Transfer of Heavy Metals from Contaminated Soil into Organs of Soybean Crops [*Glycine max* (L.) Merr.] in Córdoba, Argentina

Diploma Thesis

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Confirmation

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Stuttgart, December 5, 2011 LYDIA STEFFAN

Abstract

The present thesis investigated into the transfer of heavy metals from contaminated soil into organs of soybean crops [*Glycine Max* (L.) Merr.], in the peri-urban municipality of Bouwer in Córdoba Province, Argentina.

Top soil and plant samples were taken from 15 sampling points at different distances to a former lead smelter. The concentrations of Cd, Pb and Zn were analyzed with flame atomic absorption spectroscopy in different soil fractions and roots, stipes, pods and seeds and were compared to threshold values of different countries. Physico-chemical soil parameters were determined and seed quality tests were carried out. The Activity Ratio, the Enrichment Factor, the Ionic Impulsion and Hazard Quotients were calculated. The transfer of heavy metals from soil to soybean organs was examined with curve models and by calculating the Transfer and Bioconcentration Factors.

The main results indicated that the detected concentrations of Cd, Pb and Zn of soil and plant samples were generally elevated above background values. To some extent they exceeded the considered thresholds for soil and soybean foodstuff. This contamination was seen to be caused by the overall environmental pollution in the municipality of Bouwer. The sampling site nearest to the former smelter was found to be significantly affected by a contamination with Pb. This appears to be caused by the former smelting activities. Phytotoxic effects could be observed and a potential risk to human health is given by the contamination level of soybean seeds.

This thesis is available in digital form at <http://marmaro.de/docs/lsteffan-thesis/>.

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List of Abbreviations

AR	Activity Ratio
av	Index for the available heavy metal fraction in soil
BBodSchV	German soil quality guideline: Bundes-Bodenschutz- und Altlastenverordnung
BCF	Bioconcentration Factor
C	Index for the CONTROL sites
CAA	Argentinian foodstuff guideline: Código Alimentario Argentino
Cd	Cadmium
CDI	Chronic Daily Intake
CEC	Cation Exchange Capacity
CFHS	Chinese Food Hygiene Standard
CGS FAO	Foodstuff guideline of the FAO: Codex General Standard for Contaminants and Toxins in Food and Feed
CR EC EU	European foodstuff guideline: Commission Regulation (EC) No 1881/2006
CSQG	Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health
DEC RP	Argentinian soil quality guideline: Decreto 831/93 Residous Peligrosos
DW	Dry Weight
EC	Electrical Conductivity
EF	Enrichment Factor
EQSS	Chinese Environmental Quality Standard for Soils
FAAS	Flame Atomic Absorption Spectroscopy

FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
GV	Germination Value
HCL	Hollow Cathode Lamp
HI	Hazard Index
HM	Heavy Metal
II	Ionic Impulsion
M-W U	Mann-Whitney U-test
MAD	Median Absolute Deviation
MaxGV	Maximum Germination Value
MIP CR	Contaminated Range of HM concentrations in Metal Industry Processing affected soils
n	Sample size
ncHQ	non-cancer Hazard Quotient
OM	Organic Matter
Pb	Lead
pt	Index for the pseudo-total heavy metal fraction in soil
R	Index for the REFERENCE site
RfDo	oral Reference Dose
S	Index for the SMELTER site
SD	Standard Deviation
TF	Transfer Factor
WR UPS	World Range of HM concentrations in Unpolluted Soil
Zn	Zinc

Preface

Intention and Realization

To go abroad was my wish since teenage years, but not just for traveling. I wished to live and study there; to really get in touch with the people from the other country and their different ways of life. In a globalizing world, where people move closer every day, a valuable experience, which mediates skills, that can not be read out of a book by studying.

I remember this lecture in autumn of the year 2009, after which I had this smalltalk with one of my professors. In 15 minutes we worked out an idea which should become true within the next 2 years: to do my thesis abroad.

Since several years, a relationship due to the intention of Dr. ANDREAS KLUMPP exist between the *Department of Plant Ecology and Ecotoxicology* at the *University of Hohenheim* in Stuttgart, Germany, which is directed by Prof. Dr. ANDREAS FANGMEIER and the *Instituto Multidisciplinario de Biología Vegetal,Sección Contaminación y Bioindicadores* of the *Universidad Nacional de Córdoba* in Argentina, which is directed by Prof. Dr. MARÍA LUISA PIGNATA.

Both institutes conduct research in the area of environmental pollution and ecotoxicology. My thesis should be related with exactly these topics. Everything seemed to be perfect and I really wanted to go, but I was worried about financial aspects. Hence, I searched for possibilities of financial support to carry out a thesis abroad.

"Change by Exchange" is the slogan of the *German Academic Exchange Service* (DAAD), the largest funding organization of scholarships in the world. I got accepted in the (no longer arranged) program "Short-term study visits for theses of students". With this scholarship it was possible to carry out the field studies and laboratory analyses at the cooperating University in Córdoba, Argentina. Beside gaining self-confidence, self-reliance and advancing in a foreign language, I can confirm that this time qualified me in the "spirit of tolerance and openness" [DAAD, 2011].

Acknowledgments

Not everything was that easy liked described in brief form above. It was a long and exhausting, sometimes stony and difficult way, but more important than that is: it was also a very valuable, enriching challenge and educational experience in my life.

Thank you all who supported and accompanied me during this time!

Especially I want to thank my German advisors Prof. Dr. Andreas Fangmeier and Dr. Andreas Klumpp for accepting the topic and the supervision of this thesis. My special thanks also to my German supervisor Dr. Jürgen Franzaring for the company and support from the idea till the realization of this work; for all the scientific inputs, ideas, critics, and the excellent proofreading.

I like to express my gratitude to my supervisor in Argentina, Prof. Dr. María Luisa Pignata, for making it possible to carry out my studies in Córdoba and giving me always the feeling of being part of the team: Gabi, Caro, Clau, Anna, Hebe, Fer, Flor, Edu, Gus, Gon, Carlos and Dr. Judith Hebelen Rodriguez and María Julieta Salazar, whom I would like to thank especially for all their time, support and the knowledge transfer in the fields and laboratories. You all together made it a great and unforgettable time!

For backing this study financially with the scholarship, I want to thank the DAAD.

I am deeply grateful to my family, to the Schnalke family, and to my friends for being there during all the time; big thanks to Marta, Jassi, Kerstin, Heidje, Carsten, and Ju for the proofreading.

Dear Markus, for all your **L**ove **a**nd **T**ractive **e**verlasting **X**hilaration support I wholeheartedly thank you!

Chapter 1

Introduction

1.1 Prerequisites

Environmental Pollution

“Industrialization and urbanization have promoted socioeconomic development” [Hu and Ding, 2009]. But beside all positive effects for daily life, they have a deep impact on the environment. Nowadays, they are summarized by the term “environmental pollution”, which is defined as the consequence of releasing substances from any process into environmental media, meaning substances which are capable to affect human, organisms and environmental receptors harmfully [UK EPA, 1990].

Already a priority research topic in the 1980s, this “impact of hazardous substances on ecosystems and man” is still of concern [Adriano, 1986]. Pollution can cause a “contamination”, which means an exceedance of background or threshold concentrations. Natural pollution sources like volcanic eruptions, forest fires, lightning and rock weathering are negligible in comparison with the amount of pollution caused by anthropogenic sources [Navarro-Aviño et al., 2007]. Input by human stems primarily from industrial and agricultural activities, urbanization, vehicle exhausts, waste disposal and incineration [Cui et al., 2004; Lavado et al., 2007; McLaughlin et al., 1999].

Heavy Metals

Heavy metals (HM) are environmental contaminants of great concern, because, due to their biochemical properties, they accumulate in environmental media [Kabata-Pendias, 2011].

With respect to their toxicity, HMs can be divided into two groups: micronutrients like Fe, Mn, Mo, Cu, Ni and Zn that are essential in small amounts and the only toxic ones like Ar, Cd, Cr, Hg and Pb without any known biological function. The latter ones have the higher impact on organisms, but even the essential HMs can become toxic if a specific concentration level is exceeded [Alloway, 1999; Peralta-Videa et al., 2009].

A valid definition for the term "heavy metals" has never been established [Duffus, 2002]. Nor has the term "trace metals", which is often used synonymously, ever been defined exactly [Kabata-Pendias, 2011]. Several sources define HMs as elements with a density greater than 5 g/cm^3 [Parker, 1989; Brewer and Scott, 1983; Lozet and Mathieu, 1991; Morris, 1992]. Because all elements studied in this thesis – Cadmium (Cd) , Lead (Pb) and Zinc (Zn) – meet this definition, the term "heavy metals" is used, although it is discussed and criticized [Duffus, 2002].

Environmental Pollution with Heavy Metals

The pollution with HMs in particular has various sources, but the metallurgical industries are perceived to be the major anthropogenic ones [Kabata-Pendias, 2011]. They generate huge amounts of metal-containing fumes and production residues [Rieuwerts and Farago, 1996; Navarro-Aviño et al., 2007]. Due to the fact that industries are mainly located in peri-urban areas, often in a close coexistence with agriculture [Yan et al., 2007], this can cause a serious contamination of agricultural soils with HMs and turn them into a long-term sink [Micó et al., 2006].

This is of great concern in terms of bioaccumulation and biomagnification. "Bioaccumulation" is the uptake of chemicals "by an organism either directly from exposure to a contaminated medium or by consumption [...]", and "biomagnification" is the result of this process, which leads to increased concentrations of chemicals with each trophic level of the food chain [US EPA, 2011].

Therefore, many studies focused on the environmental contamination by mining and smelting activities. Amongst the most recent ones are Chen et al. [2009]; Stafilov et al. [2010]; Pelfrêne et al. [2010]; Lu et al. [2010]. The affect of "secondary lead smelters" (recycling of Pb from Pb-containing products, mainly batteries) in particular, was intensively studied by Brandvold et al. [1996]; Farago et al. [1999]; Rieuwerts and Farago [1996]; Cala and Kunimine [2003] and Fernandez-Turiel et al. [2001]. For instance, in the latter study, the considerable ranges of 31–8 714 mg/kg Pb, 0.27–30.68 mg/kg Cd and 44–4 637 mg/kg Zn in soil samples, taken in the vicinity of a secondary lead smelter in Lastenia, Argentina, were detected.

Heavy Metals in Soil

Soil – here the "pedosphere" – is an important component of our ecosystem. Its filter, buffer, storage, and transformation functions contribute to the regulation of the water, thermal and energy balance. In terms of food production, soils are a livelihood of human beings.

All HMs occur naturally in soils, at least in trace quantities. HMs in soils can be dissolved in the soil solution or bound to exchange sites. This fraction is seen to be phytoavailable. HMs which are either adsorbed to or complexed with organic matter (OM), secondary oxides, minerals and other chemical compounds may only become phytoavailable within time. In general, their availability depends on soil parameters and processes.

The most important soil parameters are the pH value, the organic matter (OM), the cation exchange capacity (CEC), and the clay content. In general, an increase of these parameters leads to a stronger adsorption of HMs to soil particles, which decreases their availability. Beside those, the speciation of HM-ions, the redox status, the content of iron (Fe) and manganese (Mn) oxides seem to have an influence on the behavior of HMs in soils. Furthermore, agricultural practices, season and climatic conditions can cause an enhanced or reduced mobility. Depending on the soil parameters, the processes of adsorption and desorption, complexation, precipitation, sequestration and occlusion, diffusion and migration, and metal competition can alter the concentration of available HM forms in soil [Alloway, 1999; Adriano, 1986; Kabata-Pendias, 2011].

The contamination of soil with a single HM element is rare [Shute and Macfie, 2006; Hao et al., 2011]. Especially the association of Cd, Pb and Zn is highly abundant in urban environments [Cizmecioglu and Muezzinoglu, 2008] and at smelter sites [Rieuwerts and Farago, 1996; Sterckeman et al., 2002; Cala and Kunimine, 2003; Cui et al., 2005; Lu et al., 2010].

Cd exists in soils mainly in form of cadmium sulfide (CdS) or in its ionic form Cd^{2+} (50–90 %), bound to exchange sites or organically complexed. The highest effect on the availability have the pH value, the CEC and the OM [Alloway, 1999; Kabata-Pendias, 2011]. Due to its relative high mobility, Cd moves to the root by mass flow and diffusion with the transpiration flux and is readily taken up by plants [Ingwersen and Streck, 2005].

Pb is seen to be the least mobile HM in soil, with a high tendency to adsorb to solid soil particles [Kabata-Pendias, 2011]. The largest part is included in mineral constituents or organic compounds [Alloway, 1999]. Especially sulfides and phosphates of Pb are extremely low soluble at pH values > 5.5 [Abrahams, 2002]. Its availability is mainly influenced by the pH value, the OM and the clay and mineral contents [Adriano, 1986].

The amount of **Zn** in soils is at least a magnitude bigger than the amount of other HMs. Its concentration in the soil solution is usually small in comparison with total amounts. Zn is easily complexed with OM and clay minerals, but its soluble forms are very mobile and readily available for plants, especially under slightly acid conditions [Kabata-Pendias, 2011]. The pH value, the OM, the humidity of soil and the activity of microorganisms dominate the availability of this element [Alloway, 1999].

Heavy Metals in Plants

Plants – here crops – transfer elements between abiotic and biotic media [Castaldi et al., 2009]. Thus, they play an important role in our ecosystem and provide the basis of food supply.

HMs in the plants tissue can cause phytotoxic effects. But HM contents in the edible parts can have detrimental effects to human health already before a phytotoxic limit is reached [Zheljazkov et al., 2008].

It depends primarily on the plant species and cultivar, as well as on the HM concentrations in soil and the soils properties if HMs are absorbed by the plant via the roots [Prasad and Hagemeyer, 1999]. Additionally, it is stated that the root system and surface, the root exudate's and mycorrhizal transpiration have an influence on the uptake [Sengar et al., 2008]. Changes of uptake behavior were also observed between different stages of growth, climatic conditions and seasons [Abrahams, 2002; Chojnacka et al., 2005]. The main reaction of elevated HM concentrations in the tissues is an increased production of reactive oxygen species (ROS). An elevated concentration of ROS can cause a damage to cell membranes and chloroplast pigments. This, in turn, alters the membrane potential and permeability and can inhibit photosynthesis. Visible phytotoxic effects are a stunned growth, chlorosis and necrosis [Peralta-Videa et al., 2009; Sengar et al., 2008; Prasad and Hagemeyer, 1999].

HMs are taken up via the root membranes by an active or passive transport. The main defense mechanisms take part in the rhizosphere. The roots can excrete chemical products (e.g. organic acids) to chelate or complex metal ions and thereby decrease the metal mobility by increasing the rhizosphere pH value [Alloway, 1999; Prasad and Hagemeyer, 1999; Verkleij et al., 2009]. To reach the xylem, HMs must cross the endodermis with the casparian strip. There, the passive transport of elements and compounds is normally blocked. But this border may lose its function in the presence of toxic levels.

