell coal, and steam division, which manages industrial systems from steam coal combustion. Panimex S.A produces plastic and Fumaric acid. Gasmar is a company dedicated to the marketing of liquefied gas. Minera Montecarmelo is a plant for treating industrial wastes. Oxiquim is a maritime terminal. Cordex is a big factory for production asphalt and is a fuel terminal also.

Studies about environmental impact of industrial activities on the elemental soil levels in the Puchuncaví-Ventanas area are scarce. Ginocchio (2000) analyzed the effect of copper smelter on grassland in terms of physicochemical soil characteristics, plant species diversity and abundance, founding a significant impact of industrial activities on plant species regeneration capabilities. De Gregori et al (2002) carried out the redox speciation of selenium present at ultratrace levels in rainwater collected at the area. The same group (De Gregori et al., 2003) conducted a work aimed to the monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile including the Puchuncaví-Ventanas industrial area. The high concentrations measured in impacted soils from Puchuncaví-Ventanas (300 mg Kg⁻¹ Cu, 34.5 mg Kg⁻¹ As and 5.3 mg Kg⁻¹ Sb) clearly showed the impact produced in this zone by the industrial and mining activities developed in their proximities. Ginocchio et al (2004) reported Cu, Zn, Pb and Cd concentrations (among other parameters) in different soil layers around the area. The levels found in the 0-5 cm layer were 361.6 mg Kg⁻¹ for Cu, 157.8 mg Kg⁻¹ for Zn, 79.9 mg Kg⁻¹ for Pb and 0.8 mg Kg⁻¹ for Cd. Copper mobility in soil around the copper smelter was investigated by Neaman (2009). The same author reported results about the effectiveness of lime and

compost for situ immobilization of trace elements in soil by using earthworms as bioindicators of toxicity (Neaman et al., 2009). These previous works in the area focused on a limited numbers of trace elements analyzed after single sampling campaigns. A more detailed evaluation of trace elements in soils, focused on the study of their distribution in particle size fractions has been recently published by Parra et al (2014). In the present work, we describe the results of a systematic, long-term investigation about ecological risk from multi element soil pollution at the Puchuncaví-Ventanas area, comprising spatial and temporal variability, source assignment and ecological risk indexes calculations.

2. Materials and methods

2.1. Sampling area and soil characteristics

The Puchuncaví-Ventanas industrial area is located in a district (Fig. 1) belonging to the V Region of Chile, Valparaíso Province. The area is located in the Chilean mainland coast ($34^{\circ} 45' S$, $71^{\circ} 29' W$), 58 km North from Valparaíso (Regional Capital Region V), 45 km North from Viña del Mar and 160 km North-West from Santiago de Chile. It comprises an area of 301 km^2 , with a population of 13,000. The main communication ways are the F-30E Road, Highway 5 North (via Catapilco), and Nogales-Puchuncaví way. Specific locations for soil sampling were selected in the area as depicted in Fig. 1. La Greda (LG) sampling point ($32^{\circ} 44' 57'' S$, $71^{\circ} 28' 30'' W$) is located 1.69 km NE from main emission sources in the area, whereas Los Maitenes (LM) sampling point ($32^{\circ} 45' 41'' S$, $71^{\circ} 27' 18'' W$) is located 2.39 km E. These two locations are expected to be the most impacted points. Puchuncaví village (PU) sampling point ($32^{\circ} 43' 17'' S$, $71^{\circ} 24' 43'' W$) is located 8 km NE from the industrial area and Valle Alegre (VA) sampling sites ($32^{\circ} 48' 30'' S$, $71^{\circ} 26' 10'' W$) is 6.72 km SE around the industrial complex. Prevalent SW winds in the zone are expected to transfer atmospheric pollutants mainly to LG, LM and PU sampling points. Maitencillo (MA) sampling area ($32^{\circ} 36' 5'' S$, $71^{\circ} 25' 56'' W$), 18 km N from

the industrial area, was selected as a reference rural area, presumably not affected by the industrial activities. The sampling points were located in undisturbed soil areas surrounding small villages. The soils at the study area are classified as Entisols. The topsoil (0-30 cm) are sandy loam (clay 13-18 %, sand 65-74 % and silt 13-17 %), a coarse texture that implies low nutrient availability and limited water holding capacity (Ginocchio, 2000; Neaman et al, 2009). Soil organic matter ranges from 1.0 to 1.8, and soil pH ranges from 4.6 to 5.5 (Ginocchio, 2000; Neaman et al, 2009).

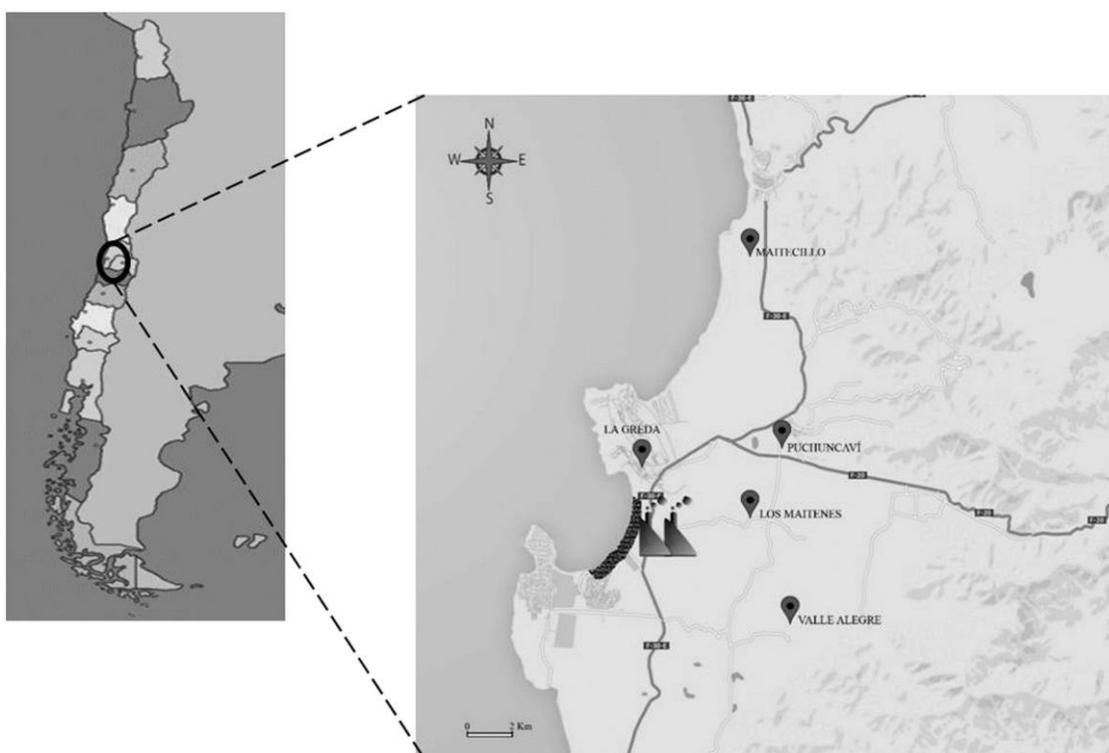


Fig. 1. Puchuncavi-Ventanas industrial area and soil sampling points.

2.2. Reagents

All chemicals used for the preparation of stock and standard solutions were of analytical grade. ICP multielemental standard solutions were obtained from PerkinElmer (Waltham, MA, USA).

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Working solutions were prepared by dilution with ultrapure water (resistivity >12M Ω) obtained from an Ultramatic system (Wasserlab, Spain).

Dilute standards and real samples were adjusted to desired pH with sub-boiled HNO₃ (69%) obtained from a quartz sub-boiling system (Kürner, Rosenheim, Germany). 1 g L⁻¹ Y (III) and In (III) standards (Parneac, Spain) were used as internal standards. HClO₄ (70%) Suprapur[®] from Merck and HF (48%) Panreac Hiperpur were used for sample digestion and pH adjustment. Standard Reference Material [®] 2710a Montana I Soil from the U.S. National Institute of Standards and Technology (NIST) was used for accuracy testing.

2.3. Soil sampling and homogenization

A total of 121 surface soil samples were collected in the study area during five monitoring campaigns carried out in winter 2007, 2008, 2009, 2010 and 2011. These activities are in the frame of a large scale and long term ongoing environmental monitoring program in the area. 5 samples were taken at randomly selected points around each designed sampling zone for each sampling campaign. For sampling, 1 m² surface was delimited and the extraneous matter (stones, leafs, seeds or roots) was eliminated. Soil samples were collected to a depth of 5-10 cm by using a hand polypropylene drill. Approximately 3 Kg of sample were extracted with a plastic spade and transferred to a conditioned plastic container. The containers were immediately closed and transferred to the lab.

According to ISO 11464, soil samples were appropriately dried in stove by heating at 50 °C for 72 h and then sieved through a polypropylene 2 mm mesh. Soil sub-samples were mechanically homogenized in a planetary mill and manually sieved through 0.2 mm using a stainless steel mesh. The samples were then stored in the fridge (4 °C) until analysis.

2.4. Soil analysis

The digestion protocol for the soil samples was based on a previously reported method

(Palomo Marín et al., 2011). Briefly, 50 mg soil or reference material samples were placed in Teflon digestion vessels (Savillex, USA). 2.5 mL HNO₃ and 5 mL HF were added and the mix was left to react for some minutes. The vessels were then closed and heated to 90°C in a stove for 8 hours. After cooling, the vessels were open, 2.5 mL HClO₄ was added and the solution was evaporated to dryness on a plate at 200 ° C. 1 mL HNO₃ was then added and the solution was again evaporated to dryness. The samples were finally taken with 2.5 mL HNO₃ and water to a total volume of 50 ml. 50 µL of a 10 mg L⁻¹ In (III) and Y (III) solution was used as internal standards. Montana Soil Reference material (NIST code 2710a) was used to assess the accuracy of the experimental results.

2.5. Determination of elements by ICP-MS

The digested samples were assayed on PerkinElmer ELAN 9000 ICP-MS equipment by a standard protocol. The main instrumental parameters were as follows: RF power 1,000 W, Ar plasma flow rate 1 L min⁻¹, washing time 35 s, and 3 replicates per sample. Quantification was performed by internal standard calibration using PerkinElmer multi-element ICP-MS calibration standards. Blank samples were assayed and no significant concentrations of the studied elements were found. Concentrations of elements in soil samples are expressed in dry soil weight terms.

2.6. Statistical analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were performed by using the SPSS 19.0 software and XLSTAT 2009.1.02 for Windows Excel. Hierarchical cluster analysis was performed by using Euclidean distance and the Ward agglomerative algorithm. To get a better insight into the latent structure of the data, the principal component extracted correlation matrix was subjected to varimax orthogonal rotation.

2.7. Assessment of potential ecological risk

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In this study, the following indexes have been used as indicators for assessment of soil ecological risk:

-Geoaccumulation index (I_{geo}): It allows the estimation of contamination comparing preindustrial and recent metal concentrations in soils. It was originally proposed by Müller (1969) for sediments and then modified by Loska et al (2004) for soil pollution estimation. It has been widely applied to several trace metal studies in Europe (Yaylah-Abanuz, 2011).

This index is expressed by Eq. (1):

$$I_{geo} = \log_2(C_n / 1.5 B_n) \quad (1)$$

Where C_n is the measured concentration of element n in the soil sample, and B_n is the geochemical background value of element n in the upper Earth's crust (Wedepohl, 1995). The constant value 1.5 is included to consider the natural fluctuation of the concentration of a given substance.

I_{geo} values are categorized in seven groups, as follows: practically uncontaminated ($I_{geo} \leq 0$); uncontaminated to moderately contaminate ($0 < I_{geo} < 1$); moderately contaminated ($1 < I_{geo} < 2$); moderately to heavily contaminate ($2 < I_{geo} < 3$); heavily contaminated ($3 < I_{geo} < 4$); heavily to extremely contaminated ($4 < I_{geo} < 5$) and extremely contaminated ($I_{geo} > 5$).

-Enrichment factor (EF): This method is based on the standardization of an element concentration tested against a reference element (Yaylah-Abanuz, 2011). Fe, Sc, Ti, Al, Ca, or Mn are generally used as reference elements for calculation of EF (Quevauviller, 1989). Mn was selected as reference element in this study.

The value of the enrichment factor was calculated according to Eq. (2):

$$EF = [C_n(\text{sample}) / C_{ref}(\text{sample})] / [B_n(\text{background}) / B_{ref}(\text{background})] \quad (2)$$

Where:

C_n (sample) is the concentration of element n in the sample, C_{ref} (sample) is the concentration of the reference element (Mn) in the sample, B_n (background) is the concentration of element n in the upper Earth Crust (Wedepohl, 1995), and B_{ref} (background) is the concentration of the reference element in the earth crust.

Enrichment factor values are grouped in five categories (Sutherland, 2000): Deficiency to minimal enrichment ($EF < 2$); Moderate enrichment ($2 < EF < 5$); Significant enrichment ($5 < EF < 20$); Very high enrichment ($20 < EF < 40$) and extremely high enrichment ($EF > 40$).

-Contamination factor (C_f), contamination degree (C_{deg}), and integrated pollution index (IPI): These are related indexes. C_f was proposed by Håkanson (1980), as the ratio between the concentration of selected pollutants (PCBs, Hg, Cd, As, Cu, Cr, Zn, and Pb) in sediment samples and reference preindustrial concentration values. It has been later adapted to the study of soil pollution by using upper crust elemental concentrations (Wedepohl, 1995) as reference values. In our case, PCBs were not measured and for this reason we've replaced this parameter by Sb. Median lethal dose (LD_{50}) of Sb (WHO, 2003) is in the same range of PCBs (Euro chlor, 2002), so it can be assumed that both of them have similar eco-toxic effect.

The contamination factor (C_f) is calculated by Eq. (3):

$$C_f = C_n \text{ (sample)} / C_n \text{ (background)} \quad (3)$$

Håkanson (1980) divided the C_f into four categories: Low contamination ($C_f < 1$); Moderate contamination ($1 \leq C_f < 3$); Considerable contamination ($3 \leq C_f < 6$) and very high contamination ($C_f > 6$).

Contamination degree (C_{deg}) is defined as the sum of the individual contamination factors by Eq. (4):

$$C_{deg} = \sum C_f \quad (4)$$

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C_{deg} is divided into four groups as C_f (Håkanson, 1980): Low contamination ($C_{deg} < 8$); Moderate contamination ($8 \leq C_{deg} < 16$); Considerable contamination ($16 \leq C_{deg} < 32$) and very high contamination ($C_{deg} > 32$).

The integrated pollution index (IPI) is defined as the average of the contamination factors computed for each sample (Yaylah-Abanuz, 2011). IPI values are divided in four categories (Wei and Yang, 2010): Low pollution level ($IPI \leq 1$); Moderate pollution level ($1 < IPI \leq 2$); High pollution level ($2 < IPI \leq 5$) and extremely high pollution level ($IPI > 5$).

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3.1. Analytical quality control

NIST 2710a (Montana Soil) certified standard reference samples were digested and assayed using the analytical protocol for the real samples, to ensure the reliability of the results, and the results obtained are presented in Table 1. The percentage of recovery was between 80 and 120 % for all elements, showing that the analytical methodology is properly operating for the analysis of real soil samples.

Table 1. Analytical results of certified reference material (2710a, Montana Soil)

Elements	Certified values(mg/ Kg)	Measured concentration(mg/ Kg)	Mean recovery (%)
As(*)	0.154±0.010	0.166±0.039	90
Cd	12.3±0.3	12.4±3.0	84
Pb(*)	0.552±0.003	0.563±0.160	80
V	82±9	78±9	103
Mn(*)	0.214±0.006	0.229±0.033	99
Co	5.99±0.14	6.52±1.52	90
Cu(*)	0.342±0.005	0.370±0.101	86
Zn(*)	0.418±0.015	0.492±0.087	105
Sr	255±7	221±3	88
Ti(*)	0.311±0.007	0.353±0.003	114
Sb	52.5±1.6	44.0±0.4	84
Ba	792±36	736±86	91

Data are (mg/ Kg) except (*) that is (%)

3.2. Soil contamination levels

Mean, maximum and minimum concentrations of elements in the soil samples collected at the studied sampling stations are shown in Table 2, and all individual results are included in the supplementary material (Table S1). The variability of the elemental concentrations within the study area (including the sampling locations LG, LM, PU, and VA) was somewhat high. It has been reported that the dispersal of contaminants around smelters is highly dependent on the local situation, mainly on the prevailing wind direction (Ettler et al., 2011), so SW dominant winds in the study area have to be also considered for the interpretation of pollutants transfers. The global mean values including all samples were calculated as indicative of the overall pollution status of the area (Table 3). The soil elemental concentration levels in the study area were first inspected by comparing with soil quality standards. There is not a general regulation about soil environmental quality applicable in Chile so we have selected soil quality standards values published by the Australian government (Australian department of environment and conservation, 2010), Canada (Canadian council of ministers of the environment, 2007) and The Netherlands (Ministry of housing, spatial planning and the environment, 2000) as reference values for assessment of the soil elemental levels found in this study (Table 3). About the samples collected at the most impacted areas of La Greda (LG) and Los Maitenes (LM), close to the pollution sources at the Puchuncaví-Ventanas area, the most significant finding is probably the very high concentration of copper found in the soils of the study area compared with Canadian, Australian and Dutch standards. The average Cu level at LG and LM is 7 and 4 times higher, respectively, than the least restrictive Dutch value. This result can be derived from the environmental impact of the large copper smelter operating in the area. As exceeds Australian standard values but are in the range of standard values approved in Netherland. Vanadium levels in LG and LM are between approved levels in Canada and Australia. Barium is in the range of Australian standard values but less than Canadian and Dutch reference values. Pb, Cd, Ni, Hg, Zn, Sb, Cr, and Co levels measured in LG and LM don't

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exceed the international soil quality standard values selected for comparison. So the sampling areas LG and LM can be preliminarily classified as significantly impacted by industrial activities, at least for some of the element studied (especially copper). About the samples collected at the less impacted areas of Puchuncaví village (PU) and Valle Alegre (VA), most of the concentrations of the assayed element in the soil are under the limits established by the aforementioned international soil quality standards. Only As and V are within the quality standards ranges. About Cu, the mean value at the PU sampling point exceeds the reference values, showing the long distance impact of the copper smelter in the dominant wind direction (SW), but the VA values are in the range of Canadian and Australian standards, reflecting that this location is more isolated from wind transport from the emission sources. The reference sampling area at Maitencillo (MA) presents similar elemental soil concentrations that those measured in VA. Soil concentration of the elements in the study area were then compared with previously reported values found in comparable industrial areas around the world (Table 3), although direct comparison of the results is complicated due to the disparity of sampling conditions (sample number and sites), and digestion protocols. In our case, copper smelter and coal-fired power plant are the two most impacting activities in the area. We have not found reported results about case studies with these industrial activities together, so we have compared our results with other studies with separated industrial activities. E.g. Nkana smelter in Zambia (Ettler et al., 2011) and Port Kembla smelter, NSW, in Australia (Martley et al., 2004) are two comparable industrial areas affected by copper smelting activities. As mean value in the soils at our study area around Codelco copper smelter are significantly higher than those reported around Nkana and Port Kembla smelters. This effect is observed for all locations (LG, LM, PU and VA) even MA (Table 2).

Table 2. Element concentrations in soil collected at the sampling stations within the Puchuncaví-Ventanas industrial area. La Greda (LG), Los Maitenes (LM), Puchuncaví village (PU), Valle Alegre (VA) and reference station at Maitencillo (MA). All results in (mg/Kg). n is the number of samples

Elements		LG (n= 26)	LM (n= 26)	VA (n= 21)	PU (n= 25)	MA (n= 21)
As	Max-Min	93.26-22.8	110.56-9.56	15.16-8.59	49.48-8.7	36.09-6.63
	Mean	50.84	56.98	11.64	27.51	15.63
Pb	Max-Min	165.84-24.56	210.27-16.8	35.46-11.12	94.09-6.41	85.03-16.85
	Mean	84.8	89.06	19.84	45.57	18.93
Cd	Max-Min	2.83-0.52	2.84-0.45	0.56-0.14	1.46-0.05	0.33-0.17
	Mean	1.34	1.3	0.24	0.55	0.25
Ni	Max-Min	92.62-3.62	85.12-2.43	18.27-0.04	79.42-1.54	13.78-0.04
	Mean	20.02	16.76	10.19	14.17	10.22
Hg	Max-Min	3.81-0.0001	1.38-0.0001	0.49-0.0001	1.34-0.0001	0.05-0.0001
	Mean	0.77	0.31	0.08	0.2	0.01
V	Max-Min	147.9-29.77	160.94-43.31	185.55-76.28	127.41-50.06	170.57-94
	Mean	86.36	115.43	135.72	97.47	137.91
Mn	Max-Min	721.48-181.44	721.5-392.62	1178.97-573.97	1014.12-325.6	864.91-576.77
	Mean	408.13	497.65	772.47	569.19	711.06
Zn	Max-Min	540.16-41.3	309.69-80	170.31-44.73	161.93-33.28	254.6-63.11
	Mean	232.26	157.04	90.94	100.43	123.2
Sr	Max-Min	446.31-171.33	312.22-132.86	216.67-119.21	218.86-142.27	301.43-222.92
	Mean	273.52	218.98	164.53	175.97	242.25
Sb	Max-Min	9.83-1.08	15.99-0.48	2.14-0.54	7.28-0.39	1.84-0.68
	Mean	4.84	6.49	1.32	3.33	1.32
Cr	Max-Min	35.25-13.23	36.72-17.61	36.14-18.03	50.76-16.25	42.2-28.5
	Mean	24.32	26.83	27.2	33.38	34.72
Co	Max-Min	11.34-2.53	11.06-5.76	20.58-7.78	14.4-7.04	11.26-8.61
	Mean	7.45	7.96	12.28	9.08	9.58
Cu	Max-Min	2872.75-452.77	1786.77-237.19	173.82-50.56	629.91-40.37	51.74-32.22
	Mean	1403.19	771.29	93.35	284.07	42.34
K	Max-Min	12769.35-1603.85	14667.99-4381.55	14109.01-3754.94	10814.64-3512.23	11392.72-3768.36
	Mean	2499.11	9647.39	9725.81	7473.35	8277.52
Ba	Max-Min	464.93-57.14	463.83-216.78	429.06-280.5	464.46-218.37	355.56-223.65
	Mean	301.88	339.91	343.36	302.09	266.5

Higher Pb, Zn and Cr values are also observed in the soils of our study area compared with the reference smelters, whereas Cu soil level is within reported ranges. About reference coal-fired power plants, we have selected Huainan in China (Tang et al., 2013) and Afsin-Elbistan, in Turkey (Çayır et al., 2012).

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Table 3. Trace element concentration (mg/kg) in soil samples from Puchuncaví-Ventanas area compared with other case studies of polluted areas around the world, and reference soil quality standard guidelines.

Elements	Puchuncaví-Ventanas soils (this study)	Copper smelting areas		Coal fired power plants areas		Reference soil quality standards		
		Port Kembla, Nsw, Australia (Martley et al., 2004)	Copperbelt province, Zambia (Ettler et al., 2011)	Huainan, China (Tang et al., 2013)	Afsin-Elbistan, Turkey (Çayır et al., 2012)	Canada (Canadian Council of Ministers of the Environment, 2007)	Australia (Australian Department of Environment and Conservation, 2010)	Netherland (Ministry of Housing, Spatial Planning and the Environment, 2000)
As	38.12	4.1	3.1	12.77		-	20	55
Pb	62.00	29	15.3	33.79	26.7	600	1500	530
Cd	0.89	-	-	0.64	6.29	22	3	12
Ni	15.56	-	-	26.14	89.8	50	60	210
Hg	0.36	-	-	0.01	-	-	-	10
V	107.48	-	-	53.14	-	130	50	-
Mn	551.04	-	-	384.43	-	-	-	-
Zn	148.39	63	34.4	39.95	89.2	360	200	720
Sr	210.81	-	-	-	-	-	-	-
Sb	4.14	-	-	-	-	-	-	15
Cr	27.91	12	-	32.4	70.5	87	-	380
Co	9.03	-	56.8	-	-	-	-	240
Cu	669.37	76	1501	21.06	59.5	91	100	190
K	8539.64	-	-	-	-	-	-	-
Ba	320.91	-	-	-	-	2000	300	625

As, Pb, Hg, V, Mn, Zn and Cu concentration values at all sampling sites in our study area are higher than reported values in the reference coal-fired power plants areas, whereas Cd, Ni and Cr values are lower, even at the most affected sampling point at La Greda (LG).