Once within the plant, HMs are transported mostly via the apoplast or by mass flow within the xylem or phloem in a complexed form, as divalent ion or as chelate [Prasad and Hagemeyer, 1999]. During the transport, HMs get mostly bound to cell walls or get sequestrated and stored in the vacuoles. Also, an allocation to the metabolically less active epidermal cells or to deciduous organs in senescence is stated [Verkleij et al., 2009].

The uptake of **Cd** might be influenced by Zn. Due to their chemical similarity, antagonistic and synergistic effects have been observed [Lambert et al., 2007; Shute and Macfie, 2006]. After an uptake, Cd is readily translocated within the plant [Ingwersen and Streck, 2005]. According to Kloke et al. [1984] this element is an "accumulative poison". It can accumulate in plants without apparent effects, but in concentrations which might already be harmful to human health.

The uptake of **Pb** depends mainly on root interception [Alloway, 1999]. Once within the plant, it is mainly accumulated in the roots, where it is stored in the cell walls. Pb is also an "accumulative poison" [Kloke et al., 1984].

The uptake of **Zn** is essential for plants and happens actively through root membrane carriers. In the cells, Zn is part of metallo-enzymes, stabilizes cytoplasmic ribosomes, catalyzes oxidation processes and is involved in the protein- and carbohydrate metabolism [Alloway, 1999]. In reference to Kloke et al. [1984] this element belongs to the "passage poisons", which means it has a higher phytotoxic potential than it is harmful for human health.

Heavy Metals in Human

Beside a direct intake via soil particles or air, the main pathway of HM intake by human beings is via the food chain [Peralta-Videa et al., 2009].

The toxicity of **Cd** is well known since the Itai-Itai disease in the 1960s. An acute poisoning can cause a sore throat, nausea, vomiting, abdominal cramps, headaches, diarrhea or even a shock. Fever, cough and chest tight can follow. A long-term ingestion leads to liver and kidney damage and dysfunction, impairments of the reproductive system, brittleness, collapse of the skeleton and may even cause cancer [Reilly, 1991; WHO, 1992].

Pb is ranked as the most hazardous metal to human health [ATSDR, 2007]. In case of an acute poisoning, anorexia, dyspepsia, cramps and constipation were observed. A chronic intake can cause anemia, encephalopathy, kidney diseases and leads to miscarriages and still births. Up to now, there is no prove that Pb causes cancer [WHO, 1995; Reilly, 1991].

Of all nutritional metals, **Zn** is the most important one. Thus, it is relatively non-toxic for human. In case of an excessive intake, one can suffer from nausea, vomiting and abdominal pain. A long-term exposure to toxic concentrations can lead to hypercholesterolemia [Reilly, 1991; WHO, 2001].

Summarized

Agricultural soils are the basis of food supply. A contamination with HMs might not only affect the natural soil functions, but also the crops grown on these soils. The uptake of HMs by crops might cause phytotoxic effects. But since food consumption is perceived as the major pathway to human exposure to HMs, their uptake by crops also poses a significant health risk to human. [Zheng et al., 2007; Peralta-Videa et al., 2009]. Thus, the transfer of HMs from soil to crops is a important field of study.

1.2 State of the Art

The uptake of HMs by cultivated plants from HM contaminated soil has already been a research topic in the 1970s as shown by Vetter and Schulte [1979] and Markard [1974].

Since then, a lot of knowledge about the HM behavior in soils, uptake or exclusion mechanisms of plants and about specific influences like genotype, culture, climate or season was gained. In terms of a steadily growing population and decreasing areas of fertile and uncontaminated soil, the uptake of HMs by cultivated plants is still an important research topic.

Whereas some authors considered the uptake and accumulation of HMs in vegetables, like Cui et al. [2004]; Li et al. [2006]; Wang et al. [2006]; Yan et al. [2007] and Hu and Ding [2009], other studies centered on field crops, e.g. Dudka et al. [1996]; Liu et al. [2005]; Huang et al. [2008]; Castaldi et al. [2009] and Bermudez [2011]. But investigations about the HM uptake by soybeans in particular are few.

Studies About the Uptake of HMs by Soybean Crops

Already Borkert et al. [1998] showed in a laboratory experiment, were Zn and Cu were applied in excess, that soybeans are species, which are sensitive to a contamination of soil with HMs.

The quantified accumulation of Cd and Zn by soybean plants was investigated in a greenhouse study by Shute and Macfie [2006]. With respect to a contamination with multiple HMs, they found an increased bioavailability for Cd and a decreased one for Zn, which resulted in higher concentrations of Cd in the above-ground tissues.

The effect of atmospheric pollutants on the uptake of HMs from contaminated soil by soybean plants was studied by Rodriguez [2010] and Xiong et al. [2003]. Rodriguez [2010] reported an effective translocation of Pb up into the seeds for a treatment of soybean plants in climate chambers with elevated concentrations of CO_2 and lower proportions of fly ash in the soil. In open-top chambers, the treatments of soil with Cd, Pb and Zn already led to an increased concentration of these HMs in the seeds under ambient SO_2 concentrations, whereas an elevated SO_2 concentration promoted a stronger uptake of Cd [Xiong et al., 2003].

A closer approximation to field conditions was made by De Souza Silva [2006], who investigated the uptake of HMs and their translocation to different soybean organs. Although the studies were conducted in a greenhouse, the used soil originated from a contaminated area, which had been affected by atmospheric pollution with HMs, caused by a factory. Although the translocation of Pb was found to be limited by the roots, the shift into the seeds was enough to cause elevated concentrations there.

In the most recent study, conducted by Hao et al. [2011], a similar setting was applied, but the contaminated soil originated from a site affected by wastewater and copper smelter exhausts. A higher contamination level of soil resulted in a general higher uptake and the accumulation of Cd, Pb and Zn in soybean seeds was higher than in the edible parts of cowpea or pepper. The concentrations differed also between distinct cultivars, a fact that was already intensively studied for soybeans by Arao et al. [2003].

All of these studies were carried out under controlled conditions. Although laboratory experiments are only a prediction of the performance under field conditions, field studies were conducted only by a few scientists.

Amongst them Liu et al. [2005], who took samples from an area which is still covered with waste spills, 17 years after a collapse of a mine tailing dam. In this case, the detected concentrations in the soybean seeds remained lower than the applied threshold values.

The HM contents in soybean foliage in the vicinity of an electroplating factory were studied by Hang et al. [2010]. The detected close relationship between the concentrations in foliage and soil indicated that the contamination was caused by the factory.

Li et al. [2008] investigated the uptake and distribution of Pb in soybean, rice and maize, grown on a lead-zinc deposit. The highest concentrations were found in the organs of soybean, which followed the order root > stipe > seed.

The fact that soybean plants can absorb more HMs from soil than other crops was also shown by the investigations of Lavado et al. [2001], about the effect of different tillage systems and Lavado [2006], about the effect of the distance to typical pollution sources like roads, farms and cities.

1.3 The Present Study

Until now, there are no field studies about the uptake of HMs by soybean plants, cultivated in the vicinity of smelter industries. Parallels to mentioned studies are existing (especially to Lavado [2006] and De Souza Silva [2006]), but [Kabata-Pendias, 2011] stated that “each case of plant pollution is unique and should be studied for a specific environment”.

Several of the above mentioned studies have found, that in contrary to the state of knowledge, Pb can be translocated in considerable amounts into the seeds, even if the plants are grown in only slightly contaminated soils. Because of that, the investigation of a lead smelter site was of major interest.

A soil survey conducted in March 2006 by the *Comisión Nacional de Actividades Espaciales* (CONAE) with support from the *Departmento de Epidemiología* and the *Ministerio de Salud de la Provincia de Córdoba*, resulted in elevated Pb concentrations in the vicinity of the former secondary lead smelter in the municipality of Bouwer, near to the city of Córdoba, Argentina [CONAE, 2006]. Due to this report, a soil sampling at this site was conducted by the *Instituto Multidiciplinario de Biología Vegetal* within the framework of the dissertation of Salazar (in progress). Concentrations between 1 600 ppm and 1 800 ppm Pb were detected in all directions around the smelter [Salazar, 2011 (personal communication)]. This setting provided an optimal basis to investigate the uptake of HMs from contaminated soil by soybean plants, which are planted in the vicinity of this former smelter.

As in detail described above, HMs can cause phytotoxic effects [Prasad and Hagemeyer, 1999] and affect human health harmfully [Reilly, 1991]. Thus, this diploma thesis is a synthesis between ecotoxicology and toxicology. On the one hand, it analyzes the potential risks posed by HMs to the environment, on the other hand, it examines the effect on human health.

The **hypothesis** of the following study was:

> High concentrations of heavy metals in soil are transferred and translocated to soybean crops and ultimately accumulate in the seeds with consequences for plant quality and food safety.

To verify this assumption, the **main objects** were:

- To evaluate the concentrations of HMs in soils and soybean crops at different sampling sites.
- To study the transfer of HMs from soil into soybean organs.
- To evaluate the risks of adverse effects to the plant quality and human health, which may result from the transfer of HMs from soil to soybean organs.

Chapter 2

Material and Methods

2.1 Study Area

Argentina, an economically emerging country in South America, faces serious environmental problems. These are owing to an increasing population, an increasing number of vehicles, and industrial expansion in the major urban agglomerations, together with a lack of environmental policies [Carreras and Pignata, 2002].

Córdoba

Córdoba is the second largest city in the country, with over 1.4 million inhabitants. The city is located in central Argentina at 31°24′ latitude and 64°11′ longitude, on an average altitude of 400 m ASL [Municipalidad de Córdoba, 2011].

In reference to the updated climate classification of Köppen-Geiger by Peel et al. [2007], the climate of greater Córdoba is humid subtropical with dry winters (Cwa). The mean annual precipitation is calculated to be 834.9 mm for the period between 1981 and 2007. The mean annual temperature for the same period averages 18.0 °C [Fuerza Aérea Argentina, 2008a,b]. Despite its central position, Córdoba is still under an oceanic influence, which resulted in alleviating effects of temperature extremities.

The main wind direction is from North–East to South–West. This is a result of the southern hemisphere atmospheric circulation and the plain relief of Córdoba Province, which is bordered on the west side by the mountainous ridge of the *Sierras de Córdoba* [D.A.C.yT.S.E.M, 2003].

In these latitudes, short photoperiods are dominating. The hours of daylight differ between summer and winter by only about four hours [D.A.C.yT.S.E.M, 2003].

Concerning environmental aspects, Córdoba is one of the most polluted cities in the country [Stein and Toselli, 1996]. The important and still developing industrial areas of metallurgical industries are mainly located in its peripheral areas [Bermudez et al., 2009]. But agriculture is also residing there.

Bouwer

The suburban municipality of Bouwer is located at 31 °34′ latitude and 64°10′ longitude, about 15 km south of Córdoba city, in the department of Santa María.

Without doubt, this municipality is one the environmentally most affected areas in the province of Córdoba. The "Córdobeses" themselves call this suburban area "the waste deposit of the city". More than 2 000 residents [La Mañana, 2010] are surrounded by contamination and many of them are struggling for melioration. Their life quality is seriously affected. People are poisoned with lead and suffer from malformation, respiratory and skin afflictions and miscarriages [La Voz, 2006; Municipalidad de Bouwer, 2008].

Figure 2.1 shows the municipality of Bouwer surrounded by various pollution sources and contaminated areas.

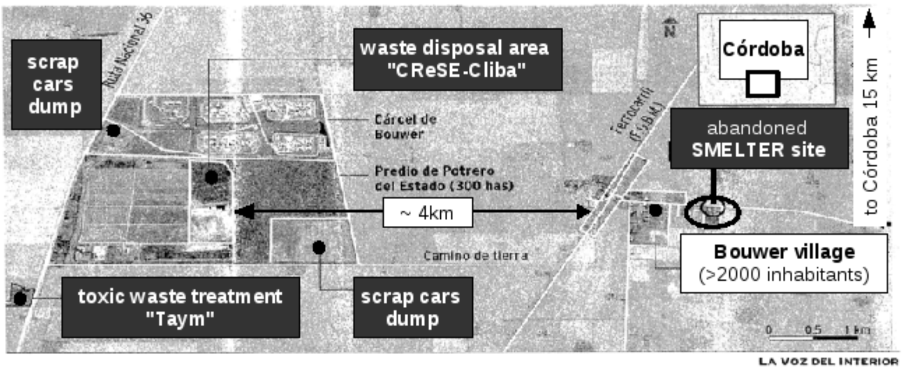

Figure 2.1: Pollution sources and contaminated areas of Bouwer municipality [Marconetti, 2008]

One of the biggest pollution sources is the waste disposal area of the company *CReSE-Cliba*. Until April 2010 the waste produced by the city was piled, buried and incinerated there. *FUNAM* (Fundación para la Defensa del Ambiente), a citizen's initiative in Bouwer, struggled for and achieved the closure. But up to now, waste heaps up to 30 m in height are still not buried, sealed or otherwise secured. A remediation of this area had been promised but never realized [Martínez, 2011].

Furthermore, the factory of *Taym* is still in operation. Toxic industrial, pharmaceutical and agrochemical residues, like Dichlorodiphenyltrichloroethane (DDT), are deposited and treated there (7 500 t/a).

Additional contamination is caused by the *Depósitos Judicial de la Provincia* dumps, where more than 15 000 scrap cars are accumulating.

Above all, from 1984 on for more than 20 years, a metallurgical company engaged in lead smelting to recover lead from old (car) batteries. Although now not longer in action, this lead smelter is a considerable contamination source [Municipalidad de Bouwer, 2008; Marconetti, 2008]. On the agricultural fields surrounding this smelter, crops like sorghum [*Sorghum bicolor* (L.) Moench], alfalfa [*Medicago sativa* L.] and soybean [*Glycine max* (L.) Merr.] are cultivated. This is the site which was the object of the investigations for this study (see Section 1.3).

2.2 Sampling Sites, Pools and Labeling

The municipality of Bouwer is under environmental investigation by the *Instituto Multidiciplinario de Biología Vegetal* since years. Beside the fields around the former lead smelter, which was the main sampling site, three other sampling sites where included into the studies. Two of them served as control. The fourth sampling site served as reference, providing background values. An overview of the in the sampling sites is given in Figure 2.3.

The Smelter Site

When the lead smelter was still in operation, it produced huge amounts of toxic fumes. They were released unfiltered directly from the smelting process into the ambient air [CONAE, 2006]. In September 2003 the company *Ingeniería Laboral y Ambiental* measured the amount of Pb released from the stack. Results showed 35 times higher values for Pb and 13 times higher values for particle matter than permitted in the province of Córdoba [La Voz, 2006].

In September 2005, a series of actions by the municipality ended in the closure of the smelter and an environmental audit [Municipalidad de Bouwer, 2008].

Since the smelter is closed, it no longer contributes to air pollution by releasing fumes. But HM-containing particle matter, aerosols and gases were already deposited on environmental targets through dry and wet deposition over decades. In addition, large quantities of slags were left around the plant without any safety precautions for soil or water protection [CONAE, 2006]. Furthermore, these leftovers from the smelting process were used to fill and level the lands surface in the vicinity [Salazar, 2011 (personal communication)]. A remediation of the area never took place [Municipalidad de Bouwer, 2008].

The pictures in Figure 2.2 give an impression of the smelter's field of activity. They indicate that the extraction process had been performed in a very primitive way.

(a) The lead smelter when it was still in action (CONAE, 2006)

(b) The abandoned smelter, surrounded by crop fields, own source

(c) Inside view of the abandoned lead smelter (Pignata, 2006)

(d) Heap of slags, showing leftovers from the smelting process. (Pignata, 2006)

Figure 2.2: Pictures from the Smelter site.

Reported health effects and former studies state that the area is indeed seriously affected by environmental contamination (see Section 2.1).

Especially the soil is regarded to be highly contaminated by HMs due to the former input via atmosphere and the enduring discharge from the remaining slags (see Section 1.3).

Because of this situation, this smelter site is a suitable investigation place to carry out field studies about the translocation and bioaccumulation of HMs in organs of soybean crops which are cultivated there.

This sampling site will be called "Smelter" ("S" when used as index), indicating that the former smelter activities and lasting influences are seen as the primary pollution source.

The Control Sites

Two additionally sites served as controls. They are about 2 km and 5 km away from the Smelter sampling site, but still located in the municipality district of Bouwer. For both sampling sites, no direct pollution source is known [Salazar, 2011 (personal communication)]. It is assumed that they represent the "general contamination level" of the municipality district of Bouwer, influenced by the overall settled industry in this suburban district. They will be called "CONTROL" in the present work ("C" when used as index).

The Reference Site

A fourth sampling site served as reference. It is situated about 40 km from the SMELTER site (53 km away from the city of Córdoba), in a rural area close to the mountainous ridge of *Sierras de Córdoba*. For this site, direct pollution by industry and traffic emissions can be excluded and contamination is not assumed [Salazar, 2011 (personal communication)]. This sampling site will be called "REFERENCE" ("R" when used as index).

Pools and Labeling

A sampling "pool" is a 5×5 m grid. Nine sub samples were taken from each grid and joined to one pool. Pools are identified by "pool codes". The code shows the affiliation of a pool to a sampling site and the relative distance to the smelter.

Figure 2.3 shows a schematically overview of the sampling sites.

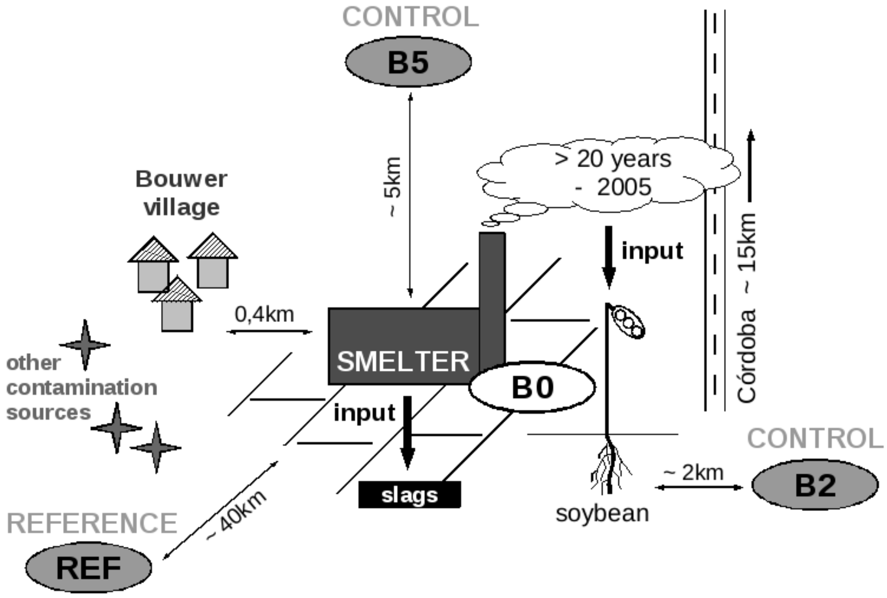

Figure 2.3: Overview of the sampling sites

The SMELTER site is identified by the label "B0". The "B" in the code stands for "Bouwer". It should imply that the samples originate form the municipality district. Samples taken from this site were labeled with "B0-x". The sampling sites CONTROL were labeled "B2" (for 2 km distance to B0) and "B5"(for 5 km distance to B0). The samples from these sites were labeled with "B2-x" and "B5-x" respectively. The "x" is replaced by a number from 1 ascending. The ascent in numbering indicates the increasing distance from the smelter and enumerates the pools within each sampling site. Additionally one pool from the sampling site REFERENCE was included. Due to the fact that only this single pool represents the whole sampling site, the pool code "REF" was chosen.

Following this scheme, labeled pools could be arranged due to their distance from the smelter in the logical order, presented in Table 2.1.

Table 2.1: Overview of coded sampling sites and pools and their location

pool code	sampling site	distance from the smelter [m]	latitude	longitude
B0-1	SMELTER	62	31°33′34.00″	64°11′06.51″
B0-2	SMELTER	65	31°33′34.76″	64°11′06.55″
B0-3	SMELTER	85	31°33′32.04″	64°11′06.52″
B0-4	SMELTER	108	31°33′33.62″	64°11′04.73″
B0-5	SMELTER	185	31°33′39.79″	64°11′08.98″
B0-6	SMELTER	200	31°33′33.82″	64°11′01.26″
B0-7	SMELTER	300	31°33′34.25″	64°10′57.49″
B2-1	CONTROL	1 980	31°33′52.49″	64°09′56.84″
B2-2	CONTROL	2 149	31°33′53.36″	64°09′50.57″
B2-3	CONTROL	2 850	31°33′55.24″	64°09′23.63″
B5-1	CONTROL	5 630	31°30′36.25″	64°10′19.90″
B5-2	CONTROL	5 640	31°30′36.36″	64°10′16.16″
B5-3	CONTROL	5 704	31°30′33.52″	64°10′19.57″
B5-4	CONTROL	5 721	31°30′33.34″	64°10′17.08″
REF	REFERENCE	38 060	31°52′01.84″	64°21′52.10″

All together, plant and soil samples from 15 grids were taken. To mitigate the influence of traffic, a potential pollution source, a distance of at least 200 m between local streets and sampling points was ensured.

2.3 Sample Material

The climate, the hydrological and thermal balance, and the physico-chemical geological characteristics for the main factors determining the genesis, distribution, condition and type of common soils in the province of Córdoba. Day length, climate and soil type are key factors for determining the agricultural land use. [D.A.C.yT.S.E.M, 2003]

2.3.1 Soil Material

Topsoil samples (0–15 cm) were taken from two adjoining geomorphological regions. The soils have similar characteristics and belong to the same soil order.

Most soil samples (B0-x, B2-x, B5-x, see Section 2.2) derived from the municipality district of Bouwer. The latter one belongs to the geomorphological region of the *Pampa lóessica alta* (transl.: "high aeolian silt deposit Pampa"). According to D.A.C.yT.S.E.M [2003], the prevailing soils are *entic Haplustolls*, belonging to the soil order of *Mollisols* [IUSS, 2007], which are comparable to *Chernozems* in the German soil classification [AG Boden, 2005]. They are characterized by a productivity index of 68 %, which indicates good soils for agriculture, just limited by climatic conditions. Other characteristics are a raised drainage, a profundity greater 100 cm, a moderate exchange capacity, a slightly acid-to-neutral reaction and a moderate content of organic matter. Under intensive agriculture this soil tends to silting. The diagnostic feature of *Mollisols* is their dark (10 YR 3/1 or darker) and mollic epipedon with a soft and granular structure [USDA, 1999]. The superficial soil type is a silty loam. Original material of the region are made up of aeolian sediments of variable grain sizes. At some locations they built up to fifty meters thick layers. The groundwater horizon is too deep to effect agriculturally used soil layers [D.A.C.yT.S.E.M, 2003].

Only the reference soil sample "REF" (see Section 2.2) belongs to the geomorphological region of the *Depresión periférica* (transl: "peripheral depression"). Profound *Mollisols* are also prevailing here. They belong to the subgroup of *typic Argiustolls*. Their productivity index is a little lower (61 %). The drainage is well. The cation exchange capacity is high and they are moderate in organic material content. The superficial soil type and the original material are the same like that of the *entic Haplutolls* in the *Pampa lóessica alta*. [D.A.C.yT.S.E.M, 2003]

2.3.2 Plant Material

Soybean plants served as plant material. The crop species (binomial name [*Glycine max* (L.) Merrill]) belongs to the legumes. The main characteristics of this annual species are a high flexibility of reproduction, a high quality protein and oil content of the seeds, and a high photoperiodical sensitivity. The latter property makes the soybean a quantitative short-day plant [Cregan and Hartwig, 1984]. Like roots of other legumes, soybean roots are infected by rhizobia (*Bradyrhizobium japonicum*), a symbiosis that makes it possible to fix atmospheric nitrogen. Under optimal conditions, the length of the principal root can reach two meters. The main inhibiting factor of growth and yield production is drought in specific growing stages. Due to their high nutritive value, soybeans are crops of increasing economic and alimentary importance on a global scale [Baigorri, 1997; Qui and Chang, 2010].

Originating from east Asia, first cultivation trials in Argentina were conducted in the year 1862. In the declaration *No. 4406* from 1972, national interest in cultivation of soybeans was officially declared. Since then, the annual production and the cultivated area is steadily increasing. Crop species like corn and sorghum are more and more replaced by soy [Giorda, 1997]. In the year 2009, nearly 31 million tons of soybean were produced, on an area of about 16 million hectares. About 90 % of the production is exported. This makes soybeans the most important commodity of the country and Argentina the major exporter of soybean cake and oil and the third biggest exporter of soybeans in the world [FAO STAT, 2011; Singh, 2010].

Córdoba is a region with very favorable conditions of climate and soil for soybean cultivation. This makes this province one of the most productive in terms of the yield by area ratio [Giorda, 1997].

98 % of the cultivated soy in Argentina is transgenic [Domínguez and Sabatino, 2005]. The sampled species were also gene-modified. The plants had either a determined or semideterminated habit. Reliable information about the agricultural practices on the sampling sites could not be obtained. But referring to Viano [2006] the use of pesticides for transgenic cultivars is huge. The harvest is sold to a local distributor, who in turn sells big amounts of agricultural products to larger distributing and processing companies [Salazar, 2011 (personal communication)].

2.4 Procedures and Processing of Sample Material

Considering the working time and extent of a diploma thesis, some source data was taken from the dissertation of Salazar, M.J. (in progress). This concerns all soil samples of the SMELTER and the REFERENCE site, as well as the plant samples of the REFERENCE site.

2.4.1 Quality Control

For the sampling procedure a stainless steel spade and woolen gloves were used. To avoid contamination during the processing of sample material, latex gloves were worn all the time. The working environment was kept clean. Furthermore, in all of the analyses Milli-Q-water and Milli-Q washed equipment was used. These preparations should ensure that the samples were not additionally contaminated during the processing.

The precision and purity of the analyses was controlled by using triplicates of samples and simultaneously processed blanks. This should detect if the measured HMs (Cd, Pb, Zn) were added during the sample preparation by accident.

The tobacco leaves "ICHJ-CTA-OTL-1" and the Chinese soil standard "GBW 07405" served as certified material. Both, blanks and certified material, were treated in exactly the same way like the corresponding material of soybean organs and soil.

All processing and analyzing steps were performed at room temperature, which was about 23 °C.

2.4.2 Sampling Procedure

Plant and soil material was sampled shortly before the harvest of the soybeans, at the end of April 2011. Nine subsamples were taken per grid (5×5 m) at distances of 2–3 m and joined to one pool. A subsample consisted of 2–5 plants and the corresponding topsoil (0–15 cm), surrounding the main root. The samples were put into plastic bags and transported to the laboratory immediately. GPS-data from the grids were stored (see Table 2.1).

2.4.3 Processing of Soil Material

The soil samples were air-dried at room temperature. Afterwards the soil was passed through a 2 mm stainless steel mesh and then sieved down to the 63 μm fraction. The sieving homogenized the sample.

Physico-chemical parameters The pH value, the electrical conductivity (EC) and the organic matter (OM) are important parameters for controlling physico-chemical processes and HM mobility in soil.

To analyze the **pH value** and the **EC** a soil-to-Milli-Q-water suspension of 1:5, according to Bäckström et al. [2004], was prepared in duplicate, shaken and reposed for 24 hours. The pH value was measured with an ALTRONIX, TPX II, the EC was measured with an OAKTON 500. Both instruments needed calibration beforehand. The temperature during measurement averaged 25 °C.

The loss on ignition represents the percentage of **OM** of a soil sample. In conformity with DIN 18128 [2004] “the method is based on the assumption that, unlike mineral constituents, the organic matter in the soil can be burned”. Therefore, the weight of the porcelain crucibles and the added soil material were noted to an accuracy of 0.01 g (OHAUS 2140). The crucibles with their contents were heated in a muffle furnace for 4 hours at 500 °C [Peltola and Åström, 2003]. After that, they were cooled down to room temperature in a freeze drier (RIFICOR L-05). The loss on ignition was calculated from the weight difference.

Metal extraction With respect to the ability of plants to extract substances from the soil, not only the total metal content is of interest. Hence, different extraction solutions and methods were used to determine the metal contents for distinct soil fractions.

To determine the **water-extractable fraction** of HMs in soil, an extraction solution of 7 g soil material and 25 ml Milli-Q-water was prepared. The suspension was shaken for 30 minutes and reposed for 24 hours, during which soil particles sedimented. The supernatant was passed through a suction strainer, containing a 2.5 μm filter (Munktell & Filtrak). The filtrate was transferred into brown glass bottles and stored in the dark at room temperature.

Many researchers (Cline and Reed [1995]; Singh et al. [1996]; Stone and Marsalek [1996]; Steele and Pichtel [1998]) applied an acid extraction with dilute HCl to partition the **available fraction** ("av" when used as index) , which contains the effective soluble, active and available HM forms [Gupta et al., 1996]. The acid extraction method is in accordance with Sutherland [2002], who applied it successfully to soil polluted by road traffic.

7 g of the sample material was mixed with 25 ml of 0.5 M HCl and was shaken for 30 minutes. The soil particles sedimented during the repose of one day. Thereafter, the supernatant was passed trough a suction strainer through filter paper two times. The first time through coarser filter paper; the second time through 2.5 μm (Munktell & Filtrak) filter paper. The filtrate was transferred into brown glass bottles and stored in the dark at room temperature.

The extraction with aqua regia was used by numerous researchers (Dragović et al. [2008]; Rodríguez et al. [2008]; Micó et al. [2006]) to determine the concentration of HMs in the **pseudo-total fraction** ("pt" when used as index), which represents the inactive and inert forms of HMs in soil [Gupta et al., 1996]. 3 g of the sample material was ashed in ceramic crucibles in a muffle furnace for four hours at 450 °C. After that, 7.5 ml of HCl 37 % and 2.5 ml HNO_3 (65 %, of quality (Merck)) were added and the samples were reposed for an hour. The digested samples were transferred from the crucibles into plastic test tubes. In doing so, the crucibles were rinsed with 2 ml of Milli-Q-water. The samples were centrifuged for 15 min at 2 000 rpm and then the supernatant was passed over a suction strainer through a filter 2.5 μm (Munktell & Filtrak). The sample-fluid was filled up to a final volume of 10 ml with Milli-Q-water and transferred into brown glass bottles where the sample solutions were stored.