Our results were then compared with the results of previous soil monitoring campaigns conducted in the area. We have found similar mean values of As and Sb soil concentrations (38.12 mg Kg⁻¹ and 4.1 mg Kg⁻¹ respectively) than those reported by De Gregory et al (2003) during a study of agricultural soils in the Puchuncaví-Ventanas area (34.5 and 5.3 mg Kg⁻¹ respectively). But we have found a mean Cu concentration in the soils (669.4 mg Kg⁻¹) that is more than double of the result reported by the 2003 study (300 mg Kg⁻¹). Our results for Cu are also higher than mean values of 361.6 mg Kg⁻¹ reported by Ginocchio et al (2004). These results may indicate a progressive enrichment of the soil due to persistent copper emissions in the

area. Our study shows similar mean results for Zn (148.4 mg Kg^{-1}), Pb (62.0 mg Kg^{-1}) and Cd (0.9 mg Kg^{-1}) soil concentrations respect to the results of Ginocchio et al (2004): 157.8 mg Kg^{-1} for Zn, 79.9 mg Kg^{-1} for Pb and 0.8 mg Kg^{-1} for Cd. The elemental soil concentration profile measured in our work is similar that the values reported by Parra et al (2014) for the same study area, considering that the sampling timeframe is different (2007-2011 vs 2007-2009, respectively). As previously mentioned, our results were produced during a long-term study comprising yearly monitoring campaigns during the 2007-2011 period, so the results were examined to identify possible temporal evolution of the most significant elemental pollutants in the soils of the study area (Fig. 2). For a better visualization, elemental concentration results were normalized, by dividing average concentration of each element during a given year by the average concentration during the whole period (2007-2011). It's difficult to extract a general conclusion, about the overall temporal trend about elemental pollution in the soils of the study area.

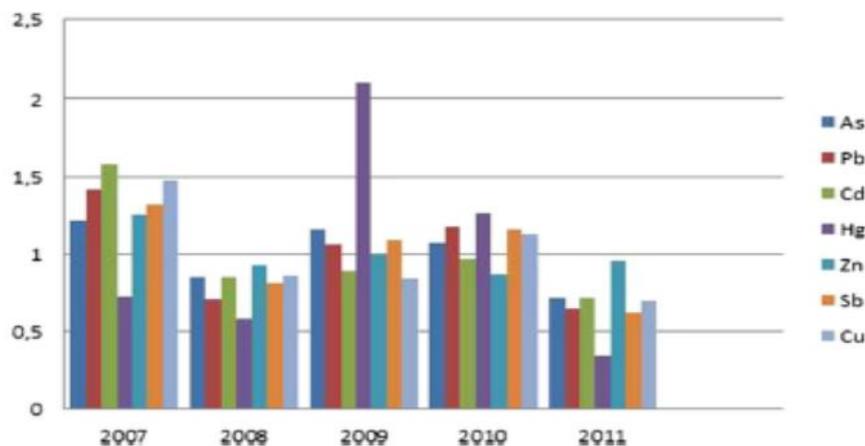


Fig. 2. Normalized concentration of trace and major elements during 2007 to 2011 sampling campaigns (refer to text for the normalization protocol used).

Most of the environmentally significant elements assayed show maximum soil concentration values in 2007 (except Hg that peaks in 2009). Then the values decrease in 2008 to peak again in 2009-2010, but reaching lower values than those measured in 2007. The values then decline in 2011 to the minimum values measured during the studied period. The observed evolution of

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soil concentrations of elemental pollutants can be related with improved environmental technologies adopted by the industries in recent years, resulting in less pollutant emission per production unit, combined to natural soil cleaning processes as wind resuspension and leaching by rain. Nevertheless, a permanent soil sampling and monitoring scheme seems essential to follow elemental concentration variability and confirm the observed trends in the medium and long term.

3.3. Cluster and PCA analysis

In order to reveal relationships among elements to identify pollution sources, cluster and PCA multivariate statistical techniques were applied to the results. The results of the cluster analysis are shown in Fig. 3, and the results of the PCA analysis are presented in Table 4. PCA factor 1, with 40% of variance, comprises As, Pb, Cd, Hg, Zn, Sb, and Cu (bold figures in Table 4) with high loadings. Elements in this factor are also grouped in cluster analysis (Fig. 3).

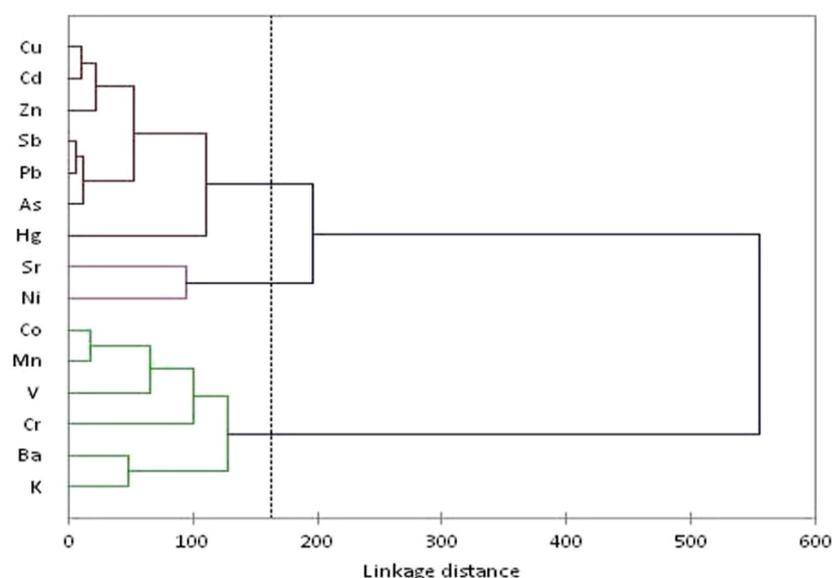


Fig. 3. Cluster analysis.

The common origin of these elements is probably related to industrial sources (Slavkovic´ et al., 2004). Our results confirm that the presence of a large copper smelter in the study area is probably a main source of these elements, as previously reported (Ginocchio et al, 2004; Parra

et al, 2014). This is also supported by the fact that the concentration of these elements in the soil is inversely correlated with the distance to the main emission sources (Table 2). These factors are related to the sources of trace elements in the studied soil samples.

Table 4. Matrix of loads for the principal component analysis (PCA). Significant values are in bold.

Elements	factor 1	factor 2	factor 3
As	0.946	0.025	0.008
Pb	0.974	0.001	-0.051
Cd	0.953	-0.124	-0.085
Ni	0.061	-0.057	-0.143
Hg	0.478	0.199	0.201
V	-0.049	0.688	0.427
Mn	-0.465	0.540	0.457
Zn	0.816	-0.034	0.141
Sr	0.190	-0.680	-0.012
Sb	0.934	0.080	-0.038
Cr	0.185	0.803	-0.058
Co	-0.212	0.645	0.568
Cu	0.903	-0.214	-0.053
K	-0.098	0.053	0.833
Ba	0.131	0.116	0.852
%Var	40	22	8

PCA factor 2 (22 % of the total variance) groups V, Mn, Cr, and Co, and these elements are also grouped by cluster analysis. The components in this factor are potentially derived from anthropogenic sources, namely traffic for V, Mn and Cr (Allen et al., 2001) and power plants for Co (ATSDR, 2004) but their ecological risk assessment indexes (see section 3.4.2) are lower than critical values considered as a proof of significant enrichment (Yay et al., 2008). Moreover the soil levels of these elements are not so dependent of distance to the industrial pollution sources that the elements belonging to factor 1 (Table 2), indicating the influence of natural or diffuse anthropogenic sources (e.g. traffic in the area). PCA factor 3 (8 % of variance) is composed of V, Mn, K and Ba, these elements being also grouped by the cluster analysis. This elemental profile suggests a common emission source related to traffic due to the presence of

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V and Mn (Allen et al., 2001), but V and Mn are also included in factor 2 so a mixing of sources cannot be excluded. K and V has been also assigned to a mineral-crystal fraction in a previous study (Parra et al, 2014). Sr and Ni appear together in the cluster analysis and they belong to factor 4 of the PCA (7 % of the variance, data not shown), so a common origin is possible, but we have insufficient data to identify that source.

3.4. Ecological risk assessment

Ecological risk assessment derived from the presence of the investigated elements in Puchuncaví-Ventanas area soils was estimated by using the geoaccumulation index (I_{geo}), the enrichment factor (EF), and finally the contamination factor (C_f), contamination degree (C_{deg}) and Integrated Pollution Index (IPI).

3.4.1. Geoaccumulation index (I_{geo})

About the index of geoaccumulation, the results of the calculations are presented in Fig. 4. Cu is the only element showing I_{geo} values above 5 (extreme contamination) in La Greda (LG) and Los Maitenes (LM), with I_{geo} values for Cu declining with distance from the copper refinery source but still high in Puchuncaví village (PU, I_{geo} value 3.7; heavy contamination).

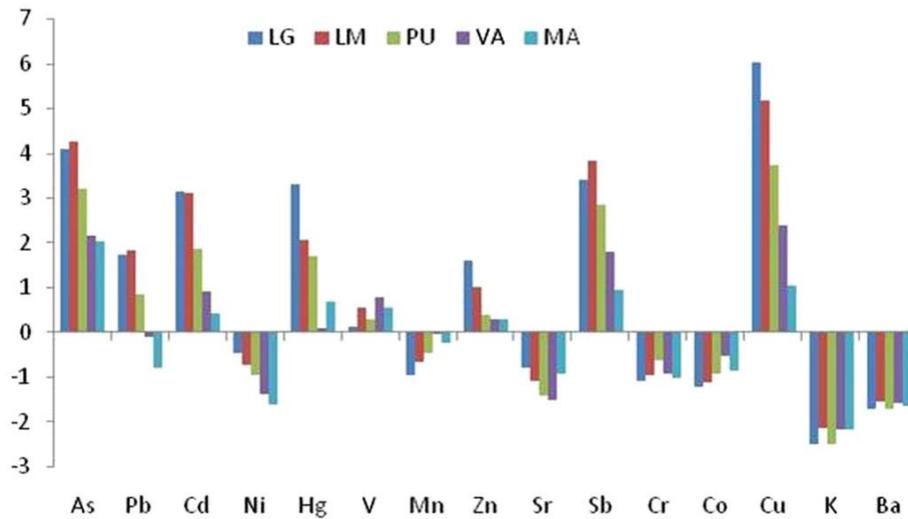


Fig. 4. Indexes of geoaccumulation (I_{geo}) for the element assayed.

Moderate Cu contamination was detected in Valle Alegre (PU, I_{geo} value 2.3), and even in the reference area of Maitencillo (PU, I_{geo} value 1.0), suggesting a long-range effect of the copper smelter in the surrounding area. As, Cd, Hg and Sb show relatively high I_{geo} values at LG and LM (above 2, moderate to heavily polluted), with lower values at the less polluted sampling areas Puchuncaví village (PU) and Valle Alegre (VA), and mostly negative I_{geo} values (no contamination) at the Maitencillo (MA) reference area. I_{geo} values for Pb, V and Zn range from no contamination to moderate, whereas Ni, Mn, Sr, Cr and Co present negative I_{geo} values (no contamination).

3.4.2. Enrichment factor (EF)

Enrichment factors (EF) values in the soils of the study area are shown in Fig. 5. Again, Cu is the only element showing extremely high index value in the locations more affected by the industrial activities (113 in LG and 78 in LM).

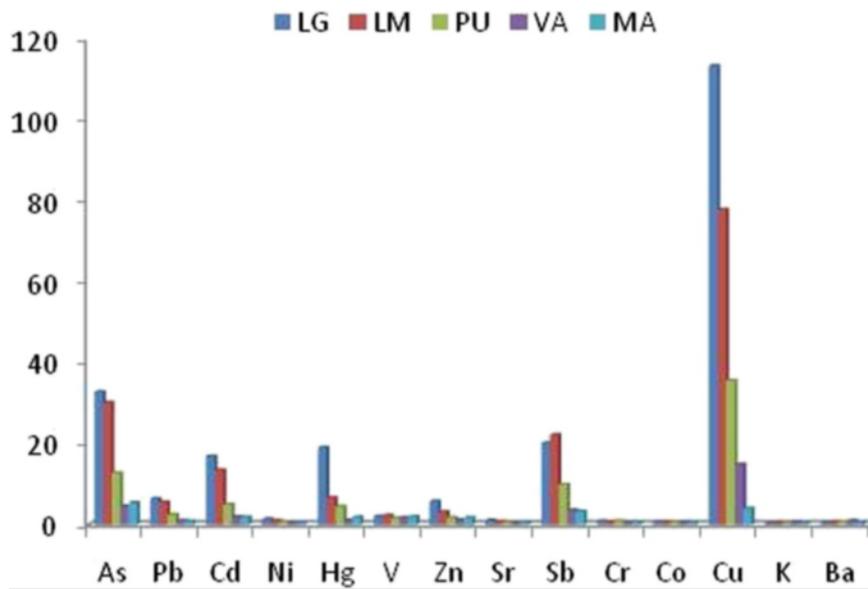


Fig. 5. Enrichment factors (EF) of the elements assayed.

EF values for Cu decrease with the distance but they show very high enrichment in Puchuncaví village (PU, EF value 36) and significant enrichment in Valle Alegre (VA, EF value 15). Even the reference area soils at Maintencillo (MA) show a moderate Cu enrichment (EF 4). EF values for the rest of the studied elements are in accordance with I_{geo} values. We inspected the overall

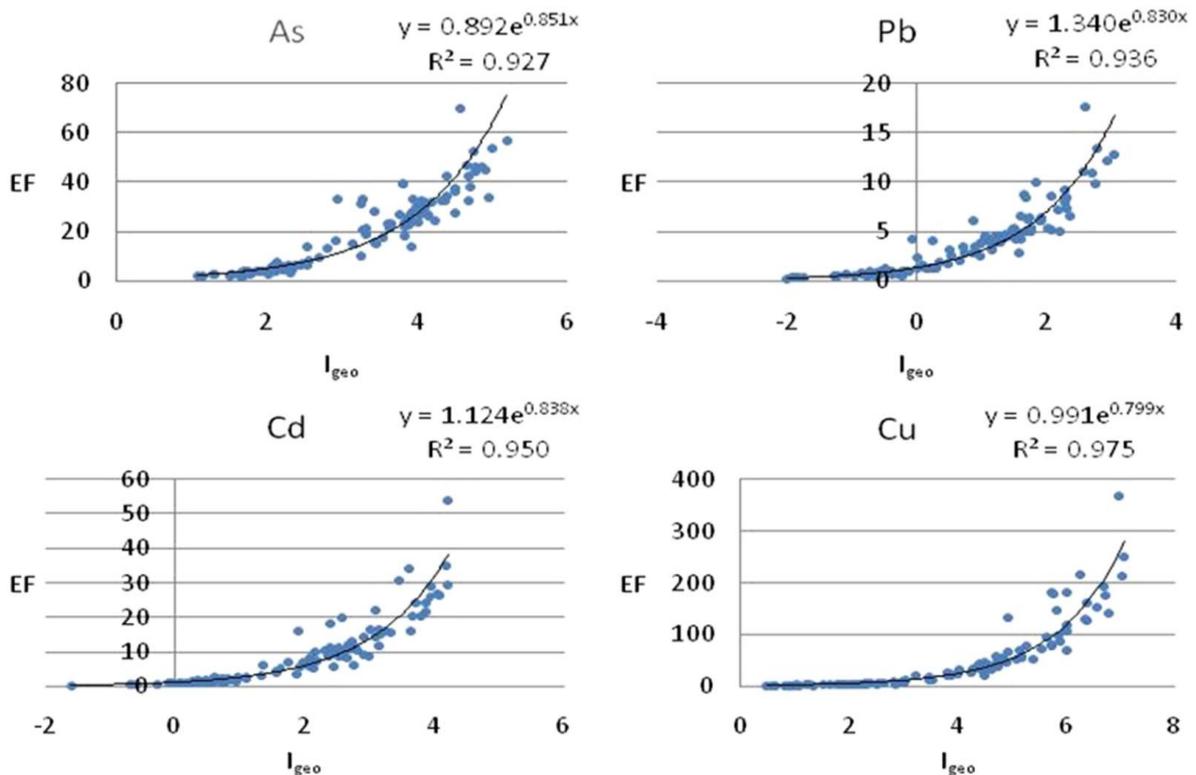


Fig. 6. Correlation between EF and I_{geo} .

correlation between EF and I_{geo} for all the individual samples assayed, and the results for representative elements are shown in Fig. 6. The indexes showed a strong exponential correlation indicating that the environmental information provided by both of them is similar.

3.4.3. Contamination factor (C_f), contamination degree (C_{deg}) and integrated pollution index (IPI)

Contamination factors (C_f) values are presented in Table 5. The results at the most impacted locations of La Greda (LG) and Los Maitenes (LM) are mostly within the category of very high contamination ($C_f > 6$), especially for Cu and As. C_f values tend to decrease with distance to the pollution sources, but they are still indicating moderate (C_f 1-3) to considerable (C_f 3-6) pollution

Table 5. Contamination factors and contamination degrees of metals of soil samples collected within the study area (Low Concentration = LC, Moderate Concentration = MC, Considerable Concentration= CC, Very High Concentration = VH).

		C_f (As)	C_f (Pb)	C_f (Cd)	C_f (Hg)	C_f (Zn)	C_f (Sb)	C_f (Cr)	C_f (Cu)	C_{deg}
LG	Value	25.42	4.99	13.12	14.76	4.47	15.62	0.7	98.13	177.2
	Contamination	VH	CC	VH	VH	CC	VH	LC	VH	VH
LM	Value	28.49	5.24	12.72	6.24	3.02	20.95	0.77	53.94	131.36
	Contamination	VH	CC	VH	VH	MC	VH	LC	VH	VH
VA	Value	6.62	1.38	2.83	1.60	1.82	5.2	0.79	7.82	28.06
	Contamination	VH	MC	MC	MC	MC	CC	LC	VH	CC
PU	Value	13.75	2.68	5.39	4.88	1.93	10.75	0.95	19.87	60.2
	Contamination	VH	CC	CC	CC	MC	VH	LC	VH	VH
MA	Value	6.02	0.87	2.00	2.38	1.82	2.88	0.73	3.04	19.74
	Contamination	VH	LC	MC	MC	MC	MC	LC	CC	CC

for some elements even at the reference area of Maitencillo. Contamination degrees (C_{deg} , sum of individual C_f s) show very high but decreasing contamination in LG, LM and PU, whereas considerable contamination is assigned to Valle Alegre (VA) and Maitencillo (MA). It seems that the contamination factor and degree are more strict ecological impact indexes than I_{geo} or EF for the assessment of the study area. C_f shows a strong linear correlation with EF (Fig. 7) and a perfect match with I_{geo} , so we can conclude that the three indexes are producing comparable environmental information.

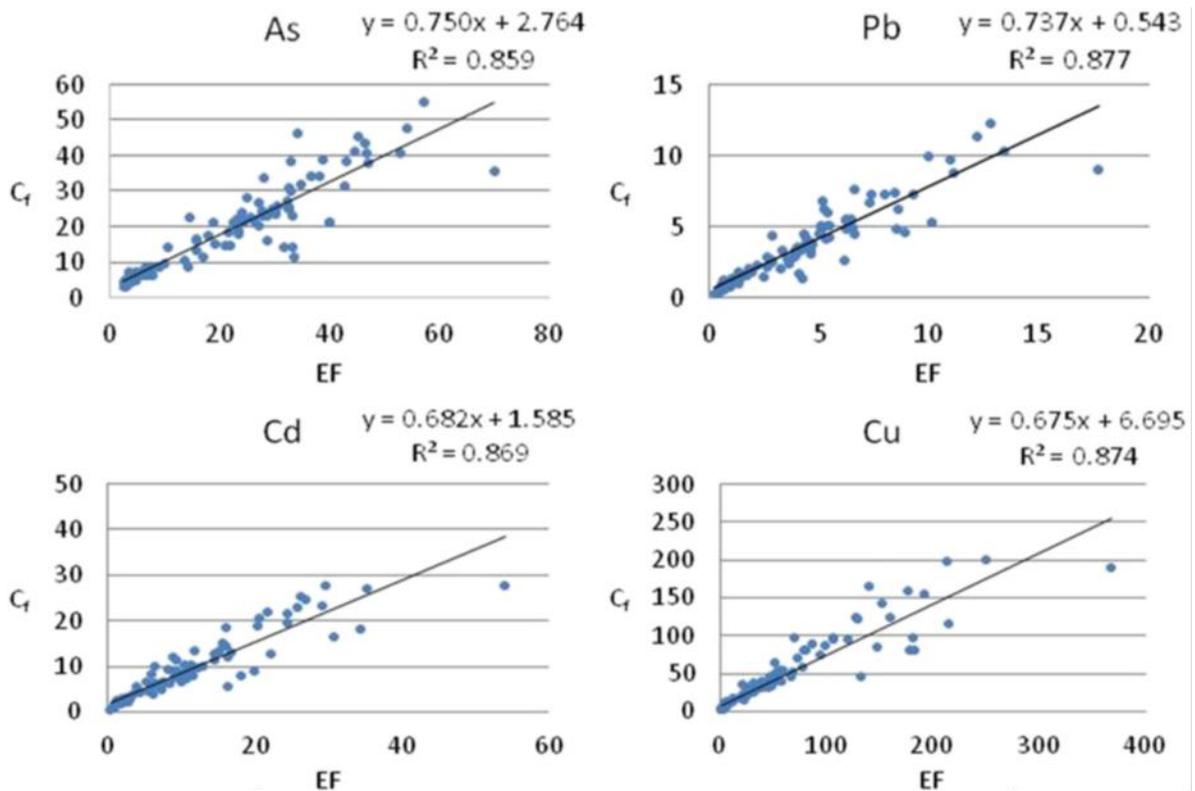


Fig. 7. Correlation between EF and Cf.

Finally, the integrated pollution index (IPI, mean of C_f values), shown in Fig. 8, clearly shows the overall level of contamination in the soil sampling sites within the study area. IPI are in the order LG > LM > PU > VA > MA. LG (IPI 20.41), PU (IPI 6.95), and LM (IPI 15.86) are classified as sites affected by extremely high level of pollution (IPI >5). VA (4.00) and MA (2.14) are classified as

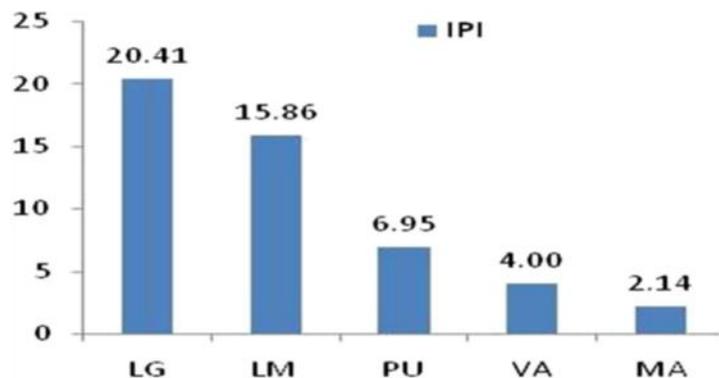


Fig. 8. Integrated pollution index of the sampling locations within the study area. La Greda (LG), Los Maitenes (LM), Puchuncaví village (PU), Valle Alegre (VA) and Maitencillo (MA).

highly polluted sites (IPI 2-5), indicating the strict nature of the IPI (similar to C_{deg}) compared to I_{geo} or EF. As previously discussed, SW wind direction can play an important role for the movement of pollutant from industrial area to sampling sites since IPI level is higher in PU (8 km to the pollution sources) than in VA (6.72 km to the pollution sources).

4. Conclusions

A long term (2007-2011) soil monitoring campaign conducted around the industrial area of Puchuncaví-Ventanas in central Chile, based on the measurement of elemental concentration profiles, PCA and cluster statistics, and application of several quantitative risk assessment indexes (geoaccumulation index, enrichment factor, contamination factor, contamination degree and integrated pollution index), has revealed significant ecological impacts, more intense at locations close to the main pollution sources and under the influence of dominant winds but also noticeable at reference background locations more than 10 km away from the industrial area. A copper refinery and a coal fired power plant complex have been identified as major pollution sources by the statistical analysis. The observed evolution of soil concentrations of elemental pollutants in the studied period, showing a decline from 2009 to 2011, can be related with improved environmental technologies adopted by the industries in recent years. A critical comparison among the ecological risk assessment indexes used showed good correlation among them, so they give comparable environmental information.

Acknowledgements

This work is supported by the Spanish Agency of International Cooperation for Development (AECID project A1/037813/11) and the Spanish Ministry of Science and Innovation (project CTQ2011-25388).