2.4.4 Processing of Plant Material

The plants from one grid were joined into one pool. Soil material sticking to the roots was removed manually. The plants were counted and separated into their organs: roots, stipes, pods and seeds. From now on, the organs were treated separately.

Leaves, as an additional organ, could not be included in the present study, because the sampling took place shortly before harvest, when the plants are without leaves. Owing to their nature, soybeans loose them in an earlier stage of growth.

To ensure that the measured concentrations of HMs originate only from the sample material, still attached dirt particles needed to be removed. After determining the fresh weight (OHAUS 2140), the organs were washed several times with tap water. Roots were treated additionally two times for 15 minutes in an ultrasonic bath (Testlab) with Milli-Q. Thereafter the sample material was put into paper bags and stored in an electrical oven at approximately 70 °C until constant weight was reached.

When constant weight was reached, the dry weight of the organs was determined (OHAUS 2140). Afterwards, the dried parts were milled in a stainless steel grinder (recco, MOC01) to homogenize the sample material. Until further processing, the material was stored in sealed plastic bags.

Dry ash digestion The determination of HMs with the flame atomic absorption spectroscopy (FAAS) (see Section 2.5.1) requires a liquid solution. Digestion methods transfer the total contents of elements from solid samples into liquid solution.

A dry ash digestion was conducted in reference to Pfeiffer and Barclay-Estrup [1992]. 3 g of each sample was ashed in ceramic crucibles in a muffle furnace for four hours at 450 °C. After that, 2.5 ml of HCl (20 %) and 0.5 ml of HNO_3 (65 %, of quality (Merck) were added to the ash, smoothly shaken and reposed for an hour.

Filtration of digestion fluid The digested samples were transferred from the crucibles into plastic test tubes. In doing so, the crucibles were rinsed with 2 ml of Milli-Q. Samples were centrifuged for 15 min at 2 000 rpm to separate solid residues. Afterwards, the supernatant was passed through a suction strainer containing a 2.5 μm filter (Munktell & Filtrak). The sample solution was diluted up to a final volume of 10 ml and transferred into brown glass bottles. The bottles were stored in the dark at room temperature.

2.5 Analyses and Tests

2.5.1 Flame Atomic Absorption Spectroscopy

The analysis of the sample material was conducted with the flame atomic absorption spectroscopy (FAAS). This technique is one of the most common, economic and frequently used ones for trace element analysis. The measurement with this technique requires calibration beforehand and is based on specific amounts of radiation, emitted or absorbed by atoms of a specific element as results of transitions between energy levels. Different techniques exist, dependent on the sensitivity and principle of measurement as well as on the atomizer instrumentation [Welz, 1997; Wright and Stuczynski, 1996].

FAAS is used to quantify particular elements in analytic solutions. The instrumentation consists of three units: The element specific light source, the sample cell and the specific light measurement units. Figure 2.4 shows the setup.

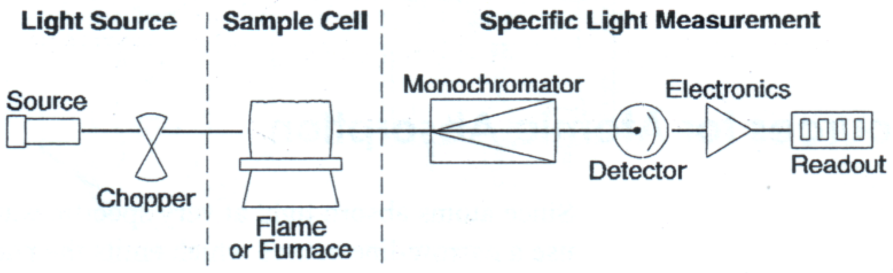

Figure 2.4: Schematic setup of an atomic absorption instrument [PerkinElmer, 1996]

The light source is a single element hollow cathode lamp (HCL) . It is made of the particular metal which is to be determined in the sample. The HCL emits light of the elemental specific wave length. A chopped light beam enters the sample cell (atomizer) where the sample solution is first dispersed and then vaporised by a flame of air-acetylene at approximately 2 300 °C. The atoms of interest absorb the specific light. In doing so, they enter an excited state and the intensity of the light beam is attenuated. After passing a monochromator, only the isolated spectral line of interest is detected by a photomultiplier. An electrical current is produced proportionally to the arriving intensity of light. The extinction is calculated by applying the Lambert-Beer law [Welz, 1997, page 95 ff]. The law states that the measured metal concentration is proportional to the transmittance and extinction. This ultimately reveals the concentration of the element of interest in the analytic solutions [PerkinElmer, 1996; Welz, 1997; Van Loon, 1985].

The following description is valid for determining one particular metal. It needs to be repeated for each element. The procedure was conducted for the detection of Cd, Pb and Zn in soil and plant solution samples. Iron (Fe) was only measured in the soil samples, with the background to use these concentrations as reference in the calculations for the Enrichment Factor (see Section 2.6.1). For the analysis a Perkin-Elmer AA3110 was used.

The applied standard conditions are listed in Table 2.2.

Preceding the measurement, a warm-up time for the HCL is required. After ten minutes a constant emission light intensity is reached. To check the correct working of the setup, a copper standard with known concentration was analyzed at first. A standard calibration was conducted with at least four calibration solutions. Quantitative measurements of particular elements in the sample solution could start after the calibration. The readout of metal concentrations in each sample solution was the resulting mean value of two 2 s

Table 2.2: Standard conditions applied in the flame atomic absorption spectroscopy, after PerkinElmer [1996]; Van Loon [1985]

Element	Wave length [nm]	Slit [nm]	Relative noise	Characteristic concentration [mg/L]	Linear range [mg/L]	Detection limit [ppb]
Cd	228.8	0.7	1.0	0.028	2.0	1.0
Fe	248.3	0.2	1.0	0.110	6.0	0.6
Pb	283.3	0.7	0.4	0.450	20.0	1.0
Zn	213.9	0.7	1.0	0.018	1.0	10.0

measurements. If the linear range of the calibration curve was passed, the samples were diluted and measured again. The latter ensures the quality of the measurement.

2.5.2 Soybean Seed Quality Tests

According to Casini et al. [1997], the parameters which most affect soybean quality are viability, germination capacity, mechanical hurts and contamination of seeds (impurities, weed seeds, fungi, bacteria) but also genetic characteristics.

To analyze the quality determining parameters of the soybean seeds, two tests were performed. The fertility and germination capacity were determined with the Germination Test. The Tetrazolium Test added information about the viability as well as the germination capacity and damages. In the evaluation the dry weight of 1000 seeds was also considered.

Germination Test

The Germination Test is performed under defined temperature and humidity conditions and lasts at least eight days [Casini et al., 1997].

The test was carried out in a greenhouse with eight hours of dark and an average temperature of 25 °C. Twenty randomly chosen seeds of each pool were immersed for ten minutes in HCl (1 %) and rinsed thereafter with distilled water. This conditioning should ensure that germination would not be disturbed by adhering bacteria, fungi or other germs. For the same reason, the seeds were sown into autoclaved sand. The seeds of each pool were sown into separate beds and put into the greenhouse. The germination was observed for two weeks. Every 24 hours, the beds were watered and the germinated plants were counted. The daily and total numbers and percentages of germinated seeds were calculated.

To compare the progress of germination, the Germination Value (GV) , according to Djavanshir and Pourbeik [1976], was calculated for each day and each pool. The GV considers the speed of germination as well as the total germination.

$$GV_d = \frac{\sum DGS}{d} \times GP \times 10 \tag{2.1}$$

with

GV : Germination Value
d : day of observation
DGS : daily germination speed
GP : germination percent [%]

Tetrazolium Test

This test was performed according to the working group of De França Neto et al. [1988, 1998]. The method was adjusted in amount and modified respectively to the conditioning of the seeds.

The method is based on a color reaction, caused by enzyme activity. During the immersion of the seeds in the colorless solution of 2,3,5-triphenyl tetrazolium chloride (TCT) (Tetrazolium) diffuses through the tissues and a reduction takes place in the viable cells. In particular, the malic acid dehydrogenases reduce the TCT, forming the red and stable reaction product of Triphenylformazan [De França Neto et al., 1998].

The observed color pattern together with a knowledge about the various seed characteristics allow a determination of presence, location and nature of damages (mechanical, bug bites, humidity or dry stress) which can occur in embryonic tissues [Moore, 1973].

Ten randomly chosen seeds per pool were watered in Milli-Q for at least 16 hours at room temperature. After removing the epidermis from the seeds, they were immersed in a tetrazolium solution of 0.075 % The immersed seeds were stored in the dark for three hours in a dryer at about 30 °C. Then the colored seeds were washed with Milli-Q, cut into halves along the embryonic axis and arranged on a petri dish for observation. The seeds which could not be observed immediately, were soaked in Milli-Q-water and stored in the fridge.

The classification was done visually. Due to their color patterns the seeds were allocated to a respective class from 1 to 8, according to the color charts in the manual of De França Neto et al. [1988]. Classes 1–3 identified the quality parameter of the vigor, classes 1–5 the germination potential and classes 6–8 the damages. Percentages of seeds for every quality parameter were calculated and evaluated using the key in Table 2.3.

Table 2.3: Evaluation key for the Tetrazolium Test, according to De França Neto et al. [1988]

vigor and germination potential	
Percentage	Evaluation
>80%	very high
70%–80%	high
50%–70%	middle
30%–50%	low
<30%	very low

damage	
Percentage	Evaluation
<6%	no quality lost
6%–10%	quality lost
>10%	high quality lost

2.6 Data Calculations

2.6.1 Soil and Soybean Factors

Activity Ratio

The *Activity Ratio* (AR) can reflect the environmental impact and the mobility of HMs in soil [Hang et al., 2010; Lopez et al., 2008]. It is the ratio of the concentration of a HM in the available soil fraction to the concentration of a HM in the pseudo-total fraction:

$$AR\ [\%] \ = \ \frac{HM_{av}}{HM_{pt}} \ \times \ 100 \tag{2.2}$$

Enrichment Factor

The relative degree of metal contamination can be estimated by comparing the metal concentrations in the soil samples to the background concentrations [Dragović et al., 2008; Hernandez et al., 2003]. This was done by calculating Enrichment Factors (EF) according to Zoller et al. [1974].

The ratio of the metal concentrations to the background concentrations of the upper continental Earth's crust (EF_{crust}) was calculated with data obtained from Wedepohl [1995].

To get an idea of the contamination degree in comparison to common loessic soils, which are dominating in the province of Córdoba, EF_{loess} was calculated according to Bermudez [2011]. Therefore, source data from Gaiero et al. [2003]; Gallet et al. [1998] and Smedley et al. [2005] was used.

In this work, iron (Fe) was used as the reference element, like applied by Dragović et al. [2008]. In Equation 2.3 the concentrations from the available fraction were used, in Equation 2.4, those from the pseudo-total fraction in mg/kg, DW.

$$EF_{crust} = \frac{HM_{topsoil} \ / \ Fe_{topsoil}}{HM_{crust} \ / \ Fe_{crust}} \tag{2.3}$$

$$EF_{loess} = \frac{HM_{topsoil} \ / \ Fe_{topsoil}}{HM_{loess} \ / \ Fe_{loess}} \tag{2.4}$$

An accepted evaluation system for the Enrichment Factors (EF) does not exist. To evaluate the obtained data anyhow, the classification suggested by Sutherland [2000] (Table 2.4) was applied.

Table 2.4: Classification key for the Enrichment Factors according to Sutherland [2000]

Enrichment	Minimal	Moderate	Significant	Very high	Extremely high
EF value	<2	2–5	5–20	20–40	>40

Ionic Impulsion

The combined effect of HMs was considered by calculating the Ionic Impulsion according to Romero et al. [1987], and the subsequent Soil Pollution Index (PI$_{soil}$) and Soybean Pollution Index (PI$_{soy}$) according to Romero et al. [1989].

This factor considers that the mixture and combination of HMs in soil may have an influence on the plants uptake [Hang et al., 2010; Romero et al., 1987, 1989].

$$II_{wx} = \sum_{m} c_m^{1/n_m} \tag{2.5}$$

with

II_{wx} : Ionic Impulsion of wx

c : HM concentration in wx [mmol/g, DW]

n : elemental oxidation number (for all 2+)

$w \in \{\text{soil, soy}\}$

$x \in \{\text{av, pt}\}$, for soil

$x \in \{\text{ro, st, po, se}\}$, for soy

$m \in \{\text{Cd, Pb, Zn}\}$

Pollution Index

The soil and plant pollution in reference to unpolluted and toxic levels was calculated according to Romero et al. [1987].

$$PI_w = \frac{II_{wx} - II_{ref}}{II_{tox} - II_{ref}} \tag{2.6}$$

with

PI_w : Pollution Index for $w \in \{\text{soy, soil}\}$
II_{wx} : Ionic Impulsion (see Equation 2.5)
II_{ref} : Ionic Impulsion of the REFERENCE site
II_{tox} : Ionic Impulsion for toxic levels
(soil: thresholds taken from soil quality guidelines (BBodSchV and DEC RP))
(soybean: reference values taken from Romero et al. [1987])

2.6.2 Transfer Factors

The assimilation, transfer, translocation and bioaccumulation of HMs were investigated by calculating Bioconcentration Factors and Transfer Factors . The calculations are in reference to other authors [Huang et al., 2008; Wang et al., 2006; Li et al., 2006], who investigated into HM transfer at contaminated sites.

To assess the quantitative transfer and accumulation, the determined concentrations were recalculated into dry matter-based values:

$$QC_o = C_o \times DM_o \tag{2.7}$$

with

QC_o : quantitative HM concentration of o [mg/kg, DW]
C_o : measured HM concentration of o [mg/kg, DW]
DM_o : dry matter of o [%]
$o \in \{\text{ro, st, po, se}\}$

Bioconcentration Factor

This factor (also termed Bioaccumulation Factor) is "the ratio of the concentration of a contaminant in an organism to the concentration in the ambient environment at steady state, where the organism can take in the contaminant through ingestion [...]" [US EPA, 2011]. This factor gives an estimation of the relative availability of HMs in soil and the plant's ability to uptake a particular metal. Most often, this factor is calculated on the basis of the total element concentration in the soil, but bioavailability of metals is highly dependent on the physico-chemical soil parameters, soil type and environmental conditions [Wang et al., 2006]. Therefore, McLaughlin et al. [2000], amongst others, suggested to use the available element concentration of soil as a better predictor for environmental risk assessment.

According to the following equation, the BCF$_{ro-av}$ (ratio between concentrations in the roots and the available HM fraction in soil), the BCF$_{ap-av}$ (ratio between concentrations in the aerial parts (stipes and pods) and the available HM fraction in soil) and the BCF$_{se-av}$ (the ratio between concentrations in the seeds and the available HM fraction in soil) were calculated.

$$BCF_{o-av}\ [\%] \ = \ \frac{HM_o\ [mg/kg,\ DW]}{HM_{av}\ [mg/kg,\ DW]} \ \times\ 100 \tag{2.8}$$

with

HM_o : HM concentration in the plant organ [mg/kg, DW]
HM_{av} : HM concentration in the available fraction of soil [mg/kg, DW]
$o \in \{\text{ro , st, po, se}\}$

Transfer Factor

With this factor the transfer of HMs within the soybean plant was studied for the considered pathway from roots to stipes, to pods, to seeds. This was done by calculating the TF$_{st-ro}$ (ratio between the concentrations in stipes and roots), the TF$_{po-st}$ (ratio between the concentrations in pods and stipes), and the TF$_{se-po}$ (ratio between the concentrations in seeds and pods).

2.6.3 Human Hazard Factors

The intake of contaminated food is the main exposure route to HMs for human beings [Peralta-Videa et al., 2009] and could cause a harm to health.

Therefore, the non-cancer health risk, deriving from HM intake via food, was estimated by calculating the non-cancer Hazard Quotient (ncHQ) [US EPA, 2000]. The ncHQ assumes that even for sensitive receptors (young children, unborn babies) harmful health effects are unlikely to expect if the estimated exposure rate (here the Chronic Daily Intake (CDI)) does not exceed the oral Reference Dose (RfDo). The latter being a "toxicity value for evaluating non-carcinogenic effects from exposures" [US EPA, 1989].

Non-cancer Hazard Quotient

The ncHQ was calculated separately for children and adults with the following equations:

$$ncHQ_{hm} = \frac{CDI_h}{RfDo_m} \tag{2.9}$$

with

CDI : average chronic daily intake [mg/kg*d] (see Equation 2.10)
$RfDo$: oral reference dose [mg/kg*d]
$h \in \{\text{child, adult}\}$
$m \in \{\text{Cd, Pb, Zn}\}$

$$CDI_h = \frac{CF \times IR \times EF \times ED_h}{BW_h \times AT} \tag{2.10}$$

with

CDI_h : average chronic daily intake [mg/kg*d] for h
CF : mean concentration of HM in soybean seed [mg/kg, DW]
IR : ingestion rate per person [kg/d]
EF : exposure frequency [d/a]
ED_h : exposure duration of h [a] (70 years for adults; 6 years for children)
BW_h : body weight of h [kg] (70 kg for adults; 30 kg for children)
AT : averaging exposure time for non-carcinogenic effects [$ED_h \times$ 365 d/a]
$h \in \{\text{child, adult}\}$

An ingestion rate of 55 g, given for pulses by Tripathi et al. [1997], was used. The RfDos (0.001 mg/kg*d for Cd and 0.3 mg/kg*d for Zn) were taken from the IRIS information system. A RfDo for Pb is still in discussion [IRIS, 2011], so the concentration of 0.004 mg/kg*d (calculated from the tolerable daily intake limit recommended by the FAO/WHO found in Huang et al. [2008] was used).

Non-cancer Hazard Index

The ncHI was calculated to explore the additive effects by more than one HM. It was calculated by summing up the ncHQs of individual elements:

$$HI_h = \sum_m ncHQ_{hm} = \frac{CDI_h}{RfDo_{Cd}} + \frac{CDI_h}{RfDo_{Pb}} + \frac{CDI_h}{RfDo_{Zn}} \tag{2.11}$$

2.7 Contamination Threshold Values

To grade the contamination levels of soil and soybean seeds, legislation thresholds of different countries were applied. In each case the legislation of Germany and Argentina was considered.

For soil, the strictest threshold values for agricultural land use can be found in the *German federal soil protection and contaminated site ordinance* [BBodSchV, 1999] . They are based on an extraction with ammonium nitrate and consider the pathway from soil to plant with regard to the plant's quality and impairments of growth. In contrast, the thresholds of the Argentinian *Decreto 831/93 Residous Peligrosos [1993]* (DEC RP) state the highest limits. Additionally, the often regarded *Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health [2007]* (CSQG) and the Chinese *Environmental Quality Standard for Soils [1995]* (EQSS) were applied. Although not part of an official legislation, the *world range of unpolluted soil* (WR UPS) [Kabata-Pendias, 2011] and the *contaminated range of metal industry processing affected soils* (CR MIPS) [Fernandez-Turiel et al., 2001] were added to complete the comparison.

For soybean foodstuff, the Argentinian *Código Alimentario Argentino [1967]* (CAA) and the also in Germany valid *Commission Regulation No 1881 [2006]* of the European Union were considered. Additionally, the *Codex Standard 193 [1995]* (CGS FAO) of the FAO and the thresholds of the *Chinese Food Hygiene Standard* (CFHS) , which were reported by Hao et al. [2011], were used for the comparison.

2.8 Statistical Analysis

The statistical analysis was conducted with the software IBM SPSS, Version 19.

First of all, descriptive statistics were calculated for investigated variables. Additionally histograms were plotted. In combination, they give a first impression of the collected data.

By converting the data into z-scores (standardized values) and plotting of box plots, outliers were identified and considered in the interpretation of the results.

Some statistical analyses are based on the assumption of normal distribution and homogeneity of variance of the data. A normal distribution of the data could not be expected due to the small sampling sizes (see Section 2.2). It is furthermore known that especially geochemical and environmental data often do not show normal distribution, but rather a multi or bimodal one [Reimann and Filzmoser, 2000]. Therefore, no tests to prove this assumption were conducted, no transformations were applied and the original data was analyzed with non-parametric procedures.

To test if the variances of compared sampling sites are equal, the Levene's test, based on means and on medians, was conducted.

Mann-Whitney U-tests (M-W U) of two independent samples were applied to compare the medians of the variables between the sampling sites SMELTER and CONTROL (see Section 3.1.3).

Bivariate correlation analysis (Kendall's Tau) were carried out and matrix scatter diagrams were plotted to study the relationships and interaction of variables. Kendall's Tau was applied, because it is recommended for small sample sizes, does not require a linear relationship between the variables and is resistant against outliers [Brosius, 2008].

Curve estimations with linear, logarithmic, inverse, power and exponential models were calculated to investigate the relationship of the HM concentrations found in the soil fractions and those detected in the soybean organs.

All tests took the probability values $p \leq 0.01$ (marked with **), $p \leq 0.05$ (marked with *) and $p \leq 0.1$ (marked with (*)) into account.

Chapter 3

Results

3.1 Evaluation Prerequisites

3.1.1 Quality Control

Replicates, Blanks and Standard Solutions

The raw data of laboratory analyses for all samples and blanks is presented in the Tables B.9 – B.14 in the appendix. The coefficient of variation within triplicates was typically below 10%, except for Cd in the roots and Pb in the roots and stipes. A higher coefficient of variation could be caused by HM concentrations out of the optimal measurement range. Root material deviations could be explained by difficulties with the homogenization of the material.

In none of the blanks any metal concentration could be determined. Thus, a contamination of the samples during the preparation or the measurement procedure can be rejected. The detected concentrations of HMs in the samples are therefore originating from the sample material.

To verify the accuracy of the instrumentation, the standard solutions were consistently analyzed between the sample solutions. In case of a deviation from the set value the calibration was repeated.

Certified Material

Table 3.1 shows the comparison between the observed values and those reported for the certified material from the FAAS analysis. The data for Cd was not attained.

The obtained values deviated much from the values given for the certified materials. A good quality of measurement could not be assumed. The results for the certified soil material "GBW 07405" were inferior compared to those for

Table 3.1: Comparison of certified and observed values from the flame atomic absorption spectroscopy analysis [mg/kg, DW]

Chinese soil GBW 07405

Element	Certified Value [mg/kg] ±SD	Observed Value [mg/kg] (n=1)	Recovery [%]
Pb	552±44	308.68	55.9
Zn	494±39	194.95	39.5

Tobacco leaves CTA-OTL-1

Element	Certified Value [mg/kg] ±SD	Observed Value [mg/kg] (n=1)	Recovery [%]
Pb	4.91±1.60	6.61	134.6
Zn	49.9±5.00	44.46	89.1

Data for Cd was not obtained.

the certified plant material "CTA-OTL-1". Due to shortage of certified material and lack of time, the processing and measurement could not be repeated.

Possible explanations for the unsatisfactory recovery percentages are various. In the processing an aqua regia extraction (HNO_3:HCl, 1:3) was applied and the FAAS was used. Although no information could be obtained about that, the certified values might have been derived from another digestion and measurement method. Furthermore, no replicates were produced because of financial aspects. Hence, the processing precision could not be verified. Concerning the certified material of the Chinese soil, remains of only about 0.5 g were processed. That was sparse in comparison with 3 g which were used for the analysis of the sample material. It could also be supposed that the remaining certified material was not in its original condition due to many uses and users over a long time. Recent measurements within the framework of other projects of the institute obtained satisfactorily results of recovery percentages for the same certified material, processed in the same manner [Bermudez, 2011] [Salazar, 2011 (personal communication)]. It is also known that the instrumentation is permanently in use and checked on correct function. During the measurement no irregularities appeared.

Considering the mentioned aspects, the handling of obtained data in the evaluation will be the following.

The obtained data of the plant material is assumed to be correct. Considering the standard deviation of the certified material, the observed values are within acceptable range. Compared to the data of other sampling points in Bouwer, the scores did not differ much [Rodriguez, 2011 (personal communication); Salazar, 2011 (personal communication)].

The obtained data for the soil material is accepted with reservation - knowing possible origins of unsatisfactory results of recovery percentages.

3.1.2 Descriptive Parameters

The descriptive parameters, which resulted from the statistical analysis, are presented in Table B.2 in the appendix. Only laboratory-gathered variables are considered. The data range, the mean, the standard deviation, the standard error of the mean and the variance were calculated. Large data ranges and a small sample size led to large standard deviations. In addition, many outliers were observed (see Table B.3 in the appendix). The Levene's test based on the median resulted in a higher homogeneity of variance (see Table B.1 in the appendix). Due to these findings, the median and the median absolute deviation (MAD) were added to the table and used in the following data explorations.

3.1.3 Grouping of Sampling Sites for Data Analysis

A Mann-Whitney U-test for data on the two control sites "B2" and "B5" showed no significant differences for any variable (see Table B.4 in the appendix). Therefore, the pools belonging to these sites were merged to one group, named "CONTROL".

A subgrouping of the sampling site "B0" was considered, but no significant differences between the pools close (B0-1 – B0-4) and far (B0-5 – B0-7) to the smelter were found. Thus, this idea was refused. A subgrouping based on the wind direction was not suitable because only one pool (B0-5) was situated south of the smelter, whereas the remaining ones were situated on the east. Hence, the sampling site "B0" with all its pools was handled as SMELTER.

3.2 Heavy Metals in Soil

Due to the state of knowledge (see Section 1.3 and Section 2.2), it has been assumed that the concentrations of HMs in the soil at the SMELTER site are higher than those at the CONTROL sites. The following data explorations were made to verify this assumption. They should present the determined concentrations of Cd, Pb and Zn in the soil fractions and investigate the availability of these HMs and their contamination level in the soils.

3.2.1 Heavy Metals in Soil Fractions

Water Extractable Fraction

The water-extractable fraction is not presented in Figure 3.1, because it was only processed and measured for the CONTROL pools (except pool B5-1) and no concentrations of Pb and Cd could be detected.

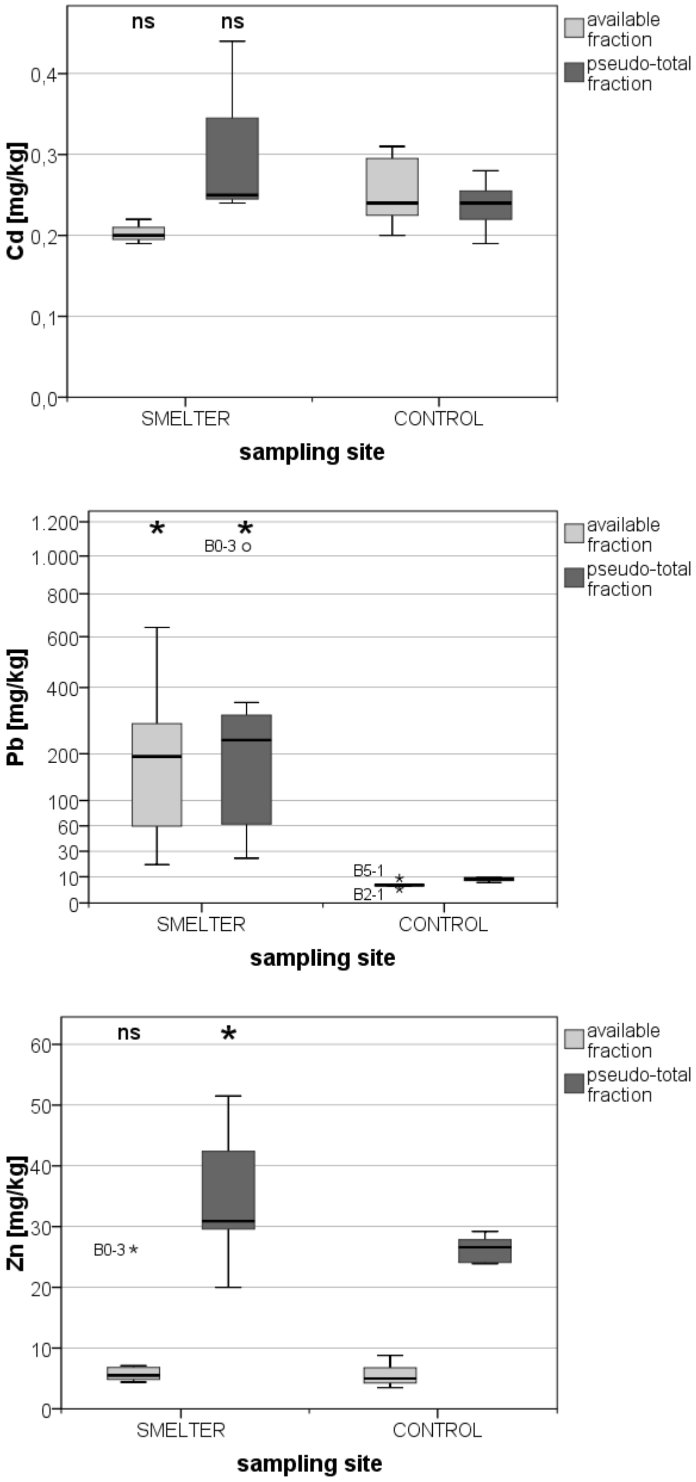

Figure 3.1: Box-and-Whisker Plots for the concentrations of Cd, Pb and Zn in the available and pseudo-total fraction of soil

The bar within the box marks the median, the box borders mark the 25th and 75th percentile and the whisker ends mark the maximum and minimum score. Outliers are presented as ○ (1.5 times height of the box) or a small ⋆ (>3 times height of the box). Significant differences ($p \leq 0.05$) between the sites are denoted with a big ★ above the plots; not significant differences ($p > 0.05$) between the sites are denoted with **ns** above the plots.

The mean Zn concentration for the Control sites in the water-extractable fraction was 0.083 mg/kg, the median 0.01 mg/kg and the range 0.00–0.02 mg/kg.