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3.2. Article 2

Environmental Pollution 218 (2016) 322-330

<http://www.sciencedirect.com/science/article/pii/S0269749116305693>

Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncaví-Ventanas industrial complex, Chile

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Abstract

The Puchuncaví Valley in central Chile, heavily affected by a range of anthropogenic emissions from a localized industrial complex, has been studied as a model environment for evaluating the spatial gradient of human health risk, which are mainly caused by trace elemental pollutants in soil. Soil elemental profiles in 121 samples from five selected locations representing different degrees of impact from the industrial source were used for human risk estimation. Distance to source dependent cumulative non-carcinogenic hazard indexes above 1 for children (max 4.4 – min 1.5) were found in the study area, ingestion being the most relevant risk pathway. The significance of health risk differences within the study area was confirmed by statistical analysis (ANOVA and HCA) of individual hazard index values at the five

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sampling locations. As was the dominant factor causing unacceptable carcinogenic risk levels for children ($< 10^{-4}$) at the two sampling locations which are closer to the industrial complex, whereas the risk was just in the tolerable range ($10^{-6} - 10^{-4}$) for children and adults in the rest of the sampling locations at the study area. Furthermore, we assessed gamma ray radiation external hazard indexes and annual effective dose rate from the natural radioactivity elements (^{226}Ra , ^{232}Th and ^{40}K) levels in the surface soils of the study area. The highest average values for the specific activity of ^{232}Th (31 Bq kg^{-1}), ^{40}K (615 Bq kg^{-1}), and ^{226}Ra (25 Bq kg^{-1}) are lower than limit recommended by OECD, so no significant radioactive risk was detected within the study area. In addition, no significant variability of radioactive risk was observed among sampling locations.

Keywords: Elemental pollutants; soil pollution; industrial pollution; human health environmental risk assessment; gamma ray radiation; natural radioactivity elements

Introduction

It is well known that some trace elements (notably heavy metals) can degrade the quality of environmental compartments as water, soil, atmosphere or living organisms. Increasing environmental concerns about the impact of trace elements on soil quality stems from the fact that they are responsible for more serious and longtime toxicity effects on soils respect to other pollutants (European Commission, 2013; Li et al., 2014). Moreover, there has been a rise in anthropogenic activities like mining, agriculture, industrialization and urbanization, all of them related to increasing of heavy metals inputs to the soils (Facchinelli et al., 2001; Li et al., 2014). Exposure to soil pollutants can affect the human body by ingestion, dermal, or inhalation contacts (Ordóñez et al., 2011 Li et al., 2014), although direct inhalation is only relevant in arid and windy locations (USEPA, 2011; Meza-Montenegro et al., 2012; Salmanighabeshi et al., 2015). Soil pollution also results on indirect impact to humans from the

food web via consumption of vegetables, especially in urban–periurban environments influenced by emerging industry (Yousaf et al, 2016).

In addition, natural radionuclides affect humans due to their presence in different environmental matrices (Ravisankar et al., 2012). Among the rock constituent minerals, there are some natural radionuclides as ^{226}Ra , ^{232}Th and ^{40}K that contribute to ionizing radiation exposure of living beings on the Earth surface (Veiga et al., 2006).

The environmental distribution of radionuclides has clear radiological consequences due to body exposure to gamma-rays (Singh et al., 2005), and particularly the assessment of radiation hazard coming direct or indirectly from natural sources, especially soil and sand. Hence, the assessment of gamma radiation dose from soil or other natural sources is important since natural radiation is the largest source of the external dose of the world population (UNSCEAR, 2000; Veiga et al., 2006). The amount of these dose rates are directly related to the concentration of natural radionuclides in soil.

Punchuncaví-Ventanas valley is an important industrial area of Chile consisting of a wide range of industrial factories and activities, which pose potential risks to the human health. The main industrial activities in this area are a Cu smelter with a yearly production capacity of around 4×10^5 metric tons of fine copper (Cochilco, 2015) and a coal-fired power plants complex (three operating units). The area suffers long-term atmospheric deposition from the industrial complex since its opening in 1964, so several researchers (Chiang et al, 1985; González and Bergvist, 1986; González and Ite, 1992; Ginocchio et al., 2004; Parra et al., 2014; Salmanighabeshi et al., 2015; Rueda-Holgado et al, 2016) have described the emission and deposition of trace elements. However, a detailed report about quantitative human risk assessment derived from exposition to soil in the area is missing; there are just some preliminary reports derived from government-supported studies. Among them, Gamboa et al (2013) found that the pollution levels in the area would justify a detailed ecological and human

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risk assessment evaluation, whereas Salgado et al (2014) found a significant carcinogenic risk from arsenic exposition (risk indexes around 10^{-4}). We aim to confirm the hypothesis that elemental pollution in the area is causing human health risk even at relatively long distance. So we present here the first detailed and systematic study on spatial gradient of health risk caused by long-term exposition of the resident people to the complex mix of elemental pollutants deposited onto the soils at the selected area, detailing the spatial distribution of the risk in different sampling locations. As a complementary study, we present also the first report about radiological risk from radioisotopes in the soils of the area.

Materials and methods

Sampling area and soil characteristics

The Puchuncaví-Ventanas industrial complex is located on the Chilean mainland coast ($34^{\circ}45'$ S, $71^{\circ}29'$ W), 160 km NW from Santiago de Chile (capital of Chile) and 58 km N from Valparaíso (Regional Capital). It is placed at the western limit of the Puchuncaví valley, which occupies 301 km² and has a population of 13,000 inhabitants (Fig. 1). Four sampling points were selected, representing different degrees of impact, taking into account distance and dominant SW winds in the area. Most impacted sites were located at La Greda (LG, $32^{\circ} 44' 57''$ S, $71^{\circ} 28' 30''$ W), 1.69 km NE from the industrial complex, and Los Maitenes (LM, $32^{\circ} 45' 41''$ S, $71^{\circ} 27' 18''$ W), 2.39 km E from the industrial complex. Less impacted sites were located at Puchuncaví village (PU, $32^{\circ} 43' 17''$ S, $71^{\circ} 24' 43''$ W), 8 km NE from the industrial complex and Valle Alegre (VA, $32^{\circ} 48' 30''$ S, $71^{\circ} 26' 10''$ W), 6.72 km SE. Maitencillo (MA) sampling area ($32^{\circ} 36' 5''$ S, $71^{\circ} 25' 56''$ W), 18 km N from the industrial sector, was selected as background area. Topsoil in the area (0–30 cm) are characterized by low nutrient availability and limited water holding capacity (coarse texture sandy loam: clay 13–18%, sand 65–74% and silt 13–17%). Soil organic matter varies from 1.0 to 1.8, and soil pH are in the range 4.6 -5.5 (Salmanighabeshi et al,

2015). Regular value of wet deposition in the area reported by Chilean meteorological services is 312.5 mm (Rueda-Holgado et al, 2016).



Fig. 1. Punchuncaví-Ventanas industrial complex and soil sampling points.

Soil sampling and analytical protocols

Sampling, acid digestion, ICP-MS assay protocols for measurement of elemental pollutant concentrations in soil (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Cu, and Ba), and analytical quality assurance have been given in detail in a previous publication (Salmanighabeshi et al., 2015). This previous work was focused on long-term (2007 to 2011) environmental assessment experimental campaigns with annual collection of soil. 121 samples were assayed (26 from LG, 26 from LM, 25 from PU, 23 from VA and 21 from MA).

Results and discussion

Samples for gamma spectrometry were dried at 80°C for 48 hours and sieved through a 2 mm mesh. 150 g of each soil sample were weighed and hermetically sealed in radon-impermeable plastic containers. The containers were kept sealed for 4 weeks in order to allow equilibrium of the ^{238}U and ^{232}Th series and their respective progeny.

Radioactivity measurements were carried out with a Ge (HP) semiconductor detector, 45% relative efficiency at 1.33 MeV, coupled to a 4096-channel analyser. The detector efficiency was calibrated using certified mixed gamma standards QCY-48, supplied by Amersham, for the energy range 60-1900 keV. The spectra were collected for 48 hours. The reliability of the spectrometer was checked by measuring the activity of an IAEA reference material, IAEA-6-SOIL. ^{232}Th activity was measured by means of the gamma emissions of ^{228}Ac (911 keV) and ^{208}Tl (583.01 keV); ^{226}Ra was measured by means of gamma emissions from ^{214}Bi (609.3 and 1764.5 keV) and ^{214}Pb (351.9 keV) assuming that both radioactive families are left in secular equilibrium. Lastly, we determined ^{40}K by using the 1460.7 keV gamma emissions. The specific activities (A) for ^{226}Ra , ^{232}Th and ^{40}K were calculated using the relation:

$$(1) \quad A = \frac{N}{t \times P \times \epsilon}$$

Where N is the net counts, t is the data collection time, P is the emission probability and ϵ is the efficiency of the detector for the corresponding peak. The error was calculated taking into account the combined uncertainty in the efficiency of the detector and the uncertainty in the net count rate. In the case of using several gamma-ray peaks for the calculations of the activity (i.e. ^{226}Ra and ^{232}Th), uncertainty in the yield is also included. The analysis of gamma spectrum obtained was performed using the software Gamma Vision (ORTEC).

Health risk assessment

Health risk from soil ingestion, dermal, and inhalation contact for adults and children were calculated according to standardized procedures. Exposure doses for ingestion, dermal, and

inhalation pathways were calculated by Eq (2), Eq (3), and Eq (4) respectively (Ordóñez et al., 2011).

Ingestion absorption:

$$(2) \quad \mathbf{ADI}_{\text{ing}} = \frac{(\mathbf{C}_s \times \mathbf{IngR} \times \mathbf{EF} \times \mathbf{ED})}{(\mathbf{BW} \times \mathbf{AT})} \times 10^{-6}$$

Where $\mathbf{ADI}_{\text{ing}}$ is the average daily intake of the element (i) from the soil (mg kg^{-1} of body weight-day); \mathbf{C}_s is the concentration of the element in soil (mg kg^{-1}); \mathbf{IngR} is the ingestion rate of soil for adults (100 mg day^{-1}), for shepherd or farmer adults (330 mg day^{-1}), and for children (200 mg day^{-1}); \mathbf{EF} is the exposure frequency ($350 \text{ days year}^{-1}$); \mathbf{ED} is the exposure duration to non-carcinogenic pollutants for adults (30 years) and for children (6 years), and for carcinogenic for adults (24 years) and for children (6 years); \mathbf{BW} is the average body weight of the exposed person for adults (70 kg) and for children (15 kg); and \mathbf{AT} is the time period over which the dose is averaged for non-carcinogenic for adults (10950 days) and for children (2190 days). Exposure data were adapted from Ordoñez et al (2011) and US Environmental Protection Agency guidelines (USEPA, 1996; USEPA, 1997).

About \mathbf{C}_s values, we averaged the concentration of the element in the samples collected at each sampling location during the yearly 2007-2011 campaigns. As we have previously published (Salmanighabeshi et al., 2015), the overall temporal trend in elemental concentrations observed in the studied area during the timeframe of the sampling is not clear, but pollutant elements concentration in soils shows a slight trend to decrease in recent years, probably due to improved environmental protection technologies adopted by the emission sources in the area.

Dermal absorption:

$$(3) \quad \mathbf{ADId}_{\text{der}} = \frac{\mathbf{C}_s \times \mathbf{SA} \times \mathbf{SL} \times \mathbf{ABS} \times \mathbf{EF} \times \mathbf{ED}}{\mathbf{BW} \times \mathbf{AT}} \times 10^{-6}$$

Results and discussion

Where ADI_{der} is the average daily intake of elements from dermal absorption ($mg\ kg^{-1}$ of body weight-day), C_s is the concentration of the element in soil ($mg\ kg^{-1}$), SA is the skin surface area available for contact, for adults ($5700\ cm^2$), for shepherd or farmer adults ($3300\ cm^2$), and for children ($2800\ cm^2$), SL is soil to skin adherence factor for adults ($0.07\ mg\ cm^{-2}\ day^{-1}$), for shepherd or farmer adults ($0.3\ mg\ cm^{-2}\ day^{-1}$) and for children ($0.2\ mg\ cm^{-2}\ day^{-1}$), ABS is dermal absorption factor, 0.03 for As and 0.001 for other elements (USEPA, 1996; USEPA, 1997; Ordóñez et al, 2011), EF , ED , BW and AT as detailed in the previous paragraph for ingestion.

Inhalation absorption:

$$(4) \quad ADI_{inh} = \frac{(C_s \times InhR \times EF \times ED)}{(PEF \times BW \times AT)}$$

Where ADI_{inh} is the average daily intake of the element (i) from soil inhalation ($mg\ kg^{-1}$ of body weight-day), C_s is the concentration of the element in soil ($mg\ kg^{-1}$), $InhR$ is the inhalation rate of soil for adults ($20\ mg\ day^{-1}$) and for children ($7.6\ mg\ day^{-1}$), PEF is particle emission factor (1.36×10^9) (USEPA, 1992). EF , ED , BW and AT as in the previous paragraph for ingestion.

Non-carcinogenic risk assessment

Non-carcinogenic risk is usually evaluated by the hazard quotient (HQ) (unitless). Hazard quotient was calculated by Eq 5 as the exposure value divided by the reference dose RfD ($mg\ kg^{-1}$ of body weight day^{-1}), which is the toxicity threshold value of each chemical. RfD values were taken from Ordóñez et al., 2011, based on the US Risk Assessment Information System (US DoE, 2010).

$$(5) \quad HQ = \frac{ADI}{RfD}$$

Adverse health effects are unlikely if $HQ \leq 1$. $HQ > 1$ indicates probable adverse health effects.

$HQ > 10$ indicates high chronic risk (USEPA, 2001; Meza-Montenegro et al., 2012).

We have calculated the total chronic hazard as the hazard index (HI) for each exposure pathway by following the procedures outlined in USEPA (1989) and USEPA (2004). The HI estimate the risk of mixing contaminants by summing individual HQ (Eq (6)) (USEPA, 1989):

$$(6) \quad \mathbf{HI = \sum HQ_i}$$

This method assumes that the health effects of the different pollutants are additive. HI > 1 indicates potential chronic effects. HI < 1 means that adverse health effects are unlikely even for sensitive populations.

Cumulative HI was calculated as the sum of the individual HI values for each of the main exposure pathways (ingestion and dermal contact, Eq (7)) (USEPA, 1989; USEPA, 2005):

$$(7) \quad \mathbf{Cumulative\ HI = \sum HI}$$

Cumulative HI ≤ 1 means that non-carcinogenic effects are unlikely for the exposed population. Cumulative HI > 1 implies a significant probability of non-carcinogenic effects, increasing with HI value. Cumulative HI can exceed the target hazard level due to one or more pollutant with HQ exceeding the target hazard level, or by the sum of several pollutants specific HQ_s under the target hazard level. The total HI might therefore overestimate the potential for non-carcinogenic health effects.

Carcinogenic risk assessment

Carcinogenic risk was calculated by Eq 8, as the probability of getting cancer through exposure to site related chemicals. The slope factor (SF) converts the estimated daily intake of a pollutant, averaged over a lifetime of exposure, directly to the incremental risk of an individual for developing cancer. SF values were taken from Ordóñez et al., 2011, based on the US Risk Assessment Information System (US DoE, 2010).

$$(8) \quad \mathbf{Risk = ADI \times SF}$$

Results and discussion

Where risk is the unitless probability of developing cancer over a lifetime due to a toxin and SF is the carcinogenicity slope factor ($\text{mg kg}^{-1} \text{ day}^{-1}$). ADI has been previously defined. Values of risks are categorized in three groups: risk $> 1.0 \times 10^{-4}$ are viewed as unacceptable, $1.0 \times 10^{-4} < \text{risk} < 1.0 \times 10^{-6}$ is considered an acceptable range depending on exposure conditions (USEPA, 2001), and risk $< 1.0 \times 10^{-6}$ is considered to pose not significant health effects. Total risk (risk_{tot}) was calculated as the sum of the risks pathways we have considered (USEPA, 2001).

Health risk from radioactivity

Gamma radiation hazards due to the specified radionuclides ^{226}Ra , ^{232}Th and ^{40}K are assessed by several different indices (OECD, 1979). Ra equivalent activity (Ra_{eq}) represents the specific activities of ^{226}Ra , ^{232}Th and ^{40}K by a single quantity, taking into account their combined radiation hazards, as defined as Beretka and Mathew (1985):

$$(9) \quad \text{Ra}_{\text{eq}} = A_{\text{Ra}} + 1.43A_{\text{Th}} + 0.077A_{\text{K}}$$

Where A_{Ra} , A_{Th} , and A_{K} (Bq.kg^{-1}) are the activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K , respectively. The convened maximum safe value of Ra_{eq} in building materials is 370 Bq kg^{-1} (Oresegun and Babalola, 1998).

Beretka and Mathew (1985) also defined indices for external and internal radiation hazards. The external hazard index is obtained from Ra_{eq} assuming that the maximum value allowed (1) corresponds to Ra_{eq} upper limit (370 Bq kg^{-1}). So the radiation exposure due to the radioactivity from construction materials is limited to 1.0 mSv y^{-1} . The external hazard index can be defined as:

$$(10) \quad \text{H}_{\text{ex}} = \frac{A_{\text{Ra}}}{370} + \frac{A_{\text{Th}}}{259} + \frac{A_{\text{K}}}{4810} \leq 1$$

To estimate the dose of radiation received for a man it is necessary to calculate the adsorbed dose rate (D). Conversion factors to transform specific activities A_{Ra} , A_{Th} , and A_{K} , in absorbed

dose rate at 1 m above the ground (in nGy h⁻¹ by Bq kg⁻¹) were calculated by Monte Carlo method (UNSCEAR, 2000), equation (11).

$$(11) \quad D \text{ (nGy h}^{-1}\text{)} = 0.0417A_K + 0.462A_{Ra} + 0.604A_{Th}$$

The absorbed doses were converted into annual effective dose E using equation (12). The conversion factor from absorbed dose to effective dose, (Q) is 0.7 Sv Gy⁻¹ for environmental exposure to gamma-rays of moderate energy and T is one year, i.e., 8760 h, as proposed in UNSCEAR (1993).

$$(12) \quad E \text{ (mSv y}^{-1}\text{)} = T \times Q \times D \times 10^{-6}$$

Statistical analysis

Hierarchical Cluster Analysis (HCA) was performed by using Euclidean distance and the ward agglomerative algorithm. Analysis of variance (ANOVA) was used to identify significant differences among hazard indexes classified by soil sampling locations. Both types of analysis were performed by using the XLSTAT for Excel (version 2009.1.02).

Results and discussion

Non-carcinogenic risk

The soils around the industrial complex of Puchuncaví-Ventanas, especially at the closer locations La Greda and Los Maitenes, are classified as impacted due to alteration of normal levels of elements, notably Cu (mainly emitted from the large Cu smelter in the area), As, V and Ba, some of them above international soil quality standards (Salmanighabeshi et al, 2015). Recent reports have also found that potentially toxic elements are present in the finest fractions of the La Greda and Los Maitenes soils (Parra et al, 2014). Hazard quotients HQ_{der} (dermal), HQ_{ing} (ingestion), and HQ_{inh} (inhalation) derived from exposition of children, adults and shepherds / farmers to individual trace elements in soil at the study area were calculated

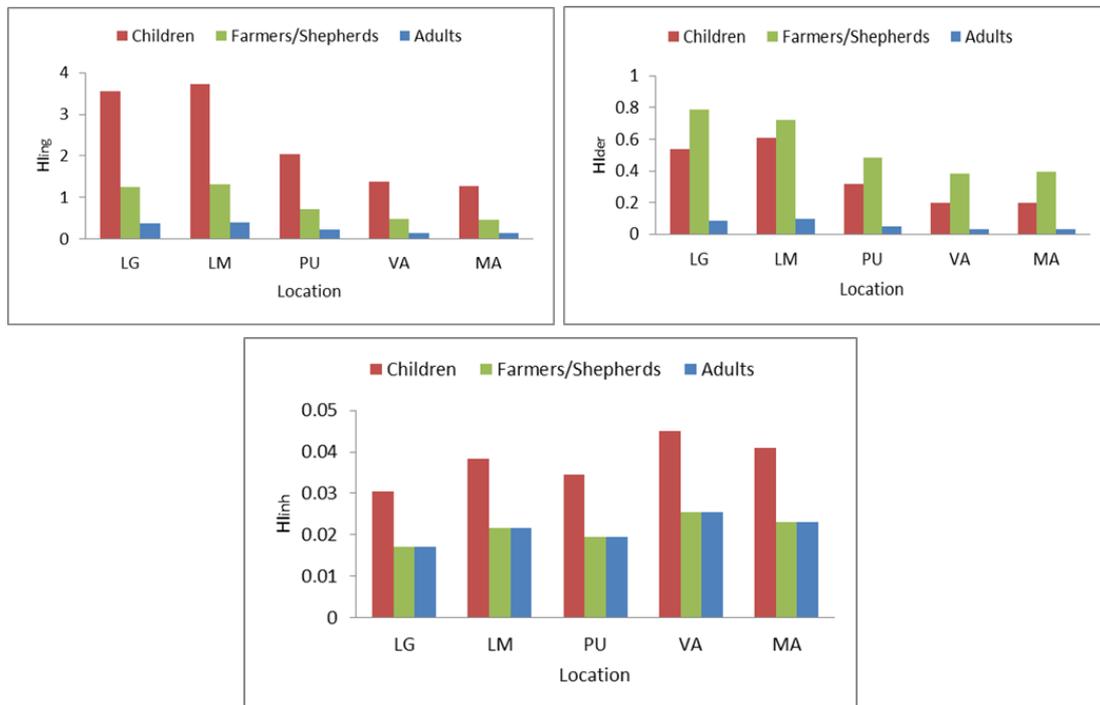
Results and discussion

as the most basic parameters for risk estimation, and the results are presented in the supplementary material (Table S1). Only As present HQ values above 1 for children exposure through soil ingestion at sampling locations La Greda ($HQ_{\text{ing-As}} = 2.17$), Los Maitenes ($HQ_{\text{ing-As}} = 2.43$) and Puchuncaví ($HQ_{\text{ing-As}} = 1.17$), indicating probable occurrence of adverse health effects. HQs for adults, shepherds or farmers are in all cases below 1. Hazard Index (HI) for ingestion, dermal and inhalation were calculated from the individual HQ values and the results are shown in Table 1 and Fig 2. The most relevant risk is ingestion with HI_{ing} values for children above 1 in all locations, meaning potential chronic effects. HI_{ing} values for farmers / shepherds are also higher than 1 in the most impacted locations of La Greda (LG) and Los Maitenes (LM). The rest of HI_{ing} values for children, adults and farmers / shepherds are below 1. The values are in the range reported for HI values around Chinese coal, copper, gold and iron mining areas (Li et al, 2014), but higher than calculated values in urban and periurban areas (Yousaf et al, 2016). HI_{ing} and HI_{der} present a similar spatial trend within the study area, with decreasing values when moving to the less exposed sites of PU, VA, and MA. In the opposite, inhalation risk did not show a clear tendency, probably due to the very low calculated values. A detailed statistical of spatial differences of ingestion risk for children was carried out by calculating HI_{ing} values for individual soil samples (total 121) and applying descriptive analysis (box-whisker plots, Figure 3), ANOVA test for significant risk differences among locations (Table 2), and hierarchical cluster analysis (Figure 4).

Table 1. HI (ingestion, dermal and inhalation) and cumulative HI values for children, adults and shepherds/farmers in soil collected at the sampling stations around the Puchuncaví-Ventanas industrial area: La Greda (LG), Los Maitenes (LM), Puchuncaví village (PU), Valle Alegre (VA) and reference station at Maitencillo (MA).

Location	Category	HI ing	HI der	HI inh	Cum HI
LG	Children	3,55E+00	5,38E-01	3,05E-02	4,12E+00
	Adults	3,80E-01	8,22E-02	1,72E-02	4,80E-01
	Farmers	1,26E+00	7,88E-01	1,72E-02	2,06E+00
LM	Children	3,73E+00	6,10E-01	3,84E-02	4,37E+00
	Adults	3,99E-01	9,32E-02	2,17E-02	5,14E-01
	Farmers	1,32E+00	7,20E-01	2,17E-02	2,06E+00
PU	Children	2,04E+00	3,19E-01	3,45E-02	2,40E+00
	Adults	2,19E-01	4,87E-02	1,94E-02	2,87E-01
	Farmers	7,22E-01	4,81E-01	1,94E-02	1,22E+00
VA	Children	1,38E+00	1,99E-01	4,49E-02	1,63E+00
	Adults	1,48E-01	3,04E-02	2,53E-02	2,04E-01
	Farmers	4,89E-01	3,83E-01	2,53E-02	8,97E-01
MA	Children	1,28E+00	1,95E-01	4,09E-02	1,51E+00
	Adults	1,37E-01	2,98E-02	2,31E-02	1,90E-01
	Farmers	4,51E-01	4,92E-01	2,31E-02	9,66E-01

Fig. 2. Hazard indexes derived from ingestion (HI_{ing}), dermal contact (HI_{der}) and inhalation (HI_{inh}) in soil sampling areas around the Puchuncaví-Ventanas industrial complex. $HI > 1$ potential chronic effects.



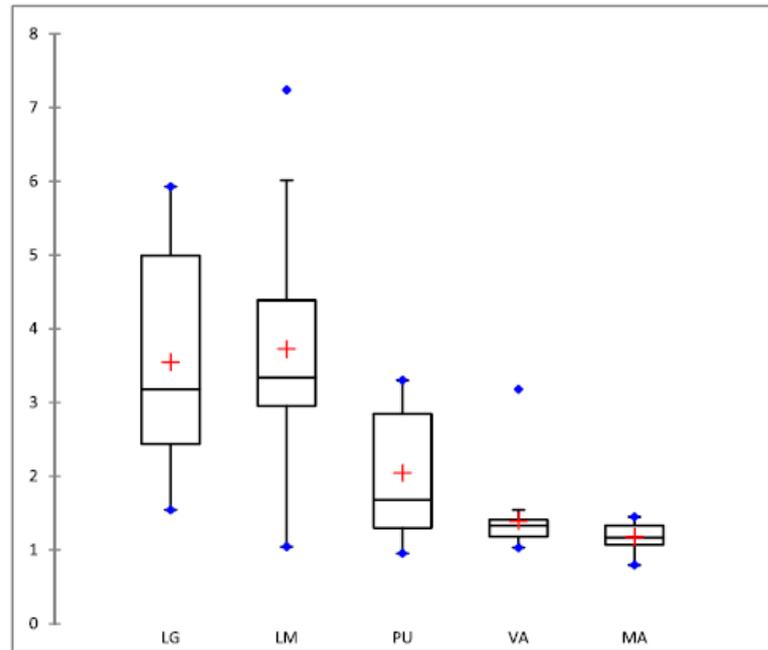


Fig. 3. Distribution of H_{ing} for children calculated from individual samples taken around the Puchuncavi-Ventanas industrial complex.

The box-whisker plot showed a higher dispersion of H_{ing} values at the more polluted sampling locations of LG and LM, probably related with temporal variability of the pollution sources. The dispersion of HI results tends to decrease with distance to the pollution sources, being very low at VA and MA sampling locations. The ANOVA test (Table 2) shows that the two most polluted sampling locations of LG and LM present a similar level of ingestion risk ($p = 0.972$), whereas the risk at LG-LM is significant higher with respect to the less polluted areas, especially to the less polluted VA-MA zone but also with respect to the intermediate pollution sampling area PU. No significant risk difference was observed between VA and MA ($p = 0.960$), or between PU and VA ($p = 0.195$), but a significant difference close to the critical value 0.05 is observed between PU and MA ($p = 0.043$). A clear risk gradient is confirmed around the industrial area, with still dangerous values of H_{ing} (above 1) for children within a range of about 20 km from the industrial sources.

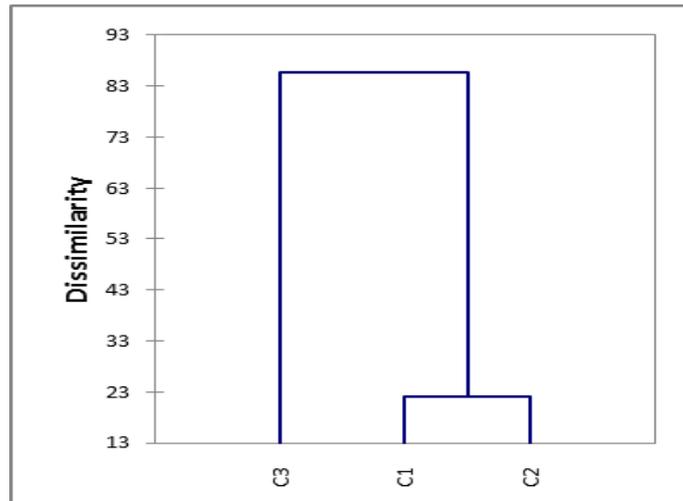


Fig. 4. Hierarchical cluster analysis of H_{ing} for children calculated from individual samples taken around the Puchuncaví-Ventanas industrial complex.

Cluster analysis (Figure 4) of individual H_{ing} values for children classified the data in three categories. Category C3 includes 9 samples from LG, 5 samples from LM, 17 samples from PU, 22 samples from VA (all samples from this location except one) and 21 samples from MA (all samples from this location). So this category is assigned to samples with low ingestion risk. Category C2 included 10 samples from LG, 15 samples from LM, 8 samples from PU and 1 sample from VA, all of them with intermediate values of H_{ing} . Finally, category C1 includes 7 samples from LG and 6 samples from LM with the highest values of H_{ing} .

Table 2. ANOVA test of H_{ing} for children calculated from individual samples taken around the Puchuncaví-Ventanas industrial complex. Significant differences are marked with *

	LG	LM	PU	VA	MA
LG		0,972	< 0,0001*	< 0,0001*	< 0,0001*
LM			< 0,0001*	< 0,0001*	< 0,0001*
PU				0,195	0,043*
VA					0,960
MA					

Results and discussion

Carcinogenic risk

Ingestion, dermal, and inhalation carcinogenic risks were calculated for exposure to As (ingestion, dermal, and inhalation) Cd and Ni (inhalation) in soil (Table 3) using available slope factors (Ordóñez et al., 2011). As represents the highest carcinogenic risk from soil exposition in the study area. Elevated levels of As around the industrial complex are mainly due to the industrial emissions, in view of previously published results about enrichment factors (Salmanighabeshi et al, 2015). There is a clear gradient of As enrichment factor around the industrial complex ranging from more than 30 (very high enrichment) in La Greda and Los Maitenes sampling points to 12 (significant enrichment) in Puchuncaví sampling point and less than 5 (moderate enrichment) in Valle Alegre and Maitencillo sampling points. The calculated carcinogenic risk indexes show extremely low carcinogenic risk through inhalation of As, Cd and Ni at all sampling locations (individual values in the range 10^{-9} to 10^{-11}), whereas dermal contact and ingestion risks are within the acceptable range (between 1.0×10^{-4} and 1.0×10^{-6}) for all locations, with higher values at the most impacted LG and LM locations. Total carcinogenic risk (Figure 5) for children is above the 10^{-4} threshold in LM and on the limit at LG. Carcinogenic risk values should be taken as approximate values due to uncertainty in the measurement of soil elemental concentrations that were previously reported (Salmanighabeshi et al, 2015). These values are comparable with carcinogenic risk levels reported at an open-cast coal mine in Inner Mongolia, China (Jia et al, 2015). Total carcinogenic risk is decreasing with distance to the industrial sources with a similar trend than observed for the non-carcinogenic risk. Nevertheless, all locations present total carcinogenic risk above the 10^{-6} no effects threshold, so the health risk exists even at 20 km from the industrial complex.

Table 3. Carcinogenic risk from exposition to soils for children and adults at sampling locations around the Pichuncavi-Ventanas industrial complex

			As	Cd	Ni
LG	RISK _{ing}	Children	8,36E-05		
		Adults	3,58E-05		
	RISK _{der}	Children	1,73E-05		
		Adults	1,06E-05		
	RISK _{inh}	Children	2,34E-10	2,58E-10	5,15E-10
		Adults	5,27E-10	5,82E-10	1,16E-09
RISK _{tot}	Children	1,01E-04			
	Adults	4,64E-05			
LM	RISK _{ing}	Children	9,37E-05		
		Adults	4,01E-05		
	RISK _{der}	Children	1,94E-05		
		Adults	1,19E-05		
	RISK _{inh}	Children	2,62E-10	2,50E-10	4,31E-10
		Adults	5,90E-10	5,65E-10	9,72E-10
RISK _{tot}	Children	1,13E-04			
	Adults	5,20E-05			
PU	RISK _{ing}	Children	4,52E-05		
		Adults	1,94E-05		
	RISK _{der}	Children	9,37E-06		
		Adults	5,72E-06		
	RISK _{inh}	Children	1,26E-10	1,06E-10	3,64E-10
		Adults	2,85E-10	2,39E-10	8,22E-10
RISK _{tot}	Children	5,46E-05			
	Adults	2,51E-05			
VA	RISK _{ing}	Children	2,18E-05		
		Adults	9,33E-06		
	RISK _{der}	Children	4,51E-06		
		Adults	2,76E-06		
	RISK _{inh}	Children	6,08E-11	5,56E-11	2,72E-10
		Adults	1,37E-10	1,25E-10	6,13E-10
RISK _{tot}	Children	2,63E-05			
	Adults	1,21E-05			
MA	RISK _{ing}	Children	2,23E-05		
		Adults	9,57E-06		
	RISK _{der}	Children	4,63E-06		
		Adults	2,82E-06		
	RISK _{inh}	Children	6,24E-11	4,68E-11	2,59E-10
		Adults	1,41E-10	1,06E-10	5,84E-10
RISK _{tot}	Children	2,69E-05			
	Adults	1,24E-05			

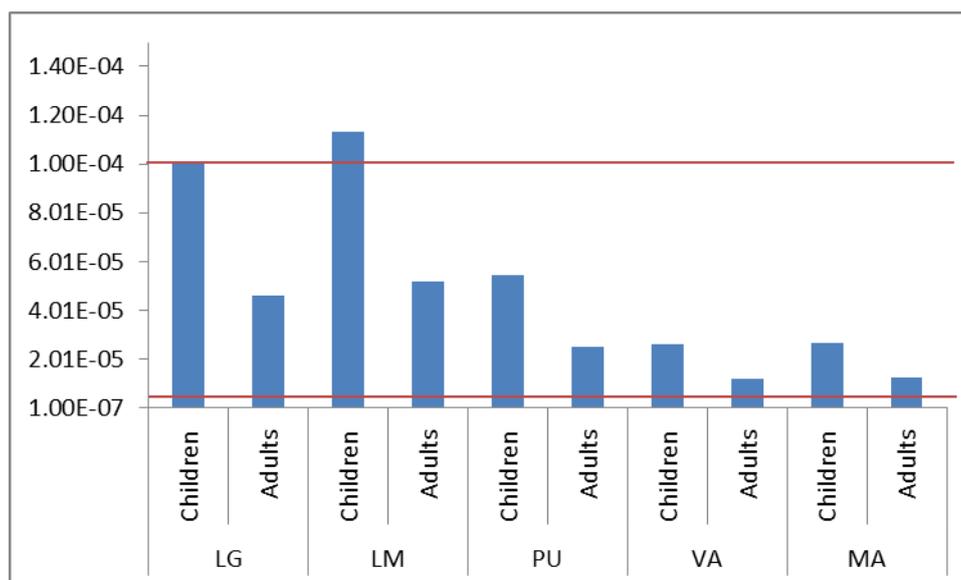


Fig.5. Total carcinogenic risk for children and adults exposition to arsenic. Risk $> 1.0 \times 10^{-4}$ is unacceptable, $1.0 \times 10^{-4} < \text{Risk} < 1.0 \times 10^{-6}$ is considered an acceptable, and Risk $< 1.0 \times 10^{-6}$ is considered to pose not significant health effects.

Health risk from radioactivity

Mean specific radioactivity values of ^{226}Ra , ^{232}Th and ^{40}K measured in the soil samples are shown in Fig 6. Similar values were obtained at the different locations sampled within the study area around the Puchuncaví-Ventanas industrial complex, so no influence of the distance to the industrial pollution sources was observed. The highest average values for the specific activity of ^{232}Th and ^{40}K are 31 and 615 Bq kg^{-1} , respectively, were measured in VA location, and the maximum value for ^{226}Ra is 25 Bq kg^{-1} were measured in MA. The lowest values of the specific activity of ^{232}Th and ^{226}Ra are 25 and 21 Bq kg^{-1} , respectively (LG station), 519 Bq kg^{-1} for ^{40}K in MA station. The values are similar to those reported for other soils of South America, e.g. Brazil, (Malanca et al., 1996) Venezuela (Sajó-Bohus et al., 1999), Argentina (Juri et al., 2008) and Uruguay (Odino Moure, 2010). The behaviour of mean values of the specific radium equivalent activities (Ra_{eq}) and the external hazard index at the different sampling locations is the same observed for ^{226}Ra , ^{232}Th and ^{40}K , with no significant differences detected for Ra_{eq} within the study area. The highest values of Ra_{eq} and H_{ex} are 114 Bq kg^{-1} and 0.31 respectively,

measured in soils from VA sampling area. These values are relevant from inspection of materials as suitable or not for bulk use in building activities.



Fig.6. Mean specific activity (Bq kg⁻¹) of ²²⁶Ra, ²³²Th and ⁴⁰K at soils from sampling locations around the Puchuncaví-Ventanas industrial complex.

The recommended maximum levels of Ra_{eq} for home building materials is 370 Bq kg⁻¹ ($H_{ex} < 1$) and 370-740 Bq kg⁻¹ for industrial buildings (Oresegun and Babalola, 1998). It is worth noting that the average Ra_{eq} values measured in Puchuncaví soils are under the limits proposed by OECD. The values of Ra_{eq} obtained in Puchuncaví soils are similar to those calculated for building materials in published results from other countries. For example, Beretka and Mathew (1985) measured values between 15 and 883 Bq kg⁻¹ in Australian building materials. Amrani and Tahtat (2001) reported data from Algerian building materials in the range from 28 and 190 Bq kg⁻¹. Ahnmed (2005) studied Egyptian building materials. He reported lowest values of about 100 Bq kg⁻¹ in mud and clay bricks and higher values of about 400 Bq kg⁻¹ in granites and marbles. Higher values have been reported at soils in other places in South America, e.g. the external hazard index (H_{ex}) was found to be 6.5 in the area around Buena Beaches, corresponding to Brazilian beach sands (Veiga et al., 2006). Higher values have been also reported in Kerala, India, about 35 times above the OECD limit (Shetty and Narayana, 2007).

Results and discussion

The radiological hazard of soils for individuals is evaluated by the annual dose rate (Eq. 12). This hazard is due to exposure to gamma rays emitted by radionuclides contained in soil. The ratio of indoor to outdoor dose rate at the Puchuncaví area was estimated as 1.0, and people were estimated to spend 20% of the time outdoors (UNSCEAR, 1993). The values of external annual effective dose rates for the sampling stations were between 0,30 and 0,33. The maximum value is 0.33 mSv γ^{-1} , obtained in Valle Alegre (VA). The values of the annual effective dose rates at the Puchuncaví study area (mean value around 0,30 mSv γ^{-1}) were lower than average worldwide exposure of 2.4 mSv γ^{-1} due to natural sources (UNSCEAR, 2000) and also under the limits published by OECD (OECD, 1979). The values found in our study are in the range of those reported from many countries in South America and from other parts of the world (Malanca et al., 1996; Sajó-Bohus et al., 1999; UNSCEAR, 2000; Gonzalez-Chornet and Gonzalez-Labajo, 2004; Lakshmi et al., 2005; Singh et al., 2005; Juri et al. 2008; Montes and Desimoni, 2011).

Conclusions

A comprehensive study of the human health risk from trace elements and radioactive pollutants in soil from the Puchuncaví Valley in central Chile, heavily affected by a range of anthropogenic emissions from a localized industrial complex, has shown a clear spatial gradient of human health risk caused by trace elemental pollutants in soil (distances ranging from 1.5 to 18 km). Distance to source dependent cumulative non-carcinogenic hazard indexes above 1 for children (max 4.4 – min 1.5) were found in the study area. The most important pathway of exposure to elements present in the soil is the ingestion, and As represents the highest carcinogenic risk from soil exposition in the study area. Total carcinogenic risk for children is above the 10^{-4} threshold in LM and just on the limit at LG, decreasing with distance to the industrial sources with a similar trend than observed for the non-carcinogenic risk. All locations present total carcinogenic risk above the 10^{-6} no effects threshold, so the health risk

impact is observed even at 20 km from the industrial complex. Therefore, more effective pollution controls should be deployed to tackle trace elements emissions from the industrial sources in the Puchuncaví-Ventanas complex in order to alleviate soil pollution in the study area, even at relatively long distance, and consequently to reduce the observed non-carcinogenic and carcinogenic health risk. In the meantime, it would be beneficial to release information about protective habits for the exposed population.

Gamma ray radiation external hazard indexes and annual effective dose rate from the natural radioactivity elements (^{226}Ra , ^{232}Th and ^{40}K) levels in the surface soils of the study area show no significant risk. The highest average values for the specific activity of ^{232}Th (31 Bq kg^{-1}), ^{40}K (615 Bq kg^{-1}), and ^{226}Ra (25 Bq kg^{-1}) are lower than the limit recommended by OECD. No significant variability of radioactive risk was observed among sampling locations, so it seems that the industrial activities in the area have produced no alteration of radioactivity levels in the soils. Our radioactivity results may be useful for the estimation of natural radioactivity reference levels along the Pacific coast of South America.

Acknowledgements

This work was supported by the Spanish Ministry of Science and Innovation (project CTQ2014-52309-P), Spanish Agency of International Cooperation for Development (AECID project A1/037813/11), Junta de Extremadura (GR15087) and FEDER. Support from AES-GENER (Puchuncaví-Ventanas) is also acknowledged. E. Bernalte acknowledges funding from Junta de Extremadura (Spain, PO 14021).

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3.3. Article 3

Trace elements levels in native plant species around the industrial site of Puchuncaví-Ventanas (central Chile): Evaluation of the phytoremediation potential

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Abstract

The present work investigates uptake of pollutants (Cu, Sb, As, Pb, Cd, Zn, Cr, Mn, Ni, V, and Co) from soil and their accumulation in the biomass samples (leaves and flowers) of three selected native plants (namely *Oenothera Affinis*, OA; *Sphaeralcea Velutina*, SV and *Argemone Subfusiformis*, AS) around an industrial area (Punchuncaví-Ventanas) located in the Puchuncaví-valley, in the central region of Chile. Main emission sources in the area come from copper refinery and coal fired power plants, and a set of other 14 different industrial facilities. Trace elements measurements in the native plants of this area and the ability of transfer of these pollutants from soil to plant (transfer factor) will be assessed in order to identify the potential use of these plant species for phytoremediation. Preliminary results showed a high concentration of trace elements in OA, SV and AS samples. However, the concentration of these elements in the plants was not inversely correlated to the distance of the main emission sources, which pointed out the influence of soil variables such as pH, in the plant uptake of trace elements. Moreover, the high concentrations of trace elements such as Cu, As, Cr and V, upon the toxic limits in the native plant species, recommend the need for continuous monitoring of the region. OA species was identified as the plant with the highest capacity for

HMs accumulation, which also showed higher accumulation potential in whole aerial parts than in leaves. However, transfer factors values suggested that these native plants had only phytoremediation potential for Ni and Cr elements. This study provides preliminary baseline information on trace element compositions of important native plants and soil in the Punchuncaví-Ventanas area for phytoremediation purposes.

Key words: plant species, industrial pollution, trace elements, transfer factor, phytoremediation.

1. Introduction

Nowadays, heavy metals and other trace elements are known as one of the most important environmental issues with harmful effects on both human and biophysical environment. They may come from atmospheric and terrestrial sources. Different anthropogenic activities such as mining, ignition of fossil fuels, metal working industries, and transport traffic can increase levels of heavy metals and other trace elements in all natural matrices like air, water, and soil (Massa et al. 2010).

Biomonitoring is an excellent and low-cost approach to monitor the quantity of HMs in soil, which provides an insight into the influence of anthropogenic factors on the obtained results. As a result, the use of plants (or their component structures) as indicators and monitors of pollution, especially in soil and air, has become more widespread nowadays. Some of the most important advantages inherent to this method include the great availability of biological material, easiness for species identification, sampling and treatment, and ubiquity of some genera, which facilitates covering large areas (Ataabadi et al. 2010).

Exclusion and accumulation are two ways that plants can tackle with high levels of HMs (Baker et al., 2000). As mentioned by Mgana et al. there are three kinds of plant-soil relationships: excluder, accumulator, and indicator plants (Mganga et al. 2011). Excluder plants are some kind of plants that limit the transport of HMs to external parts and keep the concentration of

HMs in shoot over a wide range of metal in the soil. Nevertheless they can still contain considerable amounts of metals in their roots. Accumulators are plants with tendency and ability to accumulate high amounts of metals in their external parts. Finally, indicator plants accumulate metals in their external parts and normally, the level of metals in their tissues is a reflection of the level of metals in the soil.

There are different physical and chemical methods to clean up the HMs from the soil, but normally these techniques are economically and environmentally expensive and even more, they can have bad effects on soil structure (physical, chemical, and biological) (Massa et al. 2010). In phytoremediation technique, roots absorb HMs from the soil and transfer them to the shoots and other external parts of the plants without showing any stress symptoms (Del Río et al. 2002). Therefore, knowing the characteristics of the transfer of trace elements from soil to plants (transfer factor) is really important (Zheng et al. 2007). In this sense, transfer factor values depend on different facts. Thus, transfer factors can vary according to the location, since different locations can have different soil nutrients, pH, organic matter, clay contents, and they can also differ between plant species (Mihali et al. 2012).

Small size and slow growth plants are mostly tolerant and accumulator species, which limits their applicability for phytoremediation purposes. Thus, nowadays fast growing plants and trees are more used for phytoremediation, even when they are not hyperaccumulators (Massa et al. 2010). With this fact, polluted areas can be considered as a storage of native plants with ability to accumulate HMs into their external parts (Pastor et al. 2007).

Punchuncaví-Ventanas is one of the main industrial areas of Chile, which comprises a wide range of industrial factories and activities (such as copper smelter and coal-fired power plants) implying potential risks to human and environmental health (Salmanighabeshi et al., 2015). Previous studies around the industrial site of Puchuncaví-Ventanas were focused on a limited number of HMs (mainly copper), even though this industrial site is usually contaminated by a wide variety of HMs. For instance, González et al. determined copper concentrations in 22

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different native species near the Ventanas smelter in order to compare copper concentration levels (200 and 600 mg kg⁻¹) in these species, and aiming at identifying the hyperaccumulator plants representative of the Chilean conditions. Although no hyperaccumulator native plants were found, *Oenothera affinis* was considered as a good candidate for remediation initiatives in this area (González et al., 2008). Moreover, De Gregory et al. monitored copper (9.3 and 8.6 mg kg⁻¹) and selenium (0.1 and 0.4 mg kg⁻¹) concentrations in rainwater, soils and *alfalfa* samples at various sites from three different zones of Valparaiso region including the Puchuncaví valley. In this case, a close correlation between copper concentrations in soil and *alfalfa* samples was found as a function of the distance from the smelter (De Gregory et al., 2000). Staying focused on phytoremediation purposes of Puchuncaví valley, Meier et al. studied the LMWOA (Low Molecular Weight Organic Acids) root exudation pattern and Cu accumulation capability in shoot and root tissues of two native Chilean metallophytes (*Oenothera affinis* and *Imperata condensata*) and agricultural plants (*Lupinus albus* and *Helianthus annuus*). These plants were grown under hydroponics conditions at increasing copper levels, being analyzed after harvesting. All the plant species showed high Cu accumulation, among which, the metallophyte *Oenothera affinis* was found to be the most tolerant specie (116 and 2657 mg kg⁻¹ Cu in shoots and roots, respectively). Additionally, they stated that the LMWOA root exudation is a crucial Cu tolerance mechanism developed by metallophytes that should be considered as an important factor for plant selection in phytoremediation programs (Meier et al. 2010b). Regarding the *Oenothera affinis* specie, further studies were carried out by Muena et al. in order to evaluate the effects of liming and nitrogen fertilization on its cotyledon emergence, survival, biomass production and copper accumulation. In this case, *Oenothera affinis* was grown in soil from Puchuncaví valley, but in controlled conditions. These results showed that liming decreased Cu concentration and Cu²⁺ activity in the soil solution, whereas nitrogen fertilization increased concentration of Cu in root and shoot in limed soils. Therefore, they concluded that liming is sufficient to improve soil

conditions for development of *O. affinis* in acid and metal-contaminated soils, allowing its potential use for phytostabilization (Muena et al. 2010b). Similarly, González et al. evaluated the effects of the biodegradable chelate of methylglycinediacetic acid (MGDA) on copper extraction by *Oenothera picensis* species (classified as *Oenothera affinis* by González et al. (2008)) through acid soil samples from Los Maitenes (Puchuncaví valley). In this case, MGDA application, which was added in 4 different rates: 0 (control), 2,6 and 10 mmol plant⁻¹, considerably increased biomass production and copper extraction from 0.09 mg plant⁻¹ (control) to 1.3 mg plant⁻¹ (in the 2.6 and 10 mmol plant⁻¹ treatments). In this sense, they demonstrated that applications of MGDA are an effective and environmentally safe way to improve copper extraction by *O. picensis* in these soils (González et al., 2011). Finally, Ginocchio investigated the effects of the copper smelter plant on local vegetation and grassland in the industrial Puchuncaví Valley. Grassland was quantitatively analyzed in terms of the physico-chemical properties of soil, multitude and diversity of plant species, and soil seed bank. This work pointed out that a decrease in total soil nitrogen could explain 13% of the changes detected in plant abundance, while soil pH, and 0.05 M EDTA extractable copper explained 10% and 7%, respectively. Furthermore, they found that this pollution had affected regeneration ability of the native plant species from the soil seed bank and seed distribution in soil (Ginocchio, 2000).