The results could indicate, that the water-extractable fraction does not contain any Cd or Pb and a negligible concentration of Zn. Other explanations would be that the concentrations of HMs were so low in the concerning fraction that the determination with the FAAS was no suitable method or the amount of extracted material was not enough. Therefore, the results were not included in the following evaluation.

Available and Pseudo-total Fraction

Figure 3.1 shows that the concentrations of HMs in the available fraction were in general less than in the pseudo-total fraction. This difference was high for Zn, but very small for Pb. The concentration of Cd in the available fraction of the Control sites was the only exception from this observation.

A correlation analysis (Table 3.2) revealed significant positive correlations between the Pb concentrations in the available fraction and in the pseudo-total fraction for both sampling sites ($\tau_S = 0.904^{**}$, $\tau_C = 0.714^{*}$). An increase of Pb in the available fraction was related to an increasing concentration of Zn in the selfsame ($\tau = 0.523^{(*)}$).

Table 3.2: Correlation coefficients between the heavy metal concentrations in the available and pseudo-total fraction

Smelter

	Cd_{pt}	Pb_{av}	Pb_{pt}	Zn_{av}	Zn_{pt}
Cd_{av}	∅	-0.333^{ns}	0.333^{ns}	∅	∅
Cd_{pt}	1	-0.333^{ns}	0.333^{ns}	∅	∅
Pb_{av}		1	$\mathbf{0.904}^{**}$	0.142^{ns}	0.333^{ns}
Pb_{pt}			1	0.238^{ns}	0.428^{ns}
Zn_{av}				1	0.428^{ns}

Control

	Cd_{pt}	Pb_{av}	Pb_{pt}	Zn_{av}	Zn_{pt}
Cd_{av}	0.333^{ns}	-0.238^{ns}	-0.142^{ns}	0.047^{ns}	0.487^{ns}
Cd_{pt}	1	0.238^{ns}	0.333^{ns}	0.142^{ns}	0.195^{ns}
Pb_{av}		1	$\mathbf{0.714}^{*}$	$\mathbf{0.523}^{(*)}$	0.000^{ns}
Pb_{pt}			1	0.238^{ns}	-0.097^{ns}
Zn_{av}				1	0.000^{ns}

Kendall's Tau Correlation; $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$
$n = 7$, except for Cd at the Smelter site, there $n = 3$; ∅ = no value.

The box pattern for Pb and Zn were quite similar across the sites, but not for Cd.

The median **Cd** concentrations were all in the small range of 0.19–0.44 mg/kg. The highest concentrations of **Pb** could be detected for the Smelter site (median$_{av}$: 193.34 mg/kg, median$_{pt}$: 235.85 mg/kg). The medians for the Control sites were about 40 times less. The concentrations of **Zn** between the fractions differed on average by a factor of four (mean$_{av}$: 6.82 mg/kg, mean$_{pt}$: 31.37 mg/kg). The highest scores could be observed at the Smelter site in the pseudo-total fraction.

A site comparison, revealed larger whiskers for the Smelter site than for the Control sites.

The results of the M-W U-test (Table 3.3) showed generally lower average ranks for the Control sites than for the Smelter site, with the only exception of Cd in the available fraction.

Significant differences were found for Pb in both fractions ($p = 0.001$) and Zn in the pseudo-total fraction ($p = 0.038$). Especially for Pb, the difference of the median concentrations was huge (av: 187.70 mg/kg; pt: 227.22 mg/kg).

Hence, the soil of the Smelter site contained significant more Pb and Zn than the soil of the Control sites. The Cd concentrations in soil were found to be similar across the sites.

Table 3.3: Results of the Mann-Whitney U-test, calculated for data on the heavy metal concentrations in the available and pseudo-total fraction [mg/kg, DW]

Variable	Site	n	Mean Rank	M-W U	Z	Exact. Sig.	Median
Cd_{av}	S	3	2.66	2	−1.949	0.067	0.20^a
	C	7	6.71				0.24^a
Cd_{pt}	S	3	7.00	6	−1.032	0.383	0.25^a
	C	7	4.85				0.24^a
Pb_{av}	S	7	11.00	0	−3.130	0.001	193.34^a
	C	7	4.00				5.64^b
Pb_{pt}	S	7	11.00	0	−3.130	0.001	235.85^a
	C	7	4.00				8.63^b
Zn_{av}	S	7	7.92	21.5	−0.384	0.710	5.20^a
	C	7	7.07				5.00^a
Zn_{pt}	S	7	9.85	8	−2.111	0.038	30.55^a
	C	7	5.14				26.60^b

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

3.2.2 Soil Heavy Metal Availability

Activity Ratio

As shown in Figure 3.2, the mean Activity Ratio (AR) of Pb exceeded that of Cd at the Smelter site (mean AR_{Pb}: 85.17 %, mean AR_{Cd}: 69.72 %), whereas the contrary was found for the Control sites (mean AR_{Pb}: 67.42 %, mean AR_{Cd}: 108.07 %). Between the sites, the ARs for Cd and Pb differed significantly ($p = 0.017$, $p = 0.026$, respectively). The AR of Zn was found to be low across the sites with no significant differences and a range of medians between 8.62–21.03 %.

The proposed mobility sequences were Pb > Cd >> Zn for the SMELTER site and Cd > Pb >> Zn for the CONTROL sites.

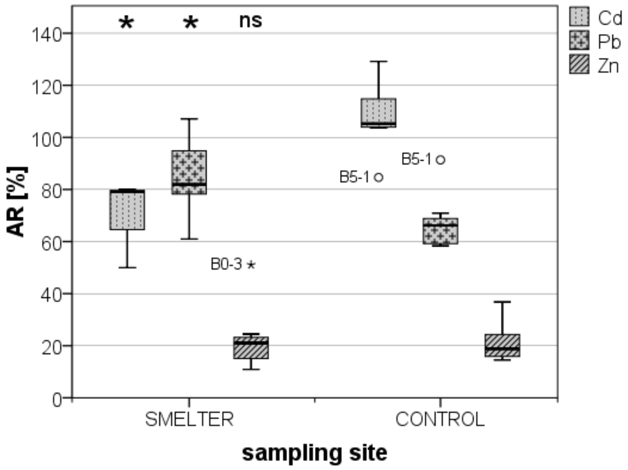

Figure 3.2: Box-and-Whisker Plots for the Activity Ratios of Cd, Pb and Zn

Labeling of the Figure follows the description in Figure 3.1

Table 3.4: Results of the Mann-Whitney U-test, calculated for data on the Activity Ratio of heavy metals

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	3	2.00	0	−2.393	0.017	79.16^a
	C	7	7.00				105.26^b
Pb	S	7	10.00	7	−2.236	0.026	81.97^a
	C	7	5.00				66.21^b
Zn	S	7	7.28	23	−0.192	0.902	21.03^a
	C	7	7.71				18.79^a

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

Physico-chemical Parameters

According to the descriptive parameters (see Table B.2), the pH value was found to be slightly acid, a bit more at the SMELTER site ($mean_S = 6.59 \pm 0.19$, $mean_C = 6.82 \pm 0.20$). The mean content of the organic matter (OM) accounted 5.28 ± 0.41 % at the SMELTER site and 5.66 ± 0.75 % at the CONTROL sites. The electrical conductivity (EC) was in the range of 46.60–404.67 μS/cm at the SMELTER site and 72.70–117.45 μS/cm at the CONTROL sites.

A correlation analysis between the different soil fractions and the physico-chemical parameters resulted in general low correlation coefficients, which were not significant. For the pseudo-total fraction the correlations coefficients were even lower than for the available fraction and therefore they were not presented.

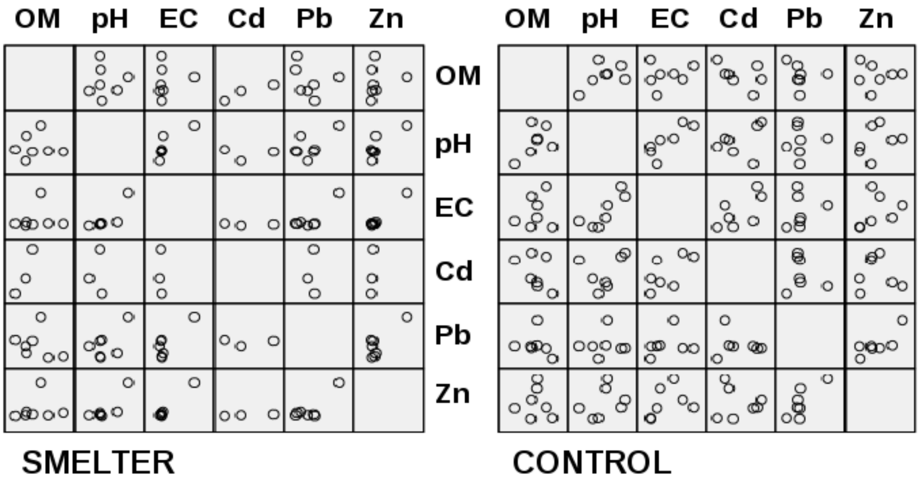

Figure 3.3: Scatter matrix of heavy metal concentrations in the available fraction and the soil's physico-chemical parameters

Table 3.5: Correlation coefficients between the heavy metal concentrations in the available fraction and the soil's physico-chemical parameters

Smelter

	n	OM	pH value	EC
Cd_{av}	3	$\emptyset$	-0.333^{ns}	-0.333^{ns}
Pb_{av}	7	-0.333^{ns}	0.292^{ns}	0.142^{ns}
Zn_{av}	7	0.333^{ns}	0.292^{ns}	0.428^{ns}
OM	7	1	0.000^{ns}	0.142^{ns}
pH value	7		1	**0.878****

Control

	n	OM	pH value	EC
Cd_{av}	7	-0.428^{ns}	0.238^{ns}	0.428^{ns}
Pb_{av}	7	-0.333^{ns}	-0.047^{ns}	0.142^{ns}
Zn_{av}	7	0.142^{ns}	0.428^{ns}	0.428^{ns}
OM	7	1	0.333^{ns}	0.142^{ns}
pH value	7		1	**0.619**$^{(*)}$

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$
$n = 7$, except for Cd_{av} at the SMELTER site, there $n = 3$; $\emptyset$ = no value.

The correlation coefficients in Table 3.5 show a significant relationship between the pH value and the EC at the SMELTER as well as at the CONTROL sites ($\tau = 0.878^{**}$, $\tau = 0.619^{(*)}$, respectively). In the scatter matrix (Figure 3.3) this relationship could be noticed by a line of dots, which might follow a linear function. No relationship was found for the OM and the pH value at the

Smelter site, whereas at the Control sites, these parameters were positively correlated ($\tau = 0.333^{ns}$).

The available fraction of **Cd** tended to increase with an increasing EC. In contrast, an increase of the OM seemed to cause a decrease of the available Cd. The influence of soil parameters on the available Cd at the Smelter site was difficult to evaluate due to a lack of data (n=3).

The available content of **Pb** decreased with an increase of the OM ($\tau = -0.333^{ns}$). The pH value seemed to have an effect on the Smelter site, but not at the Control sites.

For the available fraction of **Zn**, correlations were found for the EC and the pH value. The correlations were positive, which indicated that with an increase of EC or pH value, the concentration of Zn in the available fraction rose, too.

Amongst the metals, the highest correlation was found for Cd and Zn ($\tau = 0.358$).

The scatter matrix shows that the range of physical-chemical parameters was small, whereas the ranges of HM concentrations in soil were huge (compare to Figure 3.1). Additionally, the scatter matrix makes the outliers visible (see Table B.3), especially at the Smelter site.

To sum up, relationships between the physico-chemical soil parameters and the HM concentrations in soil were recognized, although not significant.

3.2.3 Soil Contamination

Table 3.6: Results of the Mann-Whitney U-test, calculated for data on the Enrichment Factor of heavy metals

EF$_{crust}$

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	3	2.00	0	-2.393	0.017	108.53^a
	C	7	7.00				418.34^b
Pb	S	7	10.14	6	-2.366	0.017	616.87^a
	C	7	4.85				57.87^b
Zn	S	7	5.00	7	-2.236	0.026	5.32^a
	C	7	10.00				17.65^b

EF$_{loess}$

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Pb	S	7	9.28	12	-1.597	0.128	50.82^a
	C	7	5.71				14.29^a
Zn	S	7	4.00	0	-3.130	0.001	1.97^a
	C	7	11.00				10.52^b

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

Enrichment Factors

The enrichment of HMs due to anthropogenic inputs can be estimated with the Enrichment Factors.

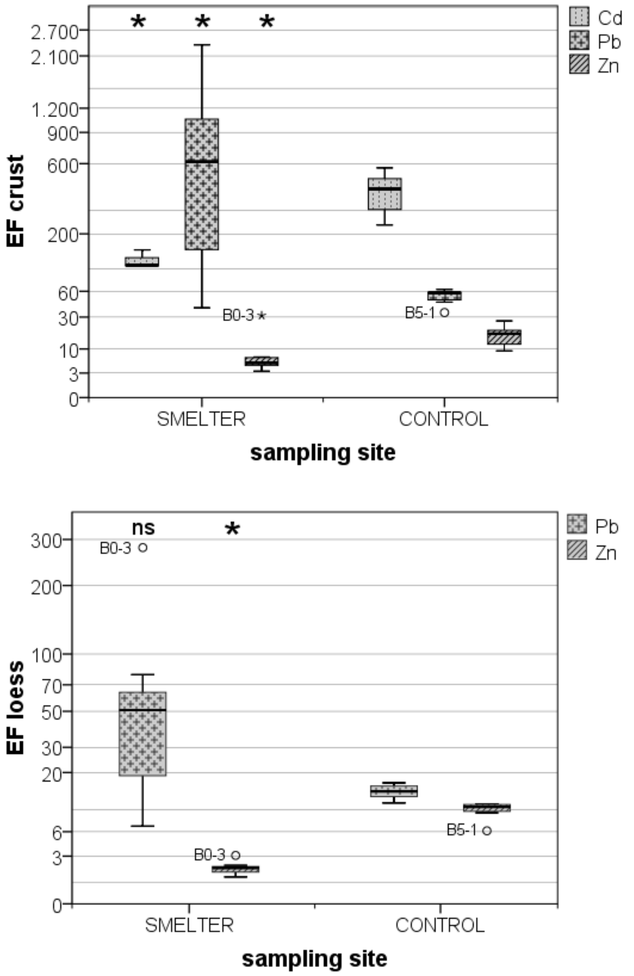

Figure 3.4: Box-and-Whisker Plot of the Enrichment Factors for Cd, Pb and Zn

Labeling of the Figure follows the description in Figure 3.1

Concerning EF$_{crust}$, Figure 3.4 shows an extremely high enrichment of Pb at the SMELTER site ($mean = 786.57$) – a value, which was far beyond the highest classification value. For this element, again a wide range between maximum and minimum score could be observed (compare to Figure 3.1). The enrichments of Cd and Zn were higher at the CONTROL sites than at the SMELTER site. Cd was also highly enriched, but less than Pb. The enrichment of Zn was the lowest among the HMs, but still evaluated as "significant" ($mean_S = 9.05$, $mean_C = 16.86$). For all elements the M-W U-test (Table 3.6) stated significant differences between the sites.