In this context, the aim of this study is to determine 11 HMs concentrations in three native plants species, OA, SV and AS, found in four sampling locations (La Greda, Los Maitenes, Valle Alegre and Cachagua) sited at a difference distance from the industrial affected area of Puchuncaví-Ventanas (Chile). The ability of these native plants to accumulate the HMs in their higher parts will be further evaluated in order to assess their potential application in phytoremediation plans of this area.

1. Materials and Methods

1.1. Site description (including vegetation)

Results and discussion

The study took place in the surroundings of Puchuncaví-Ventanas industrial complex in the coastal area of Valparaíso region in central Chile. The main emission sources operating in the complex are a copper smelter and refinery (400.000 metric tons of fine copper per year) (Cochilco, 2014) and a coal thermoelectric plant (544 MW) (AES Gener, 2008). The soil of this zone has received polluted atmospheric deposition since 1964, mainly metal-rich particulate matter and acid rain due the SO₂ emissions from the copper refinery. As a consequence, the soils around the industrial complex have now an acid pH and high concentrations of heavy metals and other elements (Ginocchio et al., 2004; Ginocchio, 2000; González et al., 2014; Parra et al., 2014; Salmanighabeshi et al., 2015). Accordingly, the ecosystems associated with these soils have become seriously degraded and eroded, characterized by a bare and sparsely vegetation (Ginocchio, 2000). Just since 1999, i.e., after 35 years of production, the emissions were reduced significantly thanks the incorporations of abatement measures required for the entry into force of the new state environmental regulations (Folchi, 2006).



Figure 1. Geographical locations of monitoring sites according to GPS records.

Three sampling points were selected for the present study: La Greda (LG) (32°45'14"S-71°28'17"W), Los Maitenes (LM) (32°46'20"S-71°28'13"W) and Valle Alegre (VA) (32°48'27"S - 71°26'5"W) located respectively 1.2 km NE, 1.5 km SE and 6.5 km SE from the industrial complex. Additional samples were collected in Cachagua (CH) (32°34'52"S -71°26'55"W), located 20.3 km N from Ventanas. These sites were selected according to previous soil contamination records (Parra et al. 2014), (Salmanighabeshi et al., 2015) that may follow an expected concentration gradient pattern. La Greda and Los Maitenes are the nearest to the emission sources and therefore, they are expected to be the most impacted sites, whereas Valle Alegre is located in an intermediate impact position, and Cachagua is the farthest location northwards and so nearly unaffected by industrial impact (Fig.1).

The vegetation at these sampling sites corresponds to typical Mediterranean ecosystems with predominance of grassland and bushes (Ginocchio et al., 2004). In the study area, especially the zone about 2-3 km around the emission sources, the vegetation cover decreases gradually until almost disappearance at the bottom of the stack of smelting slag (González et al., 2008).

2.2. Plant sampling and identification

The plant species selected for the present study were: *Oenothera Affinis* (OA), a native biannual herb and hemicryptophyte that grows up to 0.5-0.8 m (González et al., 2008), *Sphaeralcea Velutina* (SV), an endemic small, round and dense shrub (up to 0.7 m tall) nanophanerophyte plant (González et al., 2008; Ginocchio et al., 2004), and *Argemone Subfusiformis* (AS), a native annual herb and therophyte that has erect and prickly stems and leaves and reaches up to 0.7 - 1.2 m high. *Oenothera Affinis* was previously described as a potential copper metallophyte to survey plant diversity in Puchuncaví-Ventanas area (Meier et al., 2010). They are all drought resistance with high tolerance to metal contamination and soil acidity (González et al., 2008).

All samples were collected during the same day (on November 8, 2012) at each sampling point to maximize the comparability of the results. For each identified species two individuals were

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sampled, located no more than 3 m away from each. Both individuals were considered the same sample and representative of the same soil. However, a different number of species were collected at each location according to their natural availability. That is, in LM three plant species (one sample from each species) were collected. Meanwhile, in VA one sample of AS, and in LG one sample of OA were collected. Finally, in CH (reference area) two samples corresponding to OA and SV species (one of each) were selected.

Soil samples were taken around each site of plant sampling according to a previously described protocol (Salmanighabeshi et al., 2015).

2.3 Reagents

All chemicals used for the preparation of stock and standard solutions were of analytical grade. ICP multielemental standard solutions were obtained from PerkinElmer (Waltham, MA, USA) and Panreac (Spain). Working solutions were prepared by dilution with ultrapure water (resistivity >12M Ω) obtained from an ultramatic system (Wasserlab, Spain).

Hiperpure-Plus HCl (35%) and HF (48%) (Parneac, Spain) was used for plant digestion. Dilute standards and digested solutions from real samples and reference material were adjusted to desired pH with sub-boiled HNO₃ (69%) obtained from a quartz sub-boiling system (Kürner, Rosenheim, Germany). 1 g L⁻¹ Ge (II) and In (III) standards (Parneac, Spain) were used as internal standards. Standard Reference Material[®] 1573a Tomato Leaves from the U.S. National Institute of Standards and Technology (NIST 1573a) and Rye Grass No 281 from the Community Bureau of Reference (BCR 281) were used for accuracy testing.

2.4 Plant and soil analysis

After field collection, all samples were carefully washed (twice) with tap water and further with distilled water (twice) to remove soil particles. Subsequently the samples were left to dry on the lab bench at room temperature and then, they were dried out for 48 h at 40 °C in a stove after drying, leaves and flowers were picked from main branches. Finally, leaves and flowers were manually triturated and individually kept into polyethylene bags until analysis.

Plant sub-samples were mechanically homogenized in a planetary mill and manually sieved through 0.2 mm using a stainless steel mesh. A microwave system (MARS-X 1200W Microwave Accelerated Reaction System, CEM, Mathews, NC, USA) was used for samples digestion following the methodology described by (Wiseman et al. 2013). 150 mg of real sample (mixture of flower and leaves, and only leaves) and 300 mg of reference material were weighed and placed into Teflon digestion vessels. Afterwards, samples were pre-digested overnight with 4.5 mL HNO₃ and 1.5 mL HCL (9 mL HNO₃ and 3 mL HCL for reference material samples). Pre-digested samples were then filtered 2 or 3 times before digestion in a microwave at 160 °C (600 W, ramp time 15 min, and 15 minutes holding time). Then, samples were evaporated on a heater at 95 °C until near dryness. 0.5 mL of concentrated HF was added to the real samples (1 mL for reference material samples) to remove silicates, and the samples were heated until they almost dried. After that, 1 mL of concentrated HNO₃ was added to the real samples (2 mL for reference material samples) and they were evaporated until near dryness. This last step was repeated twice. Finally, the real samples were diluted to a total volume of 10 mL with 0.5 mL HNO₃ and water, whereas reference material samples were brought up to a total volume of 50 mL using 2.5 mL HNO₃ and water.

The digested samples were analysed using an ICP-MS equipment (PerkinElmer ELAN 9000). The instrumental settings were RF power 1000 W, plasma gas flow rate 1 L of argon min⁻¹ and 35 s of washing time. As internal standard, 50 µL of a solution containing In (III) and Ge (II) (10 mg L⁻¹) was used. Each sample was assayed in triplicate.

Soil samples were assayed according to a previously described protocol based on acid digestion and ICP-MS measurement (Salmanighabeshi et al., 2015).

2.5. Quality assurance/ quality control

Acid blanks were run with all sample series for quality control and no significant concentrations of the studied elements were found.

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NIST 1573a (tomato leaves) and BCR 281 (rye grass) were assayed to ensure the reliability of results and to validate the method performance. The results obtained (Table 1) show that all samples have the percentage recovery between 80% and 130% for NIST 1573a (tomato leaves) and 75% and 130% for BCR 281 (rye grass) except for some elements (As and Sb) due to an ICP-MS overestimation. This recovery percentage confirmed that the analytical methodology is properly operating for the analysis of real plant samples.

2.6. Transfer factor calculations

Transfer factor (TF) was calculated according to the following equation (Chojnacka et al., 2005; Kassaye et al., 2012; Cheng et al., 2015):

$$(Eq.1) \quad TF = C_{plant} / C_{soil}$$

Where C_{plant} is element concentration in the plant tissue ($mg\ kg^{-1}$) and C_{soil} is element concentration in the soil ($mg\ kg^{-1}$). The results are categorised based on their TF to low (less than 0.09), medium (0.1-0.3), and high (1-2) levels. In addition, $TF < 1$ indicate low uptake by plants compared to the soil reservoir and $TF > 1$ indicate high uptake by plants (Kassaye et al. 2012a).

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3.1. HMs concentrations in plant species

All the collected plants were characterized for HMs concentrations in a mixture of leaves and flower samples, except for *Oenothera Affinis* (OA), for which an additional leaves sample was also collected in La Greda (LG (leaves)). **Table 2** summarizes HMs concentrations according to the different species and sampling locations. HMs concentration were calculated on a dry weight basis and defined as toxic or non-toxic for the plant according to the values reported by (Massa et al. 2010), which are also summarized in **Table 2**.

Compared the measured trace elements concentrations in samples with results have mentioned by Greger doesn't recognise any hyperaccumulator plants in any location (Greger

1999). However, among the three different selected species, *Oenothera Affinis* (OA) showed the highest concentrations of HMs in their component structures (leaves and flowers). The average heavy metal concentrations in this species were in decreased in the order (Table 2): Cu>Mn>Zn>Cr for LM and LG, but Mn>Zn>Cu>V for CH, following a slightly similar but not exactly trend to the HMs concentrations in the soil samples: Cu>Mn>Zn>Pb/V for LM and LG, and Mn>V>Zn>Cu for CH (Table 3). In this species (OA), the highest HMs concentrations were found in Los Maitenes (LM), except for Mn and Zn, whose highest concentrations were reached in La Greda (LG).

Table 1. Results summary for the standard reference materials NIST 1573a (tomato leaves) and BCR 281 (rye grass)

Elements	SRM	Certified values (mg/kg) ±SD	Measured concentration (mg/kg) ±SD
Cr	NIST 1573a	1.99±0.06	1.44 ± 0.13
	BCR 281	-----	-----
Mn	NIST 1573a	246±8	199±12
	BCR 281	81.6±2.6	62.6±0.1
Ni	NIST 1573a	1.59±0.07	1.74±0.12
	BCR 281	3.00±0.17	2.25±0.003
Cu	NIST 1573a	4.70±0.14	4.03±0.25
	BCR 281	9.65±0.68	9.17±0.02
Zn	BCR 281	31.5±1.4	30.6±0.05
As	NIST 1573a	0.112±0.004	0.24±0.03
	BCR 281	0.057±0.004	0.095±0.00
Cd	NIST 1573a	1.52±0.04	1.77±0.20
	BCR 281	0.120±0.003	0.165±0.000
Pb	NIST 1573a	-----	-----
	BCR 281	2.38±0.11	2.71±0.00
Sb	NIST 1573a	0.063±0.006	0.088±0.008
	BCR 281	0.047±0.005	0.062±0.000
V	NIST 1573a	0.835±0.01	0.879±0.05
	BCR 281	-----	-----
Co	NIST 1573a	0.57±0.02	0.38±0.10
	BCR 281	-----	-----

The difference in the HMs concentrations between these sampling sites is probably related to the soil chemical characteristics and HMs concentrations in soil samples (Table 3). Thus, Mn and Zn concentrations were significantly higher in LG and consequently, the potential to accumulate these metals by OA species may be greater in this site. But interestingly, OA species tended to accumulate a higher quantity of Cu in LM, even though Cu concentration in

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soil was lower than the one in LG. This fact may be explained by the different soil characteristics of these locations, since the behaviour, phytoavailability and toxicity of HMs are also influenced by the physicochemical properties of the soil.

Table 2. Element concentrations (mg kg^{-1}) in plants collected at the sampling stations within the Puchuncavi-Ventanas industrial area. *Oenothera affinis* (OA), *Sphaeralcea velutina* (SV), and *Argemone subfusiformis* (AS). Underlined values are within the toxic range for plants, while those in bold are above the toxic limit.

Elements	Cr	Mn	Ni	Cu	Zn	Cd	Pb	V	Co	Concentrations (mg kg^{-1})	
										Non-toxic	Toxic
Location	0.1-0.5	30-300	0.1-5	5-30	27-150	0.05-0.2	5-10	0.2-1.5	0.02-1		
	5-30	400-1000	10-100	20-100	100-400	5-30	30-300	5-10	15-50		
LM	30.64-35.46 (33.77)	83.67-99.24 (92.34)	<u>19.70</u> 21.68 (21.00)	467.92-548.44 (507.77)	55.24-66.66 (60.35)	0.36-0.45 (0.40)	<u>34.03-78.84</u> (49.64)	11.78-14.44 (13.29)	2.03-2.22 (2.11)		
LG	<u>23.01-26.16</u> (24.95)	114.99-126.46 (120.70)	<u>16.26</u> 17.90 (17.24)	390.53-407.94 (399.41)	<u>115.46</u> 119.26 (117.87)	0.30-0.33 (0.31)	11.36-12.73 (11.99)	<u>7.51-8.92</u> (8.15)	1.38-1.67 (1.49)		
LG (leaves)	<u>8.99-9.63</u> (9.20)	117.09-127.71 (123.66)	9.06-9.83 (9.40)	190.34-204.99 (196.53)	<u>135.01</u> <u>142.57</u> (138.84)	0.27-0.29 (0.28)	5.55-6.07 (5.80)	1.23-1.27 (1.25)	0.48-0.59 (0.52)		
CH	0.82-1.27 (1.02)	52.71-60.55 (56.80)	1.20-1.47 (1.30)	12.52-14.99 (13.51)	31.46-35.08 (33.34)	0.03-0.03 (0.03)	1.06-1.14 (1.11)	1.83-2.30 (2.02)	0.28-0.42 (0.35)		
LM	<u>20.73-21.80</u> (21.23)	68.90-73.29 (71.56)	<u>12.85</u> <u>13.97</u> (13.40)	347.95-364.70 (355.22)	66.54-69.88 (68.63)	0.41-0.45 (0.43)	17.96-19.10 (18.47)	4.00-4.11 (4.06)	1.02-1.11 (1.05)		
CH	<u>23.48-25.52</u> (24.42)	63.95-70.14 (67.05)	<u>14.07</u> <u>15.07</u> (14.58)	14.40-15.43 (14.84)	30.92-36.47 (33.38)	0.12-0.13 (0.12)	0.82-1.57 (1.09)	0.89-0.92 (0.91)	0.41-0.52 (0.47)		
LM	<u>5.72-7.10</u> (6.91)	24.33-26.88 (25.90)	4.81-5.05 (4.97)	<u>33.67-40.02</u> (36.52)	43.40-47.75 (45.90)	0.06-0.09 (0.07)	1.99-8.55 (6.34)	0.47-0.51 (0.49)	0.13-0.38 (0.23)		
VA	49.02-63.31 (55.72)	47.22-52.03 (49.56)	<u>30.16</u> <u>41.19</u> (35.35)	13.20-14.03 (13.75)	27.51-33.16 (30.16)	0.06-0.10 (0.08)	0.74-4.20 (1.97)	0.40-0.42 (0.41)	0.82-0.98 (0.88)		

Table 3. Physicochemical characteristics of soil samples collected at the sampling locations and reference soil quality standard guidelines for HMs in soil. *Los Maitenes* (LM), *La Greda* (LG), *Valle Alegre* (VA) and reference sampling point at *Cachagua* (CH). Bold values exceed some of the guidelines limits (Salmanighabeshi et al., 2014) for HMs in soil.

Location	pH range	Elements concentrations (mg kg ⁻¹)										
		Cr	Mn	Ni	Cu	Zn	As	Cd	Pb	Sb	V	Co
LM	4.4-5.9	37.60	472.62	13.73	843.65	194.07	84.44	1.81	110.78	9.63	109.79	6.74
LG	6.8-8.3	37.99	531.11	17.14	1255.28	283.57	63.29	1.78	95.02	6.98	104.12	9.31
VA	6.0-6.3	42.02	723.59	6.45	147.68	156.54	40.80	1.06	56.69	4.26	121.04	10.20
CH	6.8-8.3	27.31	657.49	9.40	56.25	85.59	13.82	0.64	30.06	0.96	107.76	9.56

Note: Soil pH values are taken from (González et al., 2014). Elements concentrations in soil samples correspond to a sampling campaign conducted in 2012.

In general, several studies demonstrated that HMs availability is higher in acid soils containing low concentrations of organic matter (Zeng et al. 2011). Hence, Cu is very tightly bound to the soil at high pH, as it happened in LG (pH 6.8-8.3), while Cu uptake increases within a pH range of 5 to 7, as corresponded to LM conditions (pH 4.4-5.9).

Regarding the toxicity levels, especially remarkable is the case of Cu, which also reached concentration levels 5 times higher than the toxic level concentration. Indeed, *Oenothera Affinis* (OA) showed critical HMs concentrations (above toxicity levels) in LM for chromium, copper, arsenic and vanadium, whereas Pb and Ni levels were also found in toxic concentrations. The prevalence of critical Cu concentrations in LG suggests that the impact of the copper smelter could be the main source of these elements in the area.

Even more, both sampling locations (LG and LM) had HMs concentrations much higher than the levels found at the reference site Cachagua (CH), ranging from around 2 (Mn) to 38 (Cu) times, which highlights the impact of the industrial activities in the area. However, the concentration of these elements in the plants was not inversely correlated to the distance of the main emission sources, as happened in other soil samples studies (Salmanighabeshi, et al., 2015). This pointed out again the influence of soil variables in the plant uptake of HMs.

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Comparing the HMs concentrations in the different structural parts of *OA* species, the HMs levels found in the leaves were around 2-3 times lower than those measured in the flower and leaves mixtures (**Table 2**, LG and LG (leaves)) in the same location, except for some elements. In this sense, Cd, Mn and Zn concentrations were similar in both kind of samples, which may suggest that these elements could be distributed more uniformly through the plant.

Concerning the *Sphaeralcea Velutina* (*SV*) species, also critical copper concentrations were found in this plant in LM, whereas chromium, nickel and arsenic were found in toxic levels. Meanwhile, *Argemone Subfusiformis* (*AS*) was the plant species with the lowest HMs concentrations in LM, reaching concentration levels 80% lower (except for Zn) than those found in *OA* species in the same location. Moreover, this plant species presented higher HMs concentrations for chromium, manganese and nickel in VA, reaching critical levels for Cr and toxic levels for Ni. This is again probably due to the higher concentration levels of these metals in VA in conjunction with the acid soil characteristics (**Table 3**).

These results indicated that *Oenothera Affinis* (*OA*) and *Sphaeralcea Velutina* (*SV*) species exhibited medium ($200\text{-}600\text{ mg kg}^{-1}$) accumulation of copper in LM and LG for *OA*, and in LM for *SV*, respectively. This is in agreement with other reported studies (González et al., 2008), which identified *OA* species as the plant with the highest capacity for copper accumulation. However, in this work, further results about additional HMs and their concentrations in plants in different industrial affected locations is provided, which underlines the higher ability of *OA* species for HMs accumulation, for which also soil characteristics play a crucial role.

3.2. Soil to plant transfer factors for HMs

In order to better understand the role of soil and type of contaminated area on transfer of HMs to these native plants, soil to plants Transfer factor (TF) values for the 11 HMs were calculated for each of the investigated plants species (**Table 4**). These TFs were determined using the average element concentrations in plant species (**Table 2**) and soil samples (**Table 3**).

As it can be observed, TF values calculated for the different HMs varied widely among the different plant species, ranging from 0.012 to 1.622, 0.008 to 1.551, and 0.003 to 5.482 for *Oenothera Affinis* (OA), *Sphaeralcea Velutina* (SV) and *Argemone Subfusiformis* (AS) species, respectively. In general, TFs values were in the medium (0.1-0.3) or low level range (less than 0.09) for most of the HMs, indicating a low uptake by plants compared to the soil reservoir (Table 4). Based on the 11 HMs investigated, only Cr and Ni had TFs values > 1, which indicated enrichment of these elements in plants in some locations.

Table 4. Average transfer factors (TFs) values of 11 HMs for the plant species collected at the different sampling locations. *Los Maitenes* (LM), *La Greda* (LG), *Valle Alegre* (VA) and reference sampling point at *Cachagua* (CH). Underlined values indicate medium TF values (0.1-0.3), while those in bold indicate high TF values (>1).

Species	Location	Cr	Mn	Ni	Cu	Zn	As	Cd	Pb	Sb	V	Co
<i>Oenothera Affinis</i> (OA)	LM	<u>0.898</u>	<u>0.195</u>	1.529	<u>0.602</u>	<u>0.311</u>	<u>0.266</u>	<u>0.219</u>	<u>0.448</u>	<u>0.433</u>	<u>0.121</u>	<u>0.314</u>
	LG	<u>0.657</u>	<u>0.227</u>	1.006	<u>0.318</u>	<u>0.416</u>	<u>0.190</u>	<u>0.177</u>	<u>0.126</u>	<u>0.264</u>	0.078	<u>0.160</u>
	CH	0.037	0.086	<u>0.138</u>	<u>0.240</u>	<u>0.390</u>	0.042	0.047	0.037	0.092	0.019	0.036
<i>Sphaeralcea Velutina</i> (SV)	LM	<u>0.565</u>	<u>0.151</u>	<u>0.976</u>	<u>0.421</u>	<u>0.354</u>	<u>0.162</u>	<u>0.237</u>	<u>0.167</u>	<u>0.216</u>	0.037	<u>0.156</u>
	CH	<u>0.894</u>	<u>0.102</u>	1.551	<u>0.264</u>	<u>0.390</u>	0.035	<u>0.191</u>	0.036	<u>0.101</u>	0.008	0.049
<i>Argemone Subfusiformis</i> (AS)	LM	<u>0.184</u>	0.055	<u>0.362</u>	0.043	<u>0.237</u>	0.018	0.041	0.057	0.029	0.005	0.034
	VA	1.326	0.068	5.482	0.093	<u>0.193</u>	0.008	0.073	0.035	0.018	0.003	0.086

The TFs values obtained for the different plant species are illustrated in Figure 2 according to the different sampling locations. Regarding the *Oenothera affinis* (OA) species, TFs values of all elements were less than 1, except Ni in LM (1.529) and LG (1.006). Moreover, TFs values in the industrial affected locations (LM and LG) were always higher than those obtained in the reference area (CH), even though they were in the low or medium range. Figure 2.A shows a general tendency for the TFs values in most of the HMs: LM>LG>CH, except for Mn and Zn (higher values in LG related to higher concentrations in soil). As mentioned by previous works in this area (Salmanighabeshi et al., 2015) some HMs concentrations in soil in LM and LG locations (Table 3) are above the guideline values on the world (Netherland, Australia, and

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Canada), but TFs values in *Oenothera affinis* were not enough high (except Ni) to select this specie as a native plant candidate for phytoremediation.

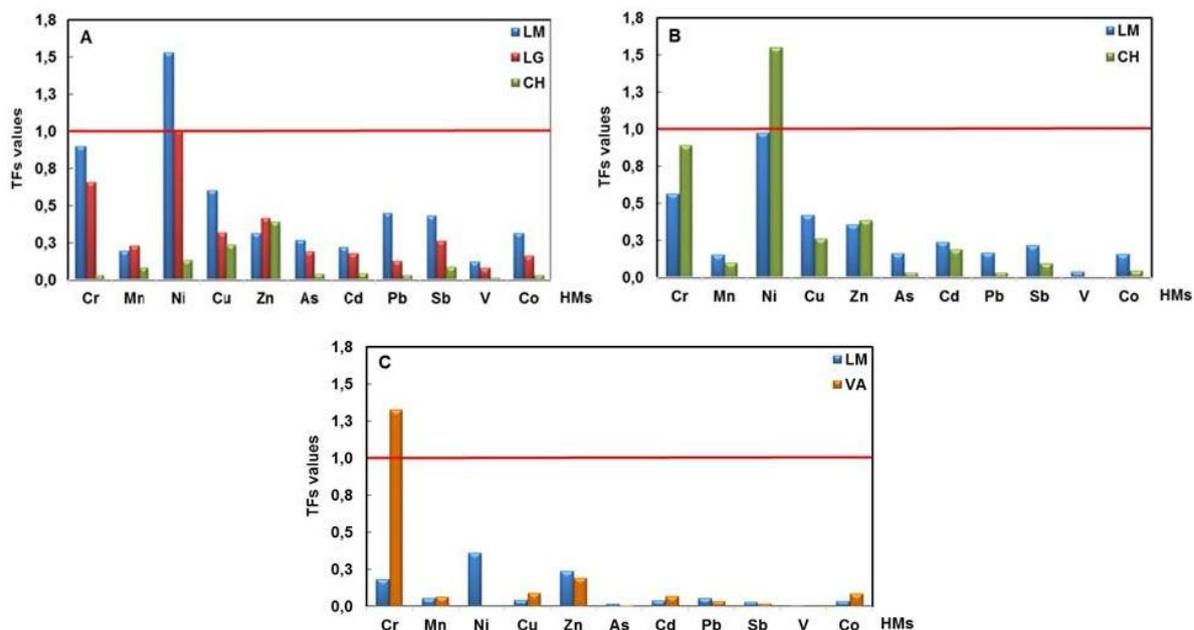


Figure 2. TFs values for the 11 HMs in each sampling location according to the plant species: (A) *Oenothera affinis*, (B) *Sphaeralcea velutina*, and (C) *Argemone subfusiformis*.