EF$_{loess}$ was only calculated for Pb and Zn, due to a lack of data for Cd. The pattern of EF$_{loess}$ was similar to EF$_{crust}$, but the scale of the enrichment was much smaller. A higher enrichment of Pb could be recorded at the SMELTER site than at the CONTROL sites and vice versa for Zn. The enrichment of Pb was classified between significant and very high; that of Zn between minimal and significant. Significant differences between the sites could only be obtained for Zn.

An estimation of the anthropogenic abundance of HMs at the SMELTER site, in comparison with the background values of the REFERENCE site, could not be made due to the lack of data for iron (Fe) for the "REF" pool.

To sum up, all investigated soils showed an enrichment of Cd, Pb and Zn in comparison to the element concentrations in the earth's crust and common loessic soils in Argentina. The enrichment sequence at the SMELTER site followed the order: Pb >> Cd >> Zn, and at the CONTROL sites: Cd >> Pb > Zn.

Soil Ionic Impulsion and Pollution Index

The Soil Ionic Impulsion (II$_{soil}$) (Figure 3.5) shows the combined effect of Cd, Pb and Zn on soils. Due to a higher concentration of a particular metal in the pseudo-total fraction (compare to Figure 3.1) also a higher II$_{soil}$ was observed for this fraction.

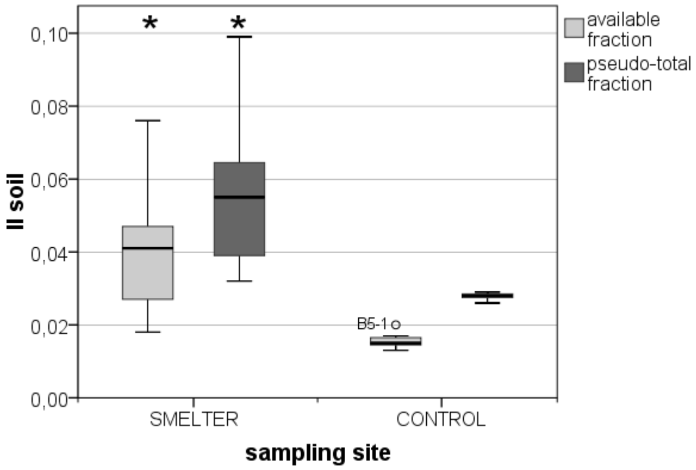

Figure 3.5: Box-and-Whisker Plots for the Soil Ionic Impulsion of heavy metals in the available and pseudo-total fraction

Labeling of the Figure follows the description in Figure 3.1

Again the wide data range between minimum and maximum score of the SMELTER site was visible (compare to Figures 3.1 and 3.4). The II$_{soil}$ at the SMELTER site was about twice as high at the CONTROL sites. This difference was significant for both fractions (see Table 3.7).

Table 3.7: Results of the Mann-Whitney U-test, calculated for data on the Soil Ionic Impulsion and the Pollution Index [%]

Soil Ionic Impulsion

Soil Fraction	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
av	S	7	10.85	1	−3.009	0.001	0.041^{a}
	C	7	4.14				0.015^{b}
pt	S	7	11.00	0	−3.148	0.001	0.055^{a}
	C	7	4.00				0.028^{b}

Soil Pollution Index

Soil Fraction	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
av_{ARG}	S	7	10.86	1	−3.003	0.001	21.33^{a}
	C	7	4.14				2.11^{b}
av_{GER}	S	7	10.86	1	−3.003	0.001	49.40^{a}
	C	7	4.14				4.88^{b}
pt_{ARG}	S	7	11.00	0	−3.130	0.001	19.75^{a}
	C	7	4.00				5.22^{b}
pt_{GER}	S	7	11.00	0	−3.130	0.001	60.84^{a}
	C	7	4.00				16.12^{b}

Different letters denote significant ($p \leq 0.05$) differences between the Smelter and the Control sites.

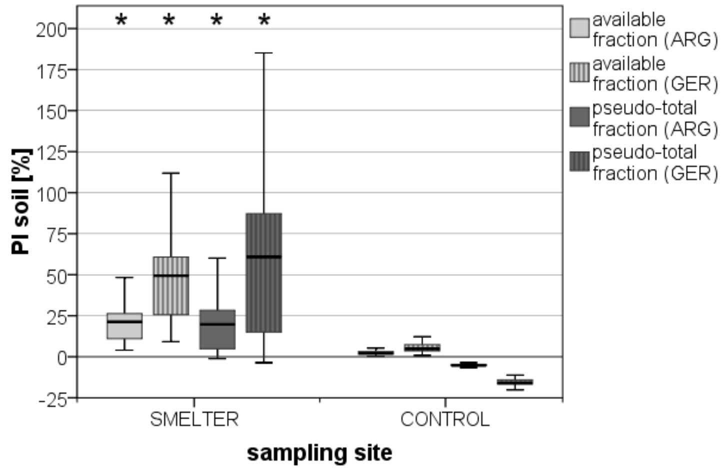

Figure 3.6: Box-and-Whisker Plots of the Soil Pollution Index of the available and pseudo-total fraction

Labeling of the Figure follows the description in Figure 3.1. "ARG" (unpatterned) and "GER" (striped) code the applied legislation (see Table 3.8)

With the Soil Pollution Index (PI_{soil}) (Figure 3.6), a relative estimate of combined metal soil contamination in relation to the REFERENCE site (II_{ref}) was attained. The threshold values of the Argentinian and German soil quality guidelines for agricultural soil were applied as toxic levels of the Ionic Impulsion (II_{tox}) in the calculations (see Table 3.8).

Independent of applied threshold values the average pollution of the SMELTER site accounted 35.11 % for the available fraction and 42.97 % for the pseudo-total fraction. The average pollution of the CONTROL sites accounted in contrast 4.04 % and 10.3 %, respectively. These differences were significant. This showed a pollution of the SMELTER site in comparison to the REFERENCE site. The negative values for the pseudo-total fraction indicated that the pollution at the CONTROL sites is even less than at the REFERENCE site.

According to the different thresholds for toxic HM levels in Argentina (ARG) and Germany (GER), the box ranges for the Argentinian legislation always show a lower PI_{soil} than those for the German legislation, whose thresholds are much stricter (see following Section).

To sum up, the combined effect of HMs significantly affects the SMELTER site. The results also showed that the term "pollution" or "contamination" is always related to and dependent on the considered threshold or reference values. Consequently, the investigated soils are seen to be more polluted in terms of the German federal soil protection ordinance (BBodSchV). The comparison with the REFERENCE site revealed that the soil of this site is also polluted. Therefore, this site can not provide background values for a comparison with the sampling sites.

Grading of Contamination Levels

To evaluate the detected concentrations in soil, threshold values from different countries were taken into account.

The check values of the *German federal soil protection and contaminated site ordinance* were exceeded at both sampling sites for all investigated HMs.

In the case of **Cd** only the mentioned exceedance for agricultural land use, referring to the BBodSchV, could be mentioned.

The pseudo-total concentrations of **Pb**, detected at the SMELTER site, were on average almost 3 000 times higher than indicated by the German check value of 0.1 mg/kg. Also the German precaution value of 70 mg/kg, which regards the natural function of soil, was exceeded. The limits of the *Canadian Soil Quality Guideline* and the Chinese *Environmental Quality Standard for Soils* were also surpassed. Only the Argentinian threshold values were not exceeded by the mean pseudo-total concentrations. The highest load of Pb (1 050.72 mg/kg) was found for pool B0-3. This maximum even exceeded the Argentinian and Canadian threshold values for industrial land-use (1 000 mg/kg and 600 mg/kg, respectively). But it was still in the range of the concentrations, detected in

Table 3.8: Threshold values of different countries for the total concentrations of Cd, Pb and Zn in agricultural soils [mg/kg, DW]

Unspecified land use

legislation	Cd	Pb	Zn
BBodSchV^{a,e,f}	1	70	150
CR MIPS	0.6–160	72–12 123	155–12 400
WR UPS	0.07–1.1	10–70	17–125

Agricultural land use

legislation	Cd	Pb	Zn
BBodSchV^{c,e,f}	0.1	0.1	2
CSQG^{b,e}	1.4	70	200
DEC RP^{b}	3	375	600
EQSS^{d}	0.6	300	250

with

BBodSchV : Bundes-Bodenschutz- und Altlastenverordnung, Germany
CR MIPS : contaminated range of metal industry processing affected soils [Fernandez-Turiel et al., 2001]
CSQG : Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, Canada
DEC RP : Decreto 831/93 Residous Peligrosos, Argentina
EQSS : Environmental Quality Standard for Soils GB15618, China
WR UPS : world range of unpolluted soil [Kabata-Pendias, 2011]
a : precaution values
b : guideline values
c : check values
d : maximum allowable concentration (MAC)
e : 63 mm fraction
f : ammonium nitrate (NH_4NO_3) extraction

Table 3.9: Concentration ranges of heavy metals in the available and pseudo-total fraction [mg/kg, DW]

Smelter

Variable	n	Range	Mean±SD
Cd$_{av}$	3	0.19–0.22	0.20±0.015
Cd$_{pt}$	3	0.24–0.44	0.31±0.11
Pb$_{av}$	7	18.23–640.71	220.20±214.98
Pb$_{pt}$	7	23.49–1 050.50	294.17±355.64
Zn$_{av}$	7	4.40–26.30	8.50±7.91
Zn$_{pt}$	7	20.00–51.50	35.20±11.62

Control

Variable	n	Range	Mean±SD
Cd$_{av}$	7	0.20–0.31	0.26±0.04
Cd$_{pt}$	7	0.19–0.28	0.24±0.03
Pb$_{av}$	7	4.01–9.07	5.85±1.54
Pb$_{pt}$	7	6.85–9.93	8.61±1.15
Zn$_{av}$	7	3.50–8.80	5.61±1.97
Zn$_{pt}$	7	23.9–29.2	26.23±2.21

soils, which were contaminated by metal processing industries. The Pb concentrations observed for the CONTROL sites did not pass any threshold, except the already mentioned check value of the BBodSchV.

The **Zn** concentrations exceeded the German check value by a factor of 18 at the SMELTER site and by a factor of 14 at the CONTROL sites.

In comparison with the world range for unpolluted soils, given by Kabata-Pendias [2011], the Cd and Zn concentrations fell within the suggested range, but Pb exceeded it.

To sum up, the exceeded thresholds for Pb and Zn at the SMELTER site demonstrated the contamination of the investigated soil with these HMs. The German check values which indicate that the plants quality might be impaired if surpassed, were exceeded by all HM concentrations.

3.3 Heavy Metals in Soybean Plants

HM concentrations in soybean plants can reach phytotoxic levels. Furthermore, the HM concentration in the seeds can present a risk to human health, through an uptake into the food chain. The following data explorations were made to present measured concentrations of Cd, Pb and Zn in the organs of soybean crops and investigate the viability and contamination of the soybean seeds. Finally, health risks, resulting from the detected HM levels in the seeds, were calculated.

3.3.1 Heavy Metals in Soybean Organs

Figure 3.7 shows that the concentration level for each metal differed much, but concerning a particular metal, a similar sequence across the sites could be observed, except for Pb.

The concentrations of **Cd** in the soybean organs followed the order: pod > stipe > seed > root. The average concentration range across all tissues and both sites was small (0.07–0.39 mg/kg). Although not significant, the median concentration of Cd in the stipes at the SMELTER site was higher than in the stipes at the CONTROL sites (0.28 mg/kg and 0.23 mg/kg, respectively). The seeds contained 0.2 mg/kg Cd on average.

For **Pb** the order of concentrations was distinct at the considered sites. For the SMELTER site, it followed the sequence root > stipe > pods > seed, whereas for the CONTROL sites, it followed the sequence pod > seed ≈ stipe > root. Larger score ranges and outliers were visible for roots, stipes and pods at the SMELTER site (0.00–6.61 mg/kg, 1.12–4.76 mg/kg and 1.50–3.24 mg/kg, respectively, outliers excluded). The seeds contained 1.49 mg/kg Pb on average.

The concentrations of **Zn** were highest in the seeds (median_S: 30.82 mg/kg, median_C: 23.99 mg/kg). The stipes on average contained 3.28 mg/kg at the

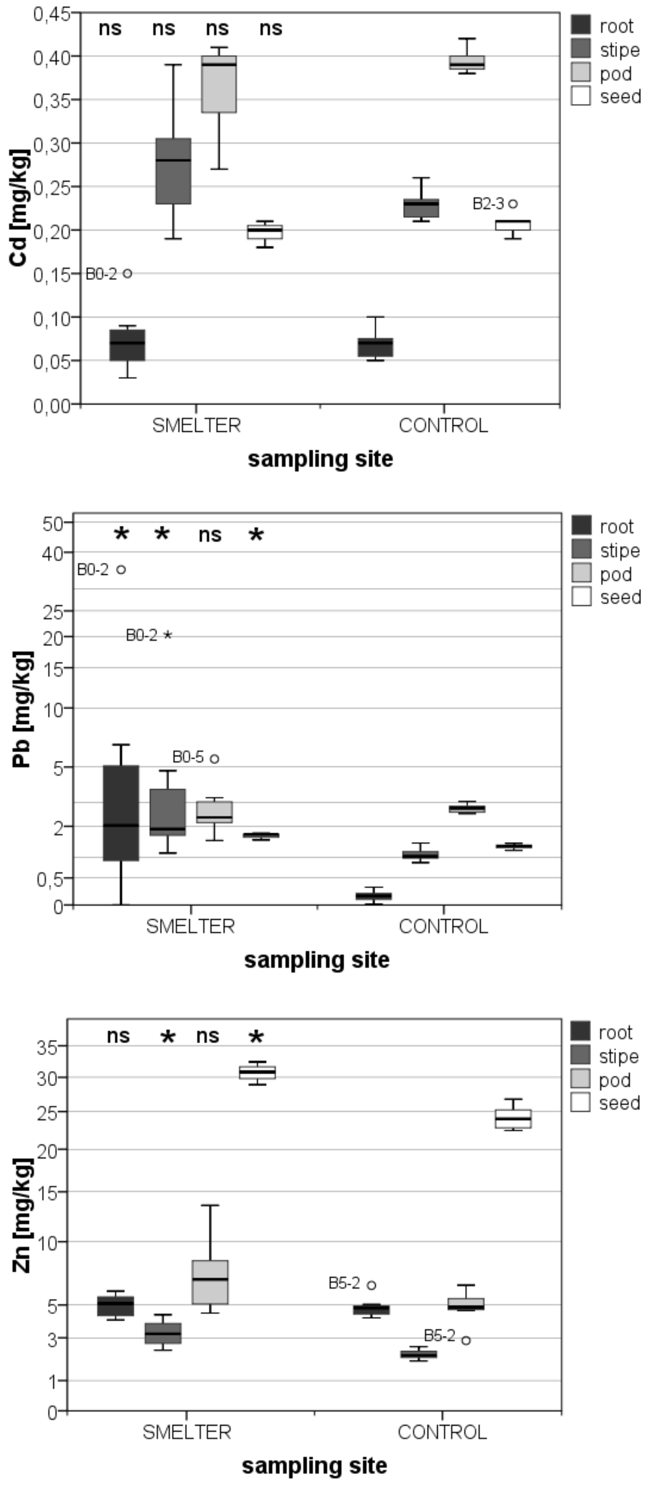

Figure 3.7: Box-and-Whisker Plots for the concentrations of Cd, Pb and Zn in the soybean organs

Labeling of the Figure follows the description in Figure 3.1

Table 3.10: Results of the Mann-Whitney U-test, calculated for data on the heavy metal concentrations in soybean organs [mg/kg, DW]

Cd

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	7.52	24	−0.064	1.000	0.07^a
	C	7	7.42				0.07^a
stipe	S	7	9.00	14	−1.349	0.209	0.28^a
	C	7	6.00				0.23^a
pod	S	7	6.71	19	−0.715	0.535	0.39^a
	C	7	8.28				0.39^a
seed	S	7	6.00	14	−1.395	0.209	0.20^a
	C	7	9.00				0.21^a

Pb

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	10.00	7	−2.239	0.026	2.03^a
	C	7	5.00				0.15^b
stipe	S	7	10.57	3	−2.750	0.004	1.90^a
	C	7	4.42				1.03^b
pod	S	7	6.71	19	−0.703	0.535	2.35^a
	C	7	8.28				2.74^a
seed	S	7	11.00	0	−3.134	0.001	1.70^a
	C	7	4.00				1.33^b

Zn

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	8.00	21	−0.447	0.710	5.10^a
	C	7	7.00				4.80^a
stipe	S	7	10.71	2	−2.878	0.002	3.22^a
	C	7	4.28				2.10^b
pod	S	7	9.28	12	−1.597	0.128	6.84^a
	C	7	5.71				4.86^a
seed	S	7	11.00	0	−3.130	0.001	30.81^a
	C	7	4.00				23.99^b

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

Smelter site and 2.15 mg/kg at the Control sites. Accordingly, the concentration order across organs was seed >> pod > root > stipe.