Similarly, TFs values obtained for the *Sphaeralcea velutina* species were above 1 only for Ni (1.551) in CH. As **Figure 2.B** shows, TFs for most of the HMs in LM were higher than those obtained for CH location (except Zn, Cr, and Ni). However, the difference in the TFs values between these two locations was not so significant, even LM has acid soil and it was also more industrial affected than CH. Likely, there are other reasons apart from soil pH for this slight difference between these two sampling locations.

Figure 2.C. illustrates the tendency in the TFs values for the *Argemone subfusiformis* species. Transfer factors of all elements were in the low and medium level, except for Ni (5.482) and Cr (1.326). However, TF value for Ni in VA seems to be abnormal compared with the rest of elements, for which further studies could be required. According to these results, the ability of this species as a native plant is enough high to absorb Ni and Cr in industrial areas.

Finally, it is worth mentioning that Cu, Zn, As or V were not accumulated in any plant species, even though their concentration in soil was higher than guideline values as mentioned by Salmanighabeshi et al. suggesting a low bioavailability or an exclusion of these HMs in these plant species. In fact, according to the TFs values calculated (most of them below 1), these plant species could be considered as excluders.

4. Conclusions

Three native plant species were collected in different locations around the industrial area of *Punchuncaví-Ventanas*, and the concentrations of 11 HMs and their corresponding transfer factors were determined. Preliminary results showed a high concentration of trace elements in *OA*, *SV* and *AS* species. However, the concentration of these elements in the plants was not inversely correlated to the distance of the main emission sources, which pointed out the influence of soil variables such as pH, on the plant uptake of HMs.

The high concentrations of trace elements such as Cu, As, Cr and V, upon the toxic limits in the native plant species of the industrial area, recommends the need for continuous monitoring of the region, given that the area is under huge pressure from industrial activities. Nevertheless, no hyperaccumulator plants were found in any location, but the results indicated that *Oenothera Affinis (OA)* and *Sphaeralcea Velutina (SV)* species exhibited medium accumulation of copper in the more industrial affected areas. In fact, *OA* was identified as the plant with the highest capacity for HMs accumulation, showing also a higher accumulation potential in whole aerial parts than in leaves.

Low bioavailability for most of the trace elements was evidenced by the low transfer factors obtained in most of the native plant species, suggesting an exclusion of these HMs. Indeed, transfer factors values suggested that these native plants had only phytoremediation potential for Ni and Cr elements.

In summary, this study provides preliminary baseline results on element compositions of important native plants and soil in the *Punchuncaví-Ventanas* areas, which can serve as the

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basis for further resource management decisions with respect to pollution assessment, plant toxicity and phytoremediation plans.

5. Acknowledgements

This work was supported by the Spanish Agency of International Cooperation for Development (AECID project A1/037813/11) the Spanish Ministry of Science and Innovation (project CTQ2014-52309-P), Gobierno de Extremadura and FEDER (GR15087), GENER a CETAM-UTFSM. Useful comments from Dr. Saioa Elcoroaristizábal are gratefully acknowledged.

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3.4. Article 4

Presence of fallout ^{236}U and plutonium isotopes, ^{239}Pu and ^{240}Pu , in soils from Southern Hemisphere.

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Abstract

Radioactivity concentrations are present in every environmental compartment, due to a variety of nuclear activities. In general, the isotopes ^{236}U , ^{239}Pu and ^{240}Pu are present in surface soils as a result of global fallout from nuclear weapons tests carried out in the 1950's and 1960's. The Southern Hemisphere has received these isotopes due to the global fallout and also an important contribution of the so-called tropospheric fallout from both the atmospheric nuclear tests performed in the French Polynesia and in Australia by France and United Kingdom, respectively. In this work we provide new data on the impact of these tests to Chile, South-America, and Mozambique and South-Africa in Africa. The obtained results point out to the presence of debris from the French tests in the Chilean soils. Instead African soils studied are only affected by global fallout corresponding to the southern hemisphere. To best knowledge of the authors the present work is the first publication on ^{236}U concentrations and $^{236}\text{U}/^{239}\text{Pu}$ atom ratios in the Africa and Southern America.

Keywords: Plutonium isotopes, U-236, AMS, fallout, soil.

1. Introduction

The atmospheric nuclear tests constitute the major source of anthropogenic radionuclides to the general environment. But the radioactive contamination is also introduced by scheduled releases from nuclear reprocessing facilities (e.g. La Hague, France; Sellafield, United Kingdom), by nuclear reactor accidents (e.g. Chernobyl, Ukraine; Fukushima, Japan) and by accidents during the transportation of nuclear weapons (Palomares, Spain, 1966; Thule, Greenland, 1968). During the period 1945-1980, about 310 Mt were detonated worldwide as a consequence of the more than 320 atmospheric nuclear tests and of the so-called safety-tests (Björklund and Goliath; 2009). Only 9% of this yield was detonated in the Southern Hemisphere, due to the French activities conducted in Mururoa and Fangataufa atolls (21°S, 128°W) (UNSCEAR, 2000), and to the British nuclear programme performed in Montebello Islands (20°S, 115°E) and in Maralinga Test Site (30°S, 135°E), in Australia (i.e., Butement et al., 1958; UNSCEAR, 2010; Tims et al., 2013b; Johansen et al., 2014). Specifically, at the atolls of Mururoa and Fangataufa, 41 atmospheric and 137 underground nuclear weapons tests were performed during 1966-1996. In addition, 5 surface and 10 underground weapons trials using conventional explosives were performed at Mururoa atoll, being dispersed about 3.5 kg of ^{239}Pu ($T_{1/2}=24110$ y) in each detonation. As a consequence, particles with $^{239+240}\text{Pu}$ activities in the 5-30 kBq range were identified in this zone (Danesi et al. 1998; IAEA, 1998). In general, the Southern Hemisphere houses about 20% of the global inventory of these radionuclides (UNSCEAR, 2000; Valković, 2000).

The most widely used radionuclide in fallout studies is the ^{137}Cs ($T_{1/2}=30.08$ y) for its abundance and easy detection by gamma spectrometry. However, since the peak of the atmospheric nuclear testing in 1963, almost two half-lives have passed and, therefore, it is difficult to analytically determine it with reasonable uncertainty. Another inconvenient is that the ^{137}Cs levels in the northern and western European countries are strongly influenced by the so-called

Chernobyl fallout (UNSCEAR, 2000). Therefore, other anthropogenic radionuclides with much longer half-lives have come into scene, such as plutonium isotopes, ^{239}Pu ($T_{1/2}=24110$ y), and ^{240}Pu ($T_{1/2}=6564$ y), (i.e. Schimmack et al., 2001; Chamizo et al., 2010; Ketterer et al., 2010; Tims et al., 2010; Wenting et al., 2014). In most recent years, many studies have been dedicated to ^{236}U ($T_{1/2}=23.42$ My), mainly due to its potential as oceanic tracer (Sakaguchi, 2012, Casacuberta, 2014). In any case, the study of different radionuclides provides complementary information about the contamination source.

The most commonly investigated Pu isotopes in terms of environmental radioactive contaminants are ^{239}Pu and ^{240}Pu . ^{239}Pu , produced by neutron capture on ^{238}U , is the most abundant one, as the latter is a mayor constituent of most nuclear fuels. The second most abundant one is ^{240}Pu , which is produced by neutron activation on ^{239}Pu . Therefore, its abundance dependence on the original composition of the nuclear fuel, the neutron flux and the burning times, in the case of nuclear reactors (Magill et al., 2006). For example, at the Trinity Test Site (New Mexico, USA), where the first American nuclear gadgets were tested, $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratios as low as 0.013 have been reported (Parekh et al., 2006); the measured integrated fallout in soils from the Southern Hemisphere (0-30°S) is 0.173 ± 0.027 (Kelley et al., 1999); and at sites influenced by the Chernobyl fallout, $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratios in the range 0.34-0.57 have been reported (Bisinger et al., 2010). Consequently, the $^{240}\text{Pu}/^{239}\text{Pu}$ atomic ratio offers very valuable information on the plutonium source. For environmental samples this ratio can only be measured using mass spectrometric (MS) techniques, because their activities are generally low and their most intense alpha emissions cannot be resolved using conventional solid-state detectors ($\Delta E= 11$ keV). Of the various MS methods, one of the most sensitive is Accelerator Mass Spectrometry (AMS) with a Pu detection limit of about 10^6 atoms with almost no matrix effects (Chamizo et al., 2008).

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^{236}U is produced by the $^{238}\text{U}(n,3n)^{236}\text{U}$ reaction in nuclear explosions and also via thermal neutron capture on ^{235}U either anthropogenically in nuclear power plants or in nature. Sakaguchi et al. (2009) estimated that up to 900 kg of ^{236}U were dispersed worldwide during the period of nuclear atmospheric testing. This inventory exceeds by a factor of 25 the estimated natural inventory, of about 35 kg (Steier et al., 2008). On the other hand, the Sellafield Nuclear Reprocessing Plant (UK), and La Hague (France), have released approximately between 60 and 130 kg of ^{236}U to the Northern Seas since 1960 (Christl et al., 2015b). Those anthropogenic sources have altered significantly the $^{236}\text{U}/^{238}\text{U}$ atom ratios in the different environmental compartments. In the terrestrial environment, a natural $^{236}\text{U}/^{238}\text{U}$ ratio of 10^{-14} has been anticipated for deeper layers of rock (Steier et al., 2008; Srncik et al., 2011a), and natural $^{236}\text{U}/^{238}\text{U}$ ratios at the order of 10^{-13} were estimated by Christl et al. (2012), including cosmogenic production. Furthermore, $^{236}\text{U}/^{238}\text{U}$ ratios up to 10^{-7} have been reported in soils from Canary Islands (Spain) solely influenced by global-fallout (Srncik et al., 2011b) In the marine environment, pre-nuclear $^{236}\text{U}/^{238}\text{U}$ atom ratios in the oceans are expected to be below 10^{-12} (Winkler et al., 2012), but ratios as high as 10^{-6} have been reported in the Irish Sea, impacted by the liquid releases from the Sellafield nuclear reprocessing plant (Lee et al., 2008). Therefore, to carry out these studies, a technique capable of analyzing $^{236}\text{U}/^{238}\text{U}$ atom ratios covering those ranges (from below 10^{-10} to 10^{-6}) is required. Of the available techniques, AMS is the most competitive one, allowing measurements of $^{236}\text{U}/^{238}\text{U}$ ratios down to 10^{-13} depending on the design of the kinematic filters, the detection system, and the accessible energy range (Vockenhuber et al., 2003). Recently, it has been demonstrated that very competitive results can be achieved with compact AMS systems, working at terminal voltages of 1 MV and below and with compact designs of the mass spectrometer (Chamizo et al., 2015; Christl et al., 2015a).

To date, the published information on the presence of ^{236}U and/or $^{239,240}\text{Pu}$ in soils from the Southern Hemisphere is very scarce. Concerning Pu isotopes, the most important data base is

due to the Department of Energy of United States (DOE), which in 1973 started to study the presence of $^{239,240}\text{Pu}$ in undisturbed soils from different areas of Africa and South America within a global scale project aimed at determining the worldwide $^{239,240}\text{Pu}$ inventory (Hardy et al., 1973). As for ^{236}U , so far only two studies have been reported: one from two former British nuclear tests sites in Australia (Emu field and Montebello Islands) (Child and Hotchkis, 2013), and a second one from northeastern Queensland, Australia, a region far from potential local sources (Srnecik et al., 2014). The main conclusion was that the $^{236}\text{U}/^{239}\text{Pu}$ global fallout ratio for soils from Southern Hemisphere might be a factor of two lower than the one for Northern Hemisphere (i.e. 0.085 ± 0.003 in Australia (Srnecik et al., 2014) against 0.23 ± 0.02 in Japan (Sakaguchi et al., 2009)). Different $^{237}\text{Np}/^{239}\text{Pu}$ global fallout atom ratios for soils from the two Hemispheres were also reported in (Kelley et al., 1999), with values for the northern latitudes of about 0.47 and for the southern ones of about 0.35. However, such remarkable differences were not observed for the $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratio, with values close to 0.18 in both cases, but with a higher variability on the Southern Hemisphere (Kelley et al., 1999). One or both of the following factors may contribute to these differences: (i) the designs of the different nuclear devices that were detonated open air, which are not well documented; and (ii) the different physico-chemical properties of the fallout particles containing the actinides and their deposition conditions. In Southern Hemisphere, it is expected a higher influence of the US thermonuclear tests conducted in the Marshall Islands (7°N approximately) and, on local or regional scales due to their much lower yield, of the French tests that were carried out Southern Pacific Ocean and of the British ones conducted in Australia. Once scavenged by rain and deposited on land, those fallout particles containing the actinides, which might be of different nature in each case, are mobilized by weathering with different rates, causing different speciation processes. It is important to mention that, despite all of those factors and the different chemical properties of uranium and plutonium (i.e. uranium is much more soluble than plutonium), similar depth profiles have been reported for ^{236}U and plutonium

isotopes in different soils solely influenced by global fallout (Sakaguchi et al., 2009; Ketterer et al., 2013; Srncik et al. 2014). This effect, which deserves further investigations, supports in many cases the determination of the $^{236}\text{U}/^{239}\text{Pu}$ average atom ratio in a specific region from surface soils studies.

In a previous work (Chamizo et al., 2011) we provide new data on the impact of nuclear tests to South America through the study of ^{239}Pu and ^{240}Pu in soils from different areas of Chile. The obtained results point out to the presence of debris from the French tests in the 20-40° Southern latitude range, with $^{240}\text{Pu}/^{239}\text{Pu}$ atomic ratios quite heterogeneous and ranging from 0.02 to 0.23. They are significantly different from the expected one for the global fallout in the Southern Hemisphere for the 30-53°S latitude range (0.185 ± 0.047), but they follow the same trend as the reported values by the Department of Energy of United States for other points with similar latitudes. The $^{239+240}\text{Pu}$ activity inventories showed as well a wider variability range in that latitude range, in agreement with the expected heterogeneity of the contamination.

The aims of the present work is (i) to extend current knowledge on the content of ^{239}Pu and ^{240}Pu in soils of remote areas from nuclear facilities in the Southern Hemisphere, specifically in Africa and South America, (ii) to provide first data of ^{236}U from these sites, and (iii) to determine isotopic ratios of the above elements to compare the fallout with other sites of the Southern Hemisphere and also with northern sites.

2. Material and Methods

2.1 Sampling locations

Soils samples were collected in the countries of Chile (South America) and Mozambique and South-Africa (Africa), during different sampling campaigns performed between 2007 and 2011 (table 1 and figure 1). La Greda, Los Maitenes, Puchuncaví and Valle Alegre sampling points are in the Puchuncaví-Ventanas valley, V region of Chile. This valley is on the Chilean mainland

coast, 160 km NW from Santiago (capital of Chile) and 58 km N from Valparaíso (Regional Capital) along the Pacific Ocean. Santa Bárbara sampling point is situated 1300 km farther south of Santiago, in the X Region of Chile. Maputo sampling point is located near of city of Maputo, capital of Mozambique (Africa), along the Indian Ocean. Durban sampling point is near of Durban city, the second most populous urban area, in South-Africa, province of KwaZulu-Natal. They were collected in undisturbed areas to a depth of 5 cm, stored on site in clean aluminium wrapping, homogenized in the laboratory, and frozen in plastic bags. For each sample, three subsamples were collected and homogenized.

Table 1. Location of sampling points

ID	Site	Country	Latitud	Longitud
1	La Greda	Chile	32° 43' S	71° 24' W
2	Los Maitenes	Chile	32° 46' S	71° 27' W
3	Puchuncaví	Chile	32° 45' S	71° 28' W
4	Valle Alegre	Chile	32° 48' S	71° 26' W
5	Santa Bárbara	Chile	42° 51' S	72° 47' W
6	Durban	South-Africa	29° 52' S	30° 57' E
7	Maputo	Mozambique	25° 57' S	32° 17' E

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2.2 Radiochemical procedure

The soil samples in this work were processed for the sequential separation of plutonium and uranium fractions. Measuring ^{236}U and $^{239,240}\text{Pu}$ from the same soil aliquots is key as long as the representation of the results is concerned, taking into account the observed variability of the Pu contamination in the soils from Chile in a previous study (Chamizo et al., 2011). About 7 g aliquots were selected from each site, spikes were added (about 2 pg of ^{242}Pu and ^{233}U) and thoroughly homogenized with a few ml of diluted HCl. These artificially added radionuclides are not present in the samples of interest, and are used to estimate the $^{239,240}\text{Pu}$ and ^{236}U concentrations, respectively, in the original soil samples, using the so-called isotope dilution method.

^{242}Pu was obtained from a standard solution supplied by National Physical Laboratories (NPL, England); ^{233}U was obtained from a standard solution provided by International Atomic Energy Agency Environment Laboratories in Monaco (IAEA-EL) (unknown supplier). Both were proved to contain insignificant amounts of the radionuclides of interest in a previous work (Chamizo et

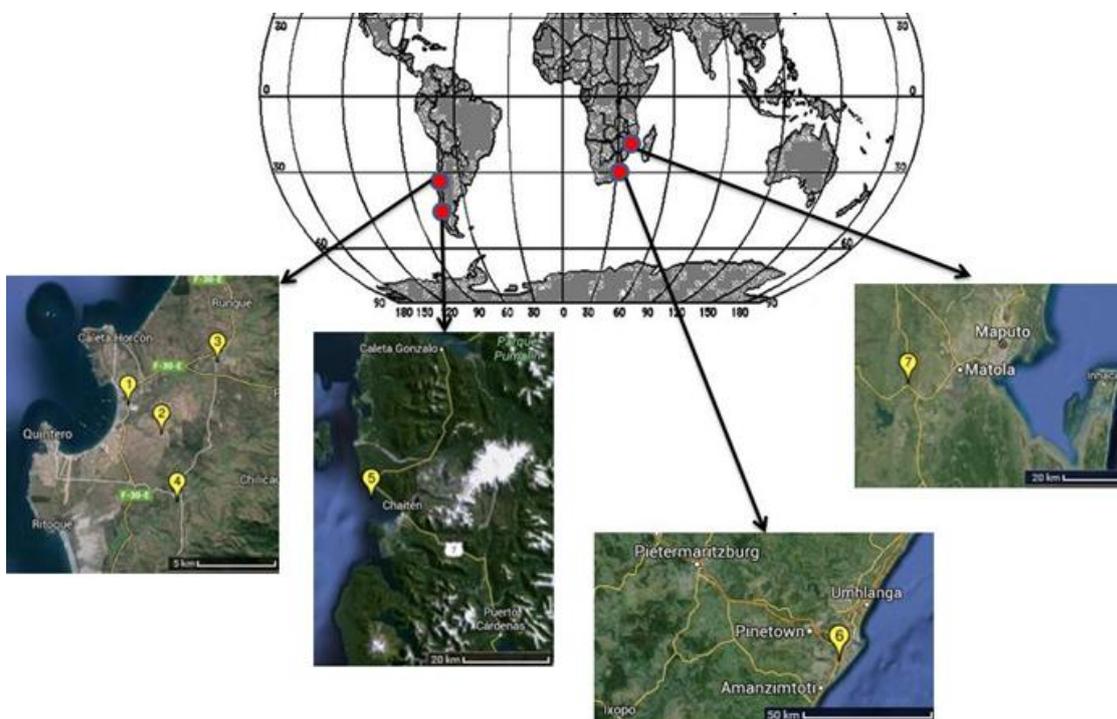


Figure 1. Locations of the sampling points

al., 2014). The samples were then calcined at 600°C in a muffle furnace for about 6 h to destroy organic material, and the ashed sample material was transferred to Teflon® vessels and leached with 8M HNO₃, 9M HCL and H₂O₂ for about 5 h at 100°C in a hot plate at atmospheric pressure. Ultra-pure HNO₃ and HCl were used, to avoid ²³⁸U contamination. That way, it is expected that fallout ²³⁶U and ^{239,240}Pu are completely transferred into solution, but only a fraction of the naturally occurring ²³⁸U (Sakaguchi et al., 2009). The resulting supernatant containing the anthropogenic uranium and plutonium was separated by centrifugation, filtered through a 1.2 µm syringe membrane, evaporated to dryness, and dissolved in about 10 ml of 0.5M HNO₃. An Fe(OH)₃ coprecipitation, using the iron present in the sample, was then applied to preconcentrate the actinides and remove the major matrix components. The resulting precipitate was then dissolved in 15 ml of 8M HNO₃, and the chemical separation of U and Pu fractions was performed using UTEVA and TEVA resins in a vacuum box following the guidelines given in Lopez-Lora et al., 2016 (in preparation). The solutions containing the purified uranium and plutonium fractions were then further processed to produce the AMS cathodes as it is explained in (Chamizo et al., 2014) . Briefly, 1 mg of Fe³⁺ (from a pure iron solution supplied by High Purity Standards (England)) was added to the solutions to coprecipitated the uranium or plutonium fractions with Fe(OH)₃. Those precipitates were transferred to quartz crucibles, dried, baked at 600°C for 1 h to convert the uranium or the plutonium to the oxide form, mixed with about 3 mg of Nb powder and pressed into appropriated aluminum cathodes.

2.3. AMS determinations

²³⁶U and plutonium isotopes, ^{239,240}Pu, were measured on the 1 MV AMS facility at Centro Nacional de Aceleradores (CNA, Seville, Spain) and on the 600 kV Tandy facility at the ETH, Zürich, Switzerland. Details about the uranium and plutonium isotopes measurement techniques have been published elsewhere (Chamizo et al., 2008; Chamizo et al., 2015; Christl

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et al., 2015a), and the most recent developments applied on the CNA system have been reported in (Scognamiglio et al., 2016). Therefore, only a brief description is given here. Plutonium or uranium isotopes are extracted from the Cs sputter ion-source as monoxide anions (e.g. $^{239}\text{Pu}^{16}\text{O}^-$); mass analyzed on a 90° sector magnet; stripped to 3+ charge state in He gas in an electrostatic tandem accelerator (e.g. $^{239}\text{Pu}^{3+}$); mass, charge and energy analyzed on different cinematic filters; and, finally, the minor isotopes (i.e. $^{233,236}\text{U}$ and $^{239,240,242}\text{Pu}$) counted in a miniaturized gas ionization chamber. ^{238}U is counted as a beam current in a Faraday Cup placed at the exit of the first sector magnet on the high energy side. For plutonium isotopes and ^{236}U , the achieved backgrounds are at the fg level (10^6 atoms, or a few μBq for $^{239,240}\text{Pu}$). The minimum $^{236}\text{U}/^{238}\text{U}$ atom ratio that can be actually determined is at the 10^{-10} level in the case of the CNA AMS facility, and below 10^{-12} in the case of the ETH Tandy system. The results of an AMS measurement are expressed as atom ratios; $^{239}\text{Pu}/^{242}\text{Pu}$ and $^{240}\text{Pu}/^{242}\text{Pu}$ in the case of plutonium isotopes, and $^{236}\text{U}/^{233}\text{U}$ and $^{236}\text{U}/^{238}\text{U}$ in the case of ^{236}U . In the case of ^{238}U , any relevant information can be obtained in this work, taking into account the leaching procedure applied to the samples, explained on the former section. ^{238}U beam current is monitored in relation to the control of the $^{236}\text{U}/^{238}\text{U}$ ratios, as they have to be above the limiting abundance sensitivity in each case to make sure that real ^{236}U atoms are counted in the detector.

3. Results

The values measured in the present work are presented in Table 2. As it can be observed, different samples were analyzed for most of the Chilean stations except for Santa Bárbara, where only one sample was analyzed. According to the previous results, it is expected a lower variability in the actinides concentrations in this area, probably less influenced by the local fallout from the Pacific tests (Chamizo et al., 2011). Therefore, the obtained result can be considered representative of the studied site. The same assumption might be true for the two African

Table 2: ^{236}U and ^{239}Pu atom concentrations and the $^{239+240}\text{Pu}$ activity concentration together with the $^{240}\text{Pu}/^{239}\text{Pu}$ and $^{236}\text{U}/^{239}\text{Pu}$ isotopic ratios. Replicate samples are indicated with a *. b.d.: below detection limit. n.m.: not measured

Site	Sample	Collected date	^{236}U ($\times 10^6$ at/g)	$^{239+240}\text{Pu}$ (mBq/g)	$^{240}\text{Pu}/^{239}\text{Pu}$	^{239}Pu ($\times 10^6$ at/g)	$^{236}\text{U}/^{239}\text{Pu}$
La Greda (Chile)	LG1/09	22/05/2009	4.97±0.42	0.0225±0.0007	0.159±0.010	15.55±0.72	0.32±0.03
	LG2/09	24/06/2009	4.10±0.44	0.0145±0.0005	0.164±0.012	9.87±0.51	0.42±0.05
	LG2/09*	24/06/2009	1.36±0.27	0.0181±0.0013	0.161±0.026	1.4±1.3	0.11±0.02
	LG1/10	05/05/2010	2.06±0.26	0.0026±0.0001	0.134±0.017	1.91±0.15	1.08±0.16
	LG2/10	02/06/2010	3.51±0.32	0.0196±0.0007	0.134±0.011	14.37±0.74	0.24±0.025
	Average ±SD		3.2±1.5	0.0141±0.0076	0.150±0.015	8.6±6.7	0.43±0.38
Los Maitenes (Chile)	LM1/07	04/07/2007	4.74±0.34	0.0373±0.0025	0.212±0.030	22.9±2.5	0.21±0.02
	LM2/09	22/05/2009	1.58±0.36	0.0180±0.0006	0.172±0.012	12.04±0.59	0.13±0.03
	LM2/09*	22/05/2009	4.22±0.52	0.0094±0.0004	0.156±0.014	6.53±0.40	0.65±0.09
	LM1/10	05/05/2010	7.86±0.54	0.0063±0.0003	0.097±0.010	5.05±0.28	1.56±0.14
	Average ±SD		4.6±2.6	0.018±0.014	0.159±0.048	11.6±8.1	0.64±0.65
Puchuncaví (Chile)	PU1/07	04/07/2007	4.73±0.87	0.0292±0.0016	0.117±0.017	22.3±1.7	0.21±0.04
	PU1/09	22/05/2009	5.47±0.52	0.0130±0.0005	0.124±0.010	9.74±0.51	0.56±0.06
	PU1/10	05/05/2010	3.92±0.39	0.0183±0.0007	0.142±0.011	13.18±0.69	0.30±0.03
	Average ±SD		4.70±0.77	0.0200±0.0083	0.128±0.013	15.1±6.5	0.36±0.18
Valle Alegre (Chile)	VA1/07	04/07/2007	9.0±1.6	0.0351±0.0026	0.123±0.024	26.4±2.6	0.34±0.07
	VA1/09	22/05/2009	b.d.	0.0073±0.0005	0.125±0.020	5.45±0.50	b.d.
	VA1/09*	22/05/2009	4.50±0.52	0.0046±0.0002	0.177±0.017	3.08±0.22	1.46±0.20
	VA1/10	05/05/2010	n.m.	0.0130±0.0005	0.165±0.014	8.81±0.54	n.m.
	Average ±SD		6.7±3.2	0.015±0.014	0.147±0.027	11±11	0.90±0.79
Santa Bárbara (Chile)	SB1/09	27/05/2009	9.1±3.1	0.0640±0.0016	0.144±0.007	45.7±1.7	0.20±0.07
Durban (South-Africa)	DU1/11	15/11/2011	b.d.	0.0180±0.0006	0.172±0.012	12.04±0.59	b.d.
Maputo (Mozambique)	MA1/11	04/10/2011	0.56±0.21	0.0078±0.0004	0.171±0.022	5.24±0.45	0.11±0.04

locations, where also one sample was analyzed. For measurements in soils of Chile, the average concentration of ^{236}U varies between a maximum of $(9.1\pm 3.1) \times 10^6$ at Santa Barbara and a minimum of (3.2 ± 1.5) (SD) $\times 10^6$ at/g at La Greda. For $^{239+240}\text{Pu}$ the range of variation is between a maximum of 0.0640 ± 0.0016 mBq/g, again at Santa Barbara, and a minimum of 0.0141 ± 0.0076 (SD) mBq/g at La Greda. For ^{239}Pu , maximum value is obtained at Santa Bárbara, being $(45.7\pm 1.7)\times 10^6$ at/g, and the minimum at La Greda, (8.6 ± 6.7) (SD) $\times 10^6$ at/g. As for the isotopic ratios for $^{240}\text{Pu}/^{239}\text{Pu}$ the range is between 0.128 ± 0.013 (SD) to 0.159 ± 0.048 (SD) for Puchuncaví and Los Maitenes, respectively. Finally the ratio $^{236}\text{U}/^{239}\text{Pu}$ varies between 0.20 ± 0.07 at Santa Barbara and 0.90 ± 0.79 (SD) at Valle Alegre. Furthermore smaller values of

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^{236}U were founded in Durban, below detection limit. $^{236}\text{U}/^{239}\text{Pu}$ ratio was higher at sample LM1/10 collected in Los Maitenes (Chile).

The comparison between the mean values obtained in Chile (South America) and values in the countries of South Africa and Mozambique (Africa) are shown in Table 3. It can be concluded from Tables 2 and 3 that for the ^{236}U larger values are obtained in Chile. And for the ^{239}Pu and $^{240} + ^{239}\text{Pu}$ values in Chile they are similar to those of South Africa and higher than in Mozambique. The $^{240}\text{Pu} / ^{239}\text{Pu}$ ratio is the same for the two African countries studied, about 0.17, and slightly lower in Chile, about 0.14. As for the relationship $^{236}\text{U} / ^{239}\text{Pu}$, the values are below the limit of detection in South-Africa, it is 0.11 ± 0.04 in Mozambique, and in Chile is much higher ranging from 0.58 ± 0.24 , at latitude 32°S and 0.20 ± 0.07 , at latitude 42°S .

4. Discussion

As discussed above, the isotopic ratio $^{240}\text{Pu}/^{239}\text{Pu}$ is a well-established indicator for the identification of the contamination source. In our samples of African continent the $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratios are 0.171 ± 0.022 and 0.172 ± 0.012 at Mozambique and South-Africa, respectively. These are all consistent within errors with the ratio expected from global fallout in Southern Hemisphere; the average composition of the $^{240}\text{Pu}/^{239}\text{Pu}$ fallout in the $30\text{-}53^{\circ}\text{S}$ latitude range, see table 3, is 0.185 with a standard deviation of 0.047 (2SD) (Kelley et al., 1999). This indicates that the observed plutonium in our samples of Africa likely originates from global fallout.

However, the $^{240}\text{Pu}/^{239}\text{Pu}$ ratio for our samples of Chile, 0.145 of average, is lower than the expected one for the global fallout in the Southern Hemisphere. They are in agreement with the reported atomic ratios observed in our previous work of samples of Chile (Chamizo et al., 2011) so, probably, they contain traces of the plutonium released in the low-yield and safety tests performed by France in Mururoa and Fangataufa atolls (22°S , 138°W), with a characteristic atom ratio ranging from 0.03 to 0.05 (Chiappini et al. 1999). Our value is similar

to these obtained by Tims et al. (2013a) across the Australian mainland, with a continental average of ~ 0.14.

Table 3: ^{236}U and ^{239}Pu atom concentrations, $^{239+240}\text{Pu}$ activity concentration and, $^{240}\text{Pu}/^{239}\text{Pu}$ and $^{236}\text{U}/^{239}\text{Pu}$ isotopic ratios. A comparison with the previous work in undisturbed areas to a depth of 5 cm is given. b.d.: below detection limit. N.D.: no data

Site (reference)	Remarks	Coordinates	^{236}U ($\times 10^6$ at/g)	$^{239+240}\text{Pu}$ (mBq/g)	$^{240}\text{Pu}/^{239}\text{Pu}$	^{239}Pu ($\times 10^6$ at/g)	$^{236}\text{U}/^{239}\text{Pu}$
Mozambique (this work)		25°S, 32°E	0.56 ± 0.21	0.0078± 0.0004	0.171± 0.022	5.24± 0.45	0.11± 0.04
South-Africa (this work)		29°S, 30°E	b.d.	0.0180± 0.0006	0.172± 0.012	12.04± 0.59	b.d.
Central of Chile (this work)		32°S, 71°W	4.81 ± 1.46	0.0171± 0.0024	0.146 ± 0.013	12.1 ± 2.0	0.58 ± 0.24
South of Chile (this work)		42°S, 72°W	9.1 ± 3.1	0.0640± 0.0016	0.144± 0.007	45.7± 1.7	0.20± 0.07
Australia (Srcnik et al., 2014)	Global Fallout (depth 5 cm)	17°S, 145°E	7.4 ± 2.5	0.077 ± 0.008	0.137± 0.007	56± 4	0.13 ± 0.04
Australia (Tims et al., 2016)	Weapons Test Sites	29° S, 131°E	128 - 6.48×10^5 (estimated)	from 9 to 1.5×10^5	from 0.01 to 0.054	from 8.9×10^3 to 1.45×10^8	1.42 (little affected) – 0.00461 (more affected)
French Polynesia (Chiappini et al., 1999)	Weapons Test Sites	22°S, 138°W	N.D.	N.D.	0.03-0.05	N.D.	N.D.
French Polynesia (Chamizo et al., 2015)	IAEA-384 (sediment from Mururoa and Fangatauf a atolls)				0.055 ± 0.001		(3.5 ± 0.4) $\times 10^{-4}$
Japan (Sakaguchi et al. 2009)	Global Fallout	34°N, 132°E,	2700-3000	1900-2200	N.D.	N.D.	0.23±0.01
USA (Ketterer et al. 2013)	Weapons Test Sites	47°N, 121°W	467±340	N.D.	0.186-0.348	2300±1600	0.19±0.04
Spain: La Palma Island (Srcnik et al. 2011b)	Global Fallout	28°N, 17°W	3558±4000	0.42±0.12	0.22±0.02	N.D.	0.20±0.02
Northern Hemisphere N. Equatorial S. Equatorial Southern Hemisphere (Kelley et al., 1999)	Global Fallout	71°-30° N	N.D.	N.D.	0.180 ± 0.014	N.D.	N.D.
		30°-0° N	N.D.	N.D.	0.178± 0.019	N.D.	N.D.
		0-30°S	N.D.	N.D.	0.173 ± 0.027	N.D.	N.D.
		30-53°S	N.D.	N.D.	0.185 ± 0.047	N.D.	N.D.
Ukrania (Kutkov et al., 1995).	Chernobyl fallout		N.D.	N.D.	0.45 - 0.52	N.D.	N.D.

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Australia was also influenced, besides the global fallout, by the local fallout consequence of the weapons development program. The United Kingdom carried out 12 atmospheric nuclear weapons tests in Australia over the period 1952 to 1957 (Butement et al., 1958). In our case, most of the French tests were conducted hundreds of meters above the sea level (Valković, 2000), so the corresponding fallout, mostly fine-size particles, may have travelled thousands of kilometers in the troposphere, reaching South America. In similar latitude but farther east, in Buenos Aires (34°S, 58°W), Argentina, values are between 0.102 and 0.180, and further south in Puerto Montt (41°S, 72°W), Chile, values between 0.145 and 0.160 have been measured (Krey et al., 1976).

The $^{240}\text{Pu}/^{239}\text{Pu}$ ratio is somewhat lower than the average Northern Hemisphere global fallout value of $0.180 \pm 0.014(2\sigma)$ in latitude 71°-30° N, and $0.178 \pm 0.019(2\sigma)$ in latitude 30°-0° N, and far below the ratio for Chernobyl of 0.45 to 0.52 (Kutkov et al., 1995)

On the other hand, ^{239}Pu concentrations vary gradually with increasing latitude. Specifically it varies a factor of 10, from 5.2×10^6 to 45.7×10^6 at/g from Mozambique (25°S) to Chile (42°S), respectively. Maximum values found in this study are similar to those in Australia (Srncik et al., 2014), about 56×10^6 at/g, also south latitude, 17°S, in areas influenced only by global fallout. In northern latitude and in areas affected by other depositions, the values reported in the literature are superior to those reported by us. For example, Ketterer et al. (2013) reported values of 2300×10^6 at/g in Washington State (47°N, 121°W) in surface soils of areas affected by atmospheric nuclear testing.

Assuming a soil density of 1.5 g/cm^3 , these results can be converted to inventories of $3.9 - 34 \times 10^7$ at/cm², from Mozambique (25°S) to Chile (37°S), in the top 5 cm of soil. These are somewhat lower than those reported by Kelley et al. (1999) close to the sampling site selected for the present study. These authors gave 124×10^7 , 46×10^7 and 40×10^7 at/cm² in the Chilean site of Santiago (33°S, 71°W), Puerto Montt (41°S, 73°W) and Punta Arenas (53°S,

71⁰W), respectively; and 62×10^7 at/cm² in Stellenbosch (31⁰S, 19⁰E), South Africa. These inconsistencies might be caused by different depth distributions of the Pu in the top soil layers and from the rough estimation of soil dry density. For instance, in soils from a specific area of Japan, about 50% of the total inventory of fallout Pu was detected in to the first 10 cm, peaking its distribution at about that depth (Sakaguchi et al., 2009); in soil cores from Washington state, between 50% and 80% of the Pu inventory is detected in the first 5 cm of soil (Ketterer et al., 2013). Therefore, in the absence of a Pu depth distribution study in our case, our results can just be considered as estimations in terms of orders of magnitude.

As in the case of the ²³⁹Pu activity concentrations, it is interesting to remark the gradual variation of ²³⁹⁺²⁴⁰Pu with increasing latitude. It varies a factor of 10, in this case from 0.0078 to 0.064 mBq/g from Mozambique (25⁰S) to Chile (37⁰S), respectively. The values found in this study are similar to those measured in Australia (Srncik et al., 2014), about 0.077 mBq/g for the first 5 cm depth, also southern latitude, 17⁰S, in areas influenced only by global fallout. In northern latitude the values reported in the literature are some superior to those reported by us. For example, Srncik et al. (2011b) gave values of about 0.4 mBq/g in surface soil of La Palma island (28⁰N, 17⁰W), Spain, situated in front of the north of Africa, and affected only by global fallout.

On the other hand and as is the case with our data of ²³⁹Pu, ²³⁶U concentrations vary gradually with increasing latitude. Specifically it varies also a factor of 10, from 0.56×10^6 to 9.1×10^6 at/g from Mozambique (25⁰S) to Chile (37⁰S), respectively. However, as it has already been mentioned in the introduction, only limited data on ²³⁶U are available in the literature. The present results on ²³⁶U are the second reported for the Southern Hemisphere from a site that is not near a nuclear weapons test site and the first for South America and Africa. The first results for the Southern Hemisphere had been reported by Srncik et al. (2014). These authors reported values of about 7×10^6 at/g in surface soil of Australia (17⁰S, 145⁰E) affected only by

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global fallout. And also in Australia but at the Maralinga test site where the majority of the United Kingdom carried out the nuclear test, we have estimated from the values of Tims et al. (2016) and for a similar depth (0-4.8 cm) that the values in the more affected site is 70×10^6 at/g.

In the northern hemisphere, Sakaguchi et al. (2009) reported values on 2000×10^6 at/g in soil, depth between 0-10 cm, in Ishikawa prefecture (34°N , 132°E), Japan, where the site is representative only for global fallout. Ketterer et al. 2013 studied the deposition of ^{236}U from atmospheric nuclear testing in four soil cores from Washington State (northwestern USA) (47°N , 121°W), reporting values the order of 500×10^6 at/g. Srncik et al. (2011b) in soil of La Palma island, Spain, affected only by global fallout, give values of 3500×10^6 at/g.

With respect to $^{236}\text{U}/^{239}\text{Pu}$ ratio we obtained values below detection limit at South-Africa, 0.11 ± 0.04 at Mozambique, 0.58 ± 0.24 in 32°S latitude, and 0.20 ± 0.07 in 42°S in latitude, at Chile. As we have discussed so far, the only data reported in the southern hemisphere in an area affected only by the global fallout is that of Srncik et al. (2014) in Australia. These authors obtained an average value of 0.085 ± 0.003 and therefore similar to the South Africa and less than our values of Chile. However, the $^{236}\text{U}/^{239}\text{Pu}$ ratio obtained in Santa Bárbara, of about 0.20, is in agreement with the global fallout ratio for the Northern Hemisphere reported in (Sakaguchi et al., 2009), of 0.23 ± 0.01 . The high values obtained for the other studied sites are not similar to other reported values in the literature. For instance, it has been estimated a $^{236}\text{U}/^{239}\text{Pu}$ ratio of 0.260 ± 0.015 in a reference soil sample affected by the so-called Chernobyl fallout (i.e. IAEA-375), and in a reference sediment sample from the French Polynesia (i.e. IAEA-384) a value of $(3.5 \pm 0.4) \times 10^{-4}$ has been published (Chamizo et al., 2015). This last ratio follows the same trend as the ones given for surface soils around two British nuclear weapons tests sites in Australia in (Tims et al., 2016), with values ranging from 5.1×10^{-4} to 1.4×10^{-2} . Therefore, $^{236}\text{U}/^{239}\text{Pu}$ atom ratios lower than the ones given for global fallout would have been

expected in advance for areas impacted by the low-yield tests conducted in Southern Hemisphere. This is not the case of the Chilean samples from 32° southern latitude. The most likely explanation is a different soil depth distribution of ^{236}U compared to ^{239}Pu , being probably the top soil layers enriched in ^{236}U . Further studies are necessary to get more information about the representation of these data.

4. Conclusion

Surface soil samples from undisturbed areas were chosen to investigate the occurrence of ^{236}U , ^{239}Pu and $^{239+240}\text{Pu}$ as well as the $^{240}\text{Pu}/^{239}\text{Pu}$ and $^{236}\text{U}/^{239}\text{Pu}$ isotopic ratios from sites in the Southern Hemisphere, Chile in South America and Mozambique and South-Africa in Africa. This study reports the first soil values of ^{236}U fallout at South-America and Africa.

The obtained $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratio is 0.17 in soils from Africa, consistent with the ratio expected from global fallout in Southern Hemisphere. However, the ratio for samples of Chile, 0.14, was lower than the expected one for the global fallout in the Southern Hemisphere so, they probably contain traces of the plutonium released in the low-yield tests performed by France in Mururoa atoll, as it was stated in a previous study. The $^{240}\text{Pu}/^{239}\text{Pu}$ ratio is somewhat lower than the average Northern Hemisphere global fallout value of 0.18 in the same latitude.

The maximum values of ^{239}Pu , $^{239+240}\text{Pu}$ and ^{236}U are similar to those in the Southern Hemisphere in areas influenced only by global fallout and lower to the values of the north latitude in areas affected by other depositions.

With respect to $^{236}\text{U}/^{239}\text{Pu}$ ratio we obtained values below detection limit at South-Africa, 0.11 ± 0.04 at Mozambique, 0.58 ± 0.24 in 32°S latitude, and 0.20 ± 0.07 in 42°S in latitude, at Chile. The values of South-Africa are similar to obtained by Srncik et al. (2014) in Australia in area affected only by the global fallout. Probably the highest values obtained of this ratio in Chilean soils are due to different depth distributions of ^{236}U and ^{239}Pu , being the soils likely

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enriched in ^{236}U in the most surface layers. Further studies are necessary to explain those high ratios. The $^{236}\text{U}/^{239}\text{Pu}$ ratio was clearly lower compared to values found in the Northern Hemisphere.

Acknowledgements.

This work has been financed thanks to the Spanish Ministry of Economy and Competitiveness (project FIS2015-69673-P), AECID (project A1/037813/11), the Spanish Ministry of Science and Innovation (project CTQ2014-52309-P), FEDER and Junta de Extremadura (GR15087) in Spain.

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3.5. General discussion

The results obtained in this thesis have allowed to enhance the scientific knowledge related to the optimization and experimental validation of strategies for the assessment of ecological risks and risks to human health, derived from exposition to environmental pollutants. Specifically, we have investigated trace elements and radioisotopes, occurring in soils and native plants as the results of atmospheric emissions from different sources, especially industrial sources.

Regarding the results obtained during risk assessment in the Puchuncaví area (Valparaíso Región, Chile), heavily impacted by industrial emissions from the beginning of the 1960s, we have generated significant improvements in the knowledge of the area in terms of ecological risk, risk for human health, and pollutants transfer from soil to vegetal biomass.

Respect to ecological risk assessment, the results that we present in the article ***“Long-term assessment of ecological risk from deposition of elemental pollutants in the vicinity of the industrial area of Puchuncaví-Ventanas, central Chile”***, published in the journal Science of the Total Environment, were obtained from soil sampling campaigns carried out at five locations representing different degree of environmental impact around the Puchuncaví-Ventanas industrial complex, from 2007 to 2011. Selected parameters (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba) were assayed in the soil samples. The variability of the elemental concentrations in soil samples within the study area (including the sampling locations La Greda, Los Maitenes, Puchuncaví and Valle Alegre) was somewhat high, depending on distance to the pollution sources. Moreover, SW dominant winds in the study area have been identified as relevant agents of pollutants transport. Soil levels of some elements such as Pb, Cd, Ni, Hg, Zn, Sb, Cr, and Co in industrial affected areas (La Greda and Los Maitenes) don't exceed the international soil quality standard (e.g. Australia, Netherland and Canada) values selected for comparison but some elements especially Cu level at Los Maitenes and La Greda is 4 and 7

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times higher, respectively. These results can be derived from the environmental impact of the copper smelter operating in the area. Our results were compared with the results of previous soil monitoring campaigns conducted in the area. Some elements like As and Sb have similar concentration with previous study, but some element like Cu concentration in the soil samples is more than double of the results reported on 2003. This result may indicate a progressive enrichment of the soil due to persistence copper emissions in the area. For a better understanding of trace element levels evolution, the results were examined to identify possible temporal evolution of the most significant elemental pollutants in the soils of the study area. Most of the environmentally significant elements assayed show maximum soil concentration values in 2007 (except Hg that peaks in 2009), the values then decline in 2011 to the minimum values measured during the studied period.

Cluster analysis and principle component analysis (PCA) technics were used to identify probable pollution sources in this area. These analysis have shown industrial activities (especially activities of the copper smelter and power plants), anthropogenic activities (especially traffic), and mineral –crustal fraction.

Ecological impact risk was assessed by enrichment factor (EF), geoaccumulation index (I_{geo}), contamination factor (C_f), contamination degree (C_{deg}), and integrated pollution index (IPI). Cu is the only element showing I_{geo} extreme contamination in La Greda and Los Maitenes, with I_{geo} values for Cu declining with distance from the copper refinery source but still high in Puchuncaví village. Moderate Cu contamination was detected in Valle Alegre, and even in the unpolluted reference area of Maitencillo. As, Cd, Hg and Sb show relatively high I_{geo} values at La Greda and Los Maitenes. About the enrichment factor (EF) index, Cu is the only element showing an extremely high index value in the locations more affected by the industrial activities, EF values for Cu decrease with the distance but they show a very high enrichment in Puchuncaví village and a significant enrichment in Valle Alegre. Even Maitencillo shows a moderate Cu enrichment. EF values for the studied elements are in accordance with I_{geo} values.

Regarding the contamination factor (C_f), the results at the most impacted locations of La Greda and Los Maitenes are mostly within the category of very high contamination, especially for Cu and As. C_f values tend to decrease with distance to the pollution sources. Contamination degrees (C_{deg}) results show very high but decreasing contamination in La Greda, Los Maitenes and Puchuncaví, whereas considerable contamination is assigned to Valle Alegre and Maitencillo. Contamination factor and degree are more strict ecological impact indexes than I_{geo} or EF for the assessment of the study area. C_f shows a strong linear correlation with EF and a perfect match with I_{geo} . The integrated pollution index (IPI), clearly shows the overall level of contamination in the soil sampling sites within the study area. IPIs are in the order LG > LM > PU > VA > MA.

Respect to human health risk assessment, the results are presented in the article ***“Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncaví-Ventanas industrial complex, Chile”***, published in the journal Environmental Pollution. Human health risk (non-carcinogenic and carcinogenic risks) for children, adults, and farmers/shepherds was assessed by different standard indexes. Non-carcinogenic risk by due to exposition to trace elements were evaluated by hazard quotient (HQ), hazard index (HI), and cumulative HI. Ingestion HQ of arsenic (HQ_{ing-As}) for children is above 1 (probable adverse health effects) in La Greda, Los Maitenes and Puchuncaví. HQs for adults, shepherds/farmers are in all cases below 1 (adverse health effects are unlikely). Ingestion hazard Index (HI) for children in all locations indicates potential chronic effects (above 1) and for adults and farmers/shepherds in La Greda and Los Maitenes are above 1 too. A detailed statistical of spatial differences of ingestion risk for children was carried out by calculating HI_{ing} values for individual soil samples and applying descriptive analysis (box-whisker plots), ANOVA test for significant risk differences among locations, and hierarchical cluster analysis. The box-whisker plot showed a higher dispersion of HI_{ing} values at the more polluted sampling locations of La Greda and Los Maitenes. The ANOVA test shows that the two

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most polluted sampling locations of La Greda and Los Maitenes present a similar level of ingestion risk. Cluster analysis of individual HI_{ing} values for children classified the data in three categories; category with low ingestion risk, category with intermediate values of HI_{ing} , and category with the highest values of HI_{ing} . The calculated carcinogenic risk indexes show extremely low carcinogenic risk through inhalation (Cd and Ni) between ranges 10^{-9} to 10^{-11} (considered as not significant health effect), whereas dermal and ingestion risks are between 1.0×10^{-4} and 1.0×10^{-6} (consider as acceptable range). Total carcinogenic risk just for children is above the 10^{-4} (unacceptable) in Los Maitenes and on the limit at La Greda.

We have assessed gamma ray radiation external hazard indexes and annual effective dose rate from the natural radioactivity elements (^{226}Ra , ^{232}Th and ^{40}K) levels in the surface soils around the Puchuncaví-Ventanas industrial area. It is worth highlighting that the present study is the first about radiological risk from radioisotopes in the soils of this area. Mean specific values of natural radionuclides (^{226}Ra , ^{232}Th , and ^{40}K) around the Puchuncaví-Ventanas industrial complex are generally low, and no influence of the distance to the industrial pollution sources was observed. The highest average value of radionuclide belongs to ^{40}K (615 Bq kg^{-1}) in Valle Alegre location. The highest values of Radium Equivalent Activity and External Hazard Index are 114 Bq kg^{-1} (maximum recommended value for home buildings is 370 Bq kg^{-1} and for industrial buildings $370\text{-}740 \text{ Bq kg}^{-1}$) and 0.31 (maximum value allowed 1) respectively, measured in soils from Valle Alegre sampling area. In conclusion, gamma ray radiation external hazard indexes and annual effective dose rate from the natural radioactivity elements (^{226}Ra , ^{232}Th and ^{40}K) levels in the surface soils of the study area show no significant risk. No significant variability of radioactive risk was observed among sampling locations, so it seems that the industrial activities in the area have produced no alteration of radioactivity levels in the soils. Our radioactivity results may be useful for the development of reference levels of natural radioactivity along the Pacific coast of South America.

Trace elements levels in biomass of native plants in the Puchuncaví area (namely *Oenothera Affinis*, OA; *Sphaeralcea Velutina*, SV and *Argemone Subfusiformis*, AS) were investigated to study uptake of pollutants from soil and their accumulation in the biomass samples in this industrial area, and the main results were included in the article ***“Trace elements levels in native plant species around the industrial site of Puchuncaví-Ventanas (central Chile): Evaluation of the phytoremediation potential”***, in preparation for the journal Environmental Science and Pollution Research. Moreover, the transfer of these pollutants from soil to plant (transfer factor) was assessed in order to identify the potential use of these plant species for phytoremediation. Copper has high concentration in external parts of plants (flower and leaf) in affected areas (La Greda and Los Maitenes) in agreement with Cu concentration in soil samples that were collected from same areas. On the other hand, concentration of trace elements in the plants was not inversely correlated to the distance of the main emission sources, as happened in soil samples studies. This issue pointed out the influence of soil variables such as; soil nutrients, pH, organic matter, clay contents in the plant uptake of trace elements. In order to better understand the role of soil and type of contaminated area on transfer of trace elements to the selected native plant species, soil to plants Transfer factor (TF) values for the studied trace elements were calculated for each of the investigated plants species. TF values calculated for the different trace elements varied widely among the different plant species, being in the high (1-2), medium (0.1-0.3), or low level (less than 0.09) range for most of the trace elements, indicating a low uptake by plants compared to the soil reservoir. Only Cr and Ni had TFs values > 1, which indicated enrichment of these elements in plants in some locations. Bases on TFs results, there are just phytoremediation potential for OA, AS, and SV species in polluted area.

Finally, we have developed a study about environmental distribution of uranium and plutonium radionuclides (^{236}U concentrations and $^{236}\text{U}/^{239}\text{Pu}$ ratios) in soils from selected locations in the southern hemisphere, the results being summarized in the manuscript

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“Presence of fallout ^{236}U and plutonium isotopes, ^{239}Pu and ^{240}Pu , in soils from Southern Hemisphere”, in preparation for the journal Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. To best knowledge of the authors the present work is the first publication on ^{236}U concentrations and $^{236}\text{U}/^{239}\text{Pu}$ atom ratios in the Africa and Southern America. The Southern Hemisphere has received these isotopes due to the global fallout and also an important contribution of the so-called tropospheric fallout from both the atmospheric nuclear tests performed in the French Polynesia and in Australia by France and United Kingdom, respectively. We provide new data on the impact of the selected isotopes to Chile in South America, and Mozambique and South-Africa in Africa. Surface soil samples from undisturbed areas were chosen to investigate the occurrence of these radionuclides. The obtained results point out to the presence of debris from the French tests in the Chilean soils. Instead African soils studied are only affected by global fallout corresponding to the southern hemisphere. For measurements in soils of Chile, the average concentration of ^{236}U is maximum in Santa Barbara and minimum in La Greda. $^{239+240}\text{Pu}$ has maximum value again in Santa Barbara, and minimum value in La Greda. ^{239}Pu has maximum value in Santa Bárbara and minimum in La Greda. The obtained $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratio is 0.17 in soils from Africa, consistent with the ratio expected from global fallout in Southern Hemisphere. However, the ratio for samples of Chile, 0.14, was lower than the expected one for that global fallout so they probably contain traces of the plutonium released in the low-yield tests performed by France in Mururoa atoll, as it was stated in a previous study. The $^{240}\text{Pu}/^{239}\text{Pu}$ ratio is somewhat lower than the average Northern Hemisphere with a global fallout value of 0.18 in the same latitude.

3.6. Discusión general

Los resultados obtenidos en esta tesis han permitido ampliar el conocimiento científico en relación con la optimización y validación experimental de estrategias para la evaluación de riesgos ecológicos y riesgos para la salud humana, derivados de la exposición a contaminantes ambientales. Específicamente se han investigado elementos traza y radioisótopos, presentes en suelos y plantas autóctonas como resultado de la deposición de emisiones atmosféricas desde diferentes fuentes, especialmente desde fuentes industriales.

En cuanto a los resultados obtenidos durante la evaluación de riesgos ambientales en la comuna de Puchuncaví (región de Valparaíso, Chile), fuertemente impactada por emisiones industriales desde los años sesenta del siglo XX, se han generado avances significativos en el conocimiento del entorno en cuanto a riesgos ecológicos, riesgos para la salud humana y transferencia de contaminantes desde el suelo hacia la biomasa vegetal.

En cuanto a la evaluación del riesgo ecológico, los resultados que se presentan en el artículo ***“Long-term assessment of ecological risk from deposition of elemental pollutants in the vicinity of the industrial area of Puchuncaví-Ventanas, central Chile”***, publicado en la revista Science of the Total Environment, derivan de campañas de muestreo de suelos desarrolladas en cinco puntos de muestreo representativos de diferentes grados de impacto de la zona industrial de Puchuncaví-Ventanas, entre 2007 y 2011. Se analizaron parámetros seleccionados (As, Pb, Cd, Ni, Hg, V, Mn, Zn, Sr, Sb, Cr, Co, Cu, K, and Ba) en las muestras de suelo. La variabilidad de las concentraciones de los analitos en las muestras de suelo tomadas en el área de estudio (localizaciones de muestreo de La Greda, Los Maitenes, Puchuncaví y Valle Alegre) resultó ser bastante elevada, dependiente de la distancia a las fuentes de contaminación. Además, los vientos SW dominantes en el área de estudio han sido identificados como agentes relevantes en el transporte de contaminantes.

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Los niveles en suelos de algunos elementos como Pb, Cd, Ni, Hg, Zn, Sb, Cr y Co, no exceden los estándares internacionales de calidad del suelo (p. ej. Australia, Holanda y Canadá) tomados como término de comparación, incluso en los puntos de muestreo más impactados (La Greda y Los Maitenes), pero algunos otros elementos, especialmente el Cu, presentan niveles 4 y 7 veces superiores en Los Maitenes y La Greda, respectivamente. Estos resultados indican una clara influencia de la refinera de cobre presente en la zona. Nuestros resultados se compararon con los de estudios previos de monitorización en la zona. Algunos elementos como As y Sb presentan niveles similares, pero otros como el Cu presentaron niveles superiores al doble de los registrados en estudios realizados en 2003. Estos resultados podrían indicar un progresivo enriquecimiento del suelo debido a la persistencia de las emisiones de cobre en la zona. Para una mejor comprensión de la evolución de los niveles de elementos traza, se examinaron los resultados en busca de identificar tendencias en su evolución temporal en el suelo de las áreas estudiadas. La mayoría de los elementos significativos desde el punto de vista medioambiental mostraron concentraciones máximas en el suelo en 2007 (con la excepción de ciertos picos de Hg en 2009), los valores declinaron después hasta 2011, que proporcionó los valores mínimos medidos durante el período de estudio.

Se efectuaron análisis clúster y de componentes principales (PCA) para identificar las fuentes más probables de contaminación en la zona. Estos análisis han mostrado la influencia de las actividades industriales (especialmente las actividades de la refinera de cobre y las centrales térmicas), otras actividades antropogénicas (especialmente tráfico), y fuentes minerales – crustales.

El riesgo de impacto ecológico se evaluó mediante el factor de enriquecimiento (EF), el índice de geoacumulación (I_{geo}), el factor de contaminación (C_f), el grado de contaminación (C_{deg}) y el índice de contaminación integrado (IPI). El Cu es el único elemento que muestra valores I_{geo} indicativos de contaminación extrema en La Greda y Los Maitenes, con valores decrecientes al

umentar la distancia respecto a la refinera de cobre, pero todavfa elevados en la ciudad de Puchuncavf. Se detectf una contaminaci6n moderada de Cu en Valle Alegre, e incluso en la zona de referencia no contaminada de Maitencillo. As, Cd, Hg y Sb mostraron valores relativamente elevados de I_{geo} en La Greda y Los Maitenes. En cuanto a los valores del factor de enriquecimiento (EF), el Cu es el ffnico elementos que muestra valores extremadamente elevados en las localizaciones mfs afectadas por las actividades industriales. Los valores de EF para Cu disminuyen con la distancia pero muestran un enriquecimiento todavfa muy elevado en la ciudad de Puchuncavf y un enriquecimiento significativo en Valle Alegre. Incluso Maitencillo muestra un enriquecimiento moderado de Cu. Los valores de EF para los elementos estudiados se correlacionan bien con los valores de I_{geo} . Respecto a los valores del factor de contaminaci6n (C_f), los resultados en los entornos mfs contaminados de la zona (La Greda y Los Maitenes estfn mayoritariamente dentro de la categorfa de contaminaci6n muy elevada, especialmente para Cu y As. Los valores de C_f tienden a disminuir con la distancia a las fuentes de contaminaci6n en la zona. Por su parte, los resultados del grado de contaminaci6n (C_{deg}) muestran valores muy altos en La Greda y Los Maitenes, decreciendo en Puchuncavf y mostrando valores de contaminaci6n considerable en Valle Alegre y Maitencillo. El factor de contaminaci6n y el grado de contaminaci6n han mostrado ser ffnices de impacto ecol6gico mfs estrictos que el ffnice de geoacumulaci6n o el factor de enriquecimiento. El ffnice de contaminaci6n integrado (IPI), muestra claramente el nivel general de contaminaci6n en el ffnrea de estudio, clasificfndose como La Greda > Los Maitenes > Puchuncavf > Valle Alegre > Maitencillo.

Respecto a la evaluaci6n de riesgos para la salud humana, los resultados se presentan en el artfculo ***“Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncavf-Ventanas industrial complex, Chile”***, publicado en la revista Environmental Pollution. Se evalfa el riesgo carcinogfnico y no carcinogfnico para ni os, adultos y agricultores/pastores, mediante diferentes ffnices. El

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riesgo no carcinogénico por exposición a elementos traza se evaluó mediante el cociente de riesgo (HQ), el índice de riesgo (HI), y el índice de riesgo acumulativo (cumulative HI). El HQ por ingestión (HQ_{ing-As}) para niños es superior a 1 (indicando efectos adversos probables) en La Greda, Los Maitenes y Puchuncaví. Los valores de HQ para adultos y agricultores/pastores son en todos los casos inferiores a 1 (indicando poca probabilidad de efectos adversos). El índice de riesgo por ingestión para niños (HI) en todas las localizaciones indica efectos crónicos potenciales (índice superior a 1), siendo también superior a 1 para adultos y agricultores/pastores en La Greda y Los Maitenes. Se llevó a cabo un estudio estadístico detallado de diferencias espaciales de riesgo de ingestión para niños calculando los valores HI_{ing} para muestras de suelo individuales, y aplicándoles análisis descriptivo (gráficos de cajas y bigotes), test ANOVA para detectar diferencias significativas de riesgo entre ubicaciones, y análisis clúster. Los gráficos de cajas y bigotes mostraron una dispersión elevada de los valores de HI_{ing} en las ubicaciones más impactadas de La Greda y Los Maitenes. El test ANOVA mostró que La Greda y Los Maitenes presentan niveles similares de riesgo por ingestión. El análisis clúster clasificó los datos en tres categorías de bajo, intermedio y alto riesgo. El cálculo de los índices de riesgo carcinogénico mostró valores extremadamente bajos de riesgo por inhalación (Cd y Ni) en rangos de 10^{-9} a 10^{-11} (sin impacto en la salud), mientras que los riesgos por contacto dérmico y por ingestión se encuentran entre 1.0×10^{-4} and 1.0×10^{-6} (rango aceptable). Sin embargo, el riesgo carcinogénico total para niños está por encima del valor umbral 10^{-4} en Los Maitenes (riesgo no aceptable) y justo en el límite en La Greda.

Se ha evaluado el índice de riesgo externo por radiación gamma y la dosis efectiva anual por elementos radiactivos naturales (^{226}Ra , ^{232}Th and ^{40}K) en el suelo superficial alrededor de la zona industrial de Puchuncaví-Ventanas, siendo este el primer estudio de este tipo desarrollado en la zona. Los valores medios específicos de radionúclidos naturales son generalmente bajos y no se observa una influencia significativa de la distancia a las fuentes de contaminación industrial en la zona. Los valores más elevados se observaron para ^{40}K (615 Bq

kg⁻¹) en Valle Alegre. Los valores más elevados de actividad equivalente de radio y de índice de riesgo externo fueron 114 Bq kg⁻¹ para el primero de ellos (el valor máximo recomendado para materiales de construcción de casas es 370 Bq kg⁻¹ y para construcciones industriales es 370-740 Bq kg⁻¹) y 0.31 para el segundo (valor máximo permitido 1), medidos en suelos de Valle Alegre. En conclusión, el índice de riesgo externo por radiación gamma y la dosis efectiva anual por elementos radiactivos naturales (²²⁶Ra, ²³²Th and ⁴⁰K) en los suelos de la zona no presentan riesgos significativos, y no se observa variabilidad significativa en la zona de estudio. Puede concluirse que la actividad industrial no ha producido alteración de los niveles de radioactividad en el suelo. Los niveles de radioactividad medidos pueden ser de utilidad para el establecimiento de niveles de referencia de radioactividad a lo largo de la costa del Pacífico Sudamericana.

Se investigaron los niveles de elementos traza en la biomasa de plantas autóctonas del área de Puchuncaví (*Oenothera Affinis*, OA; *Sphaeralcea Velutina*, SV y *Argemone Subfusiformis*, AS), para estudiar la incorporación de contaminantes desde el suelo y su acumulación en la biomasa, y los principales resultados se han recogido en la publicación ***“Trace elements levels in native plant species around the industrial site of Puchuncaví-Ventanas (central Chile): Evaluation of the phytoremediation potential”***, en preparación para la revista Environmental Science and Pollution Research. Además, se ha estudiado la transferencia de estos contaminantes desde el suelo a la biomasa vegetal mediante el factor de transferencia, para identificar el uso potencial de estos vegetales autóctonos en fitorremediación. El cobre presenta una elevada concentración en partes aéreas de las plantas (flores y hojas) en las localizaciones de muestreo más impactadas (La Greda y Los Maitenes), en correspondencia con los elevados niveles de concentración de Cu encontrados en los suelos de las mismas zonas. Por otra parte, la concentración de los elementos traza en las plantas no está inversamente correlacionado con la distancia a las fuentes de emisión, a diferencia de lo que ocurre con las concentraciones en los suelos. Este factor pone de manifiesto la importancia de

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las variables como nutrientes del suelo, pH, material orgánica o contenido de arcilla, sobre la transferencia de elementos traza desde el suelo hasta la biomasa vegetal. Para una mejor comprensión del papel del suelo y del tipo de área contaminada sobre la transferencia de los elementos traza hacia los vegetales, se calcularon los factores de transferencia suelo planta (TF) para los elementos estudiados en cada una de las especies vegetales investigadas. Los valores de TF variaron ampliamente entre las diferentes especies en estudio, encontrándose en rango alto (1-2), medio (0.1-0.3), bajo (menor de 0.09) para la mayoría de los elementos traza, indicando una baja transferencia de elementos desde las reservas del suelo. Sólo el Cr y el Ni tienen valores de $TF > 1$, lo cual indica el enriquecimiento de estos elementos en las plantas en algunas localizaciones. Con base en los TF obtenidos, sólo las especies OA, AS y SV en la zona contaminada tienen potencial para fitorremediación.

Finalmente, se ha desarrollado un estudio sobre la distribución ambiental de radionúclidos de uranio y plutonio (concentraciones de ^{236}U y ratios de $^{236}\text{U}/^{239}\text{Pu}$) en suelos de localizaciones seleccionadas en el hemisferio sur, cuyos resultados principales se recogen en la publicación ***“Presence of fallout ^{236}U and plutonium isotopes, ^{239}Pu and ^{240}Pu , in soils from Southern Hemisphere”***, en preparación para la revista Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. Según nuestros datos, el presente estudio ha generado la primera publicación sobre concentraciones de ^{236}U y ratios de $^{236}\text{U}/^{239}\text{Pu}$ en África y Sudamérica. Se proporcionan nuevos datos sobre el impacto de los isótopos seleccionados en Chile en Sudamérica, y en Mozambique y Sudáfrica en África. Se analizaron muestras de lugares no perturbados para investigar la presencia y concentración de los radionúclidos. Los resultados apuntan a la presencia de residuos de ensayos nucleares franceses en los suelos de Chile. Por el contrario, los suelos de África parecen estar únicamente afectados por el fallout global correspondiente al hemisferio sur. Para las medidas en suelos de Chile, la concentración media de ^{236}U es máxima en Santa Bárbara y mínima en La Greda. El ratio atómico $^{240}\text{Pu}/^{239}\text{Pu}$ es 0.17 en los suelos africanos, consistente con el ratio

esperado del fallout global en el hemisferio Sur. Sin embargo, el ratio de las muestras de Chile, 0.14, fue más bajo que el esperado del fallout global, por lo que probablemente contiene trazas de los ensayos de baja intensidad realizados por Francia en Mururoa, como se ha indicado en estudios previos. El ratio $^{240}\text{Pu}/^{239}\text{Pu}$ es algo inferior que la media del hemisferio Norte, con un fallout global de 0.18 en la misma latitud.

CHAPTER 4.

CONCLUSIONS

4. Conclusions

1. A long term (2007–2011) soil monitoring campaign conducted around the industrial area of Puchuncaví-Ventanas in central Chile, based on the measurement of elemental concentration profiles and statistical analysis (PCA and cluster statistics) shows copper refinery and a coal-fired power plant complex have been identified as major pollution sources in this area.
2. The observed evolution of soil concentrations of elemental pollutants in the studied period, showing a decline from 2009 to 2011, can be related to improved environmental technologies adopted by the industries in recent years.
3. Application of several quantitative risk assessment indexes (geoaccumulation index, enrichment factor, contamination factor, contamination degree and integrated pollution index), has revealed significant ecological impacts, more intense at locations close to the main pollution sources and under the influence of dominant winds but also noticeable at reference background locations more than 10 km away from the industrial area.
4. Human health risk assessment study from trace elements in soil from the Puchuncaví Valley, heavily affected by a range of anthropogenic emissions from a localized industrial complex, has shown a clear spatial gradient of human health risk caused by trace elemental pollutants in soil (distances ranging from 1.5 to 18 km). Distance to source dependent cumulative non-carcinogenic hazard indexes above 1 for children were found in the study area.
5. The most important pathway of exposure to elements present in the soil is the ingestion, and As represents the highest carcinogenic risk from soil exposition in the study area. Total carcinogenic risk for children is above the 10^{-4} threshold in LM and just on the limit at LG, decreasing with distance to the industrial sources with a similar trend than observed for the non-carcinogenic risk. All locations present total carcinogenic risk above the 10^{-6} no effects threshold, so the health risk impact is observed even at 20 km from the industrial complex.

Conclusions

Therefore, more effective pollution controls should be deployed to tackle trace elements emissions from the industrial sources in the Puchuncaví-Ventanas complex in order to alleviate soil pollution in the study area, even at relatively long distance, and consequently to reduce the observed noncarcinogenic and carcinogenic health risk.

6. Gamma ray radiation external hazard indexes and annual effective dose rate from the natural radioactivity elements (^{226}Ra , ^{232}Th and ^{40}K) levels in the surface soils of the study area show no significant risk. The highest average values for the specific activity of ^{232}Th , ^{40}K , and ^{226}Ra are lower than the limit recommended by OECD. No significant variability of radioactive risk was observed among sampling locations, so it seems that the industrial activities in the area have produced no alteration of radioactivity levels in the soils. Our radioactivity results may be useful for the estimation of natural radioactivity reference levels along the Pacific coast of South America.

7. Results of biomonitoring analysis showed high concentration of trace elements in *Oenothera Affinis*, *Sphaeralcea Velutina*, and *Argemone Subfusiformis* species. However, the concentration of these elements in the plants was not inversely correlated to the distance of the main emission sources, which pointed out the influence of soil variables such as pH, on the plant uptake of HMs. The high concentrations of trace elements such as Cu, As, Cr and V, upon the toxic limits in the native plant species of the industrial area, recommends the need for continuous monitoring of the region, given that the area is under huge pressure from industrial activities.

8. No hyperaccumulator plants were found in any location, but the results indicated that *Oenothera Affinis* (OA) and *Sphaeralcea Velutina* (SV) species exhibited medium accumulation of copper in the more industrial affected areas. In fact, OA was identified as the plant with the highest capacity for HMs accumulation, showing also a higher accumulation potential in whole aerial parts than in leaves. Low bioavailability for most of the trace elements was evidenced by

the low transfer factors obtained in most of the native plant species, suggesting an exclusion of these HMs. Indeed, transfer factors values suggested that these native plants had only phytoremediation potential for Ni and Cr elements.

9. The obtained $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratio is 0.17 in soils from Africa, consistent with the ratio expected from global fallout in Southern Hemisphere. However, the ratio for samples of Chile, 0.14, was lower than the expected one for the global fallout in the Southern Hemisphere so, they probably contain traces of the plutonium released in the low-yield tests performed by France in Mururoa atoll. The $^{240}\text{Pu}/^{239}\text{Pu}$ ratio is somewhat lower than the average Northern Hemisphere global fallout value of 0.18 in the same latitude. The maximum values of ^{239}Pu , $^{239+240}\text{Pu}$ and ^{236}U are similar to those in the Southern Hemisphere in areas influenced only by global fallout and lower to the values of the north latitude in areas affected by other depositions.

10. With respect to $^{236}\text{U}/^{239}\text{Pu}$ ratio we obtained values below detection limit at South-Africa. The values of South-Africa are similar to obtained by other study in Australia in area affected only by the global fallout. Probably the highest values obtained of this ratio in Chilean soils are due to different depth distributions of ^{236}U and ^{239}Pu , being the soils likely enriched in ^{236}U in the most surface layers. Further studies are necessary to explain those high ratios. The $^{236}\text{U}/^{239}\text{Pu}$ ratio was clearly lower compared to values found in the Northern Hemisphere.