Comparing the sites, the box plots for the Smelter site show larger ranges of HM concentrations. This is in accordance with the observations for HMs in soil (see Section 3.2.1).

According to the conducted M-W U-test (Table 3.10), soybean organs at the Smelter site contained significantly more Pb in roots, stipes and seeds ($p = 0.026$, $p = 0.004$ and $p = 0.001$, respectively) and Zn in stipes and seeds ($p = 0.002$ and $p = 0.001$, respectively). Like in the soil, no significant differences were found among the Cd concentrations at the different sites.

Table 3.11: Correlation coefficients between the heavy metal concentrations in the different soybean organs

Smelter

Cd	root	stipe	pod
stipe	-0.047^{ns}	1	
pod	0.000^{ns}	0.097^{ns}	1
seed	0.102^{ns}	0.205^{ns}	0.158^{ns}
Pb	root	stipe	pod
stipe	**0.714***	1	
pod	0.333^{ns}	0.428^{ns}	1
seed	-0.292^{ns}	0.000^{ns}	-0.487^{ns}
Zn	root	stipe	pod
stipe	**0.809***	1	
pod	0.238^{ns}	0.238^{ns}	1
seed	-0.142^{ns}	-0.333^{ns}	0.047^{ns}

Control

Cd	root	stipe	pod
stipe	∅	1	
pod	-0.216^{ns}	-0.216^{ns}	1
seed	0.389^{ns}	0.389^{ns}	**−0.914****
Pb	root	stipe	pod
stipe	0.15^{ns}	1	
pod	-0.292^{ns}	-0.097^{ns}	1
seed	0.292^{ns}	-0.097^{ns}	0.047^{ns}
Zn	root	stipe	pod
stipe	0.195^{ns}	1	
pod	-0.238^{ns}	-0.390^{ns}	1
seed	0.333^{ns}	0.097^{ns}	0.047^{ns}

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$, $n = 7$.

A correlation analysis (Table 3.11) revealed that the HM concentrations detected in the roots affected the concentrations in the upper plant parts in a different way across the HMs and sites.

Significant relationships between the concentrations in root and stipe were found for Pb and Zn at the SMELTER site ($\tau = 0.714^{*}$ and $\tau = 0.810^{*}$, respectively). For the CONTROL sites, mainly the concentrations in seeds seemed to be influenced by those in the roots.

No mentionable correlations for Cd could be detected at the SMELTER site. Instead, at the CONTROL sites, an increasing Cd content in the pods led to a significant decrease of Cd in the seeds ($\tau = -0.915^{**}$). The same, but not significant, trend could also be observed for Pb at the SMELTER site.

Whereas the concentrations in the pods were negatively correlated with those in the roots at the CONTROL sites, a positive correlation was observed at the SMELTER site. The same inverted correlation was detected for stipes and pods.

To sum up, parallels of HM concentrations in soil and plants were discovered. Whereas the Cd contents were within a small range and did not differ significantly across the sites, Pb and Zn were found to be significantly enriched in the soil, as well as in the soybean organs, at the Smelter site (compare Section 3.2.1). The seeds contained significantly higher concentrations of Pb and Zn.

3.3.2 Soybean Vitality

Yield Parameters

Differences in the habitus and health state of the soybean plants at the different sampling sites were already noted in the fields. As shown by two typical plants in Figure 3.8, the soybean plants at the Control sites were in a better condition than those at the Smelter site.

Figure 3.8: Soybean plants from the Smelter and the Control sites

Table 3.12: Results of the M-W U-test calculated for data on the organ count ratios [n]

Organ ratio	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
pod/plant	S	7	4.57	4	-2.619	0.002	18.95^{a}
	C	7	10.53				36.04^{b}
seed/plant	S	7	4.71	5	-2.452	0.011	42.32^{a}
	C	7	10.29				75.21^{b}
seed/pod	S	7	8.43	18	-0.832	0.456	2.23^{a}
	C	7	6.57				2.14^{a}

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

Table 3.13: Results of the Mann-Whitney U-test, calculated for data on the dry matter of soybean organs [%]

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	10.43	4	-2.619	0.007	11.16^a
	C	7	4.57				6.69^b
stipe	S	7	10.43	4	$-2.6.19$	0.007	37.39^a
	C	7	4.57				21.78^b
pod	S	7	6.14	15	-1.214	0.259	16.70^a
	C	7	8.86				18.27^a
seed	S	7	4.43	3	-2.747	0.004	34.22^a
	C	7	10.57				52.98^b

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

Table 3.14: Results of Mann-Whitney U-test, calculated for data on the 1000-seed weight [g]

Variable	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
1000-seed weight	S	7	4.57	4	-2.619	0.007	109.76^a
	C	7	10.53				140.58^b

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

Table 3.15: Correlation coefficients between the heavy metal concentrations in the seeds and the yield determining variables

Smelter

Element	Yield parameters			1000-seed weight
	pods/plant	seeds/plant	dry matter seed	
Pb	-0.390^{ns}	-0.390^{ns}	-0.683^{*}	-0.683^{*}
Zn	-0.333^{ns}	-0.333^{ns}	-0.238^{ns}	-0.333^{ns}

Control

Element	Yield parameters			1000-seed weight
	pods/plant	seeds/plant	dry matter seed	
Pb	-0.142^{ns}	-0.047^{ns}	-0.142^{ns}	0.047^{ns}
Zn	-0.047^{ns}	0.047^{ns}	0.142^{ns}	-0.238^{ns}

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$, $n = 7$.

M-W U-tests were conducted for the organ count ratios (Table 3.12) and the dry matter of soybean organs (Table 3.13). They revealed that the yield determining variables (pods/plant, seeds/plant, dry matter of seeds) were significantly affected at the SMELTER site. Due to the M-W U-test in Table 3.14, a significantly reduced yield at the SMELTER site could be stated.

Based on these findings, a correlation analysis (Table 3.15) was performed to evaluate if the significant higher contents of Pb and Zn in the seeds at the SMELTER site (see Section 3.3.1) were correlated with the adversely affected yield parameters.

The correlation coefficients showed that the contents of Pb and Zn in the seeds at the CONTROL sites seemed to have no influence on the yield parameters ($\tau \leq -0.238$). At the SMELTER site, the content of Pb in the seeds led to a significantly decrease of the seeds dry matter and the 1000-seed weight.

To sum up, the yield parameters were significantly affected by the concentrations of Pb in the seeds at the SMELTER site. The adverse effects were also visible to the eye.

Germination Test

The main aspects to evaluate the results of the Germination Test are the final germination of the seeds, as well as the score of the Maximum Germination Value (MaxGV) and the time it took until it was reached.

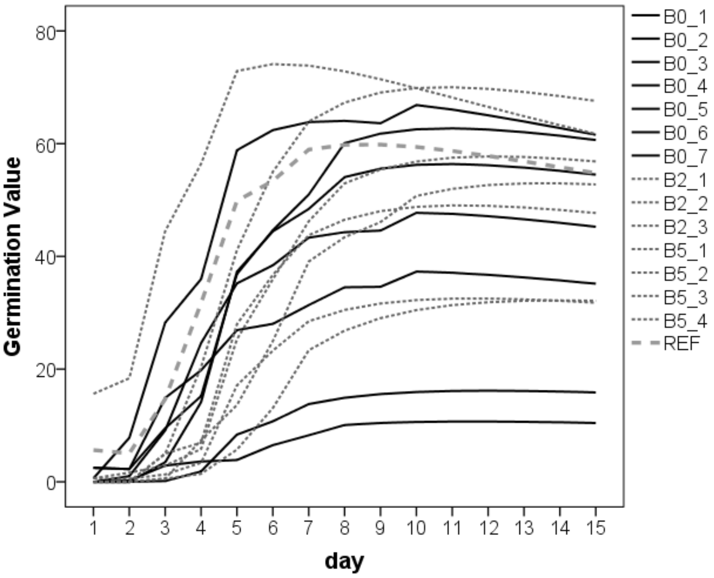

Figure 3.9: Progress of observed germination on the basis of calculated Germination Values according to Djavanshir and Pourbeik (1976)

Table 3.16: Results of the Mann-Whitney U-test, calculated for data on the final germination [%], the maximum Germination Value and the day of maximum Germination Value [n]

Variable	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Final Germination	S	7	6.21	15.50	-1.159	0.259	80.00^a
	C	7	8.79				85.00^a
MaxGV	S	7	6.57	18	-0.831	0.456	42.72^a
	C	7	4.57				52.96^a
Day of MaxGV	S	7	5.86	13	-1.519	0.165	11.00^a
	C	7	9.14				12.00^a

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

The M-W U-test (Table 3.16) confirmed the results shown in Figure 3.9. The progress of germination was not significantly different across sites. This was caused by a high variation within the sites. For both sites, pools with a rapid germination and high MaxGVs (B0-5, B2-1) were observed, as well as slow germination progresses with low MaxGVs (B0-2, B5-4). Nevertheless, a trend is visible. The most favorable germination progresses were found for pools from the CONTROL sites; the most unfavorable ones were found for pools from the SMELTER site. This trend was also reflected by the median percentage of the final germination, which was lower by 5 % on the SMELTER site.

To sum up, the seeds from the SMELTER site showed by trend a lower seed viability, which caused slower germination progresses and lower MaxGVs.

Tetrazolium Test

The Tetrazolium Test provides information about the viability, the vigor and the germination potential as well as about damages and their causes. Figure 3.10 shows some of the seed color patterns derived from the test.

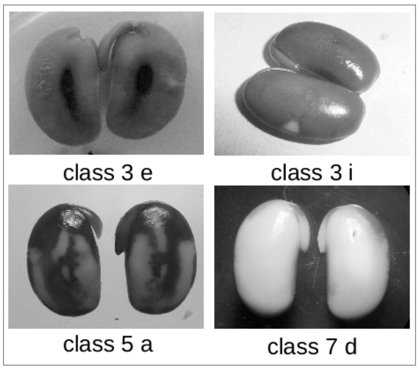

Figure 3.10: Pictures of seed color patterns resulting from the Tetrazolium Test

The viable tissue gets colored in a clear carmine red. A more intense red indicates that the tissue is in process of deterioration. In inviable cell tissue no reaction takes place, making it appear unpigmented in a yellowish white [De França Neto et al., 1998]. Percentages of seeds for the respective classes, representing the quality parameters, were calculated.

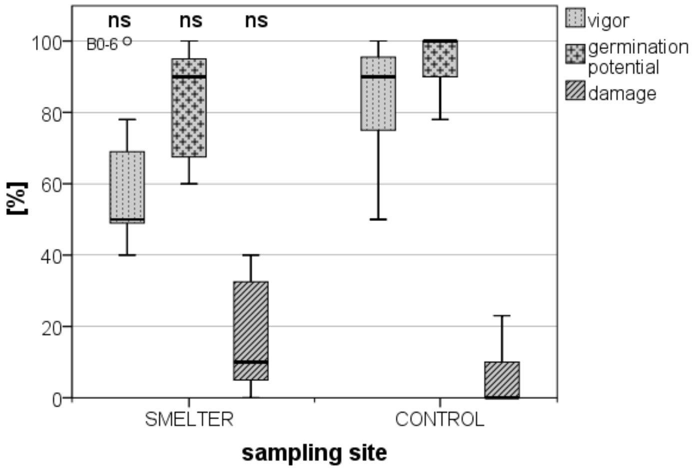

Figure 3.11: Box-and-Whisker Plot for the Tetrazolium quality parameters

Labeling of the Figure follows the description in Figure 3.1

Significant differences between the SMELTER and the CONTROL sites could not be detected (Table 3.17), but the probability value of 0.073 for the medians of 50 % and 90 % vigor indicated that differences exist for this parameter. Although the seeds from the CONTROL sites showed small damages, the vigor and the germination potential were still evaluated to be very high. The viability of the seeds from the SMELTER site was more affected. Damages > 10 % were seen to cause a high quality lost.

Table 3.17: Results of the Mann-Whitney U-test, calculated for data on the Tetrazolium quality parameters [%]

Quality parameter	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
vigor	S	7	5.43	10	-1.869	0.073	50.00^a
	C	7	9.57				90.00^a
germination potential	S	7	6.00	14	-1.413	0.209	90.00^a
	C	7	9.00				100.00^a
damage	S	7	9.00	14	-1.413	0.209	10.00^a
	C	7	6.00				0.00^a

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

A correlation analysis was conducted for the quality parameters and the concentrations of the HMs in the seeds (Table 3.18). It revealed high values for

Pb and Zn with the germination potential and the damages at the Smelter site. Although not significant, the Pb contents in the seeds could have an adverse effect on the germination potential and increased the damages. This finding was in accordance with the results in Section 3.3.2, where the Pb concentrations in the seeds were found to have a negative effect on the seeds dry matter. In contrast, Zn seemed to stimulate the germination potential in a positive way. The Cd concentrations in the seeds were found to be positively correlated with the germination potential and the damage at the Control sites ($\tau = \pm 0.453$).

Table 3.18: Correlation coefficients between the Tetrazolium quality parameters and the concentrations of heavy metals in the seeds

Smelter

Element	vigor	germination potential	damage
Cd	0.052^{ns}	-0.162^{ns}	0.162^{ns}
Pb	0.050^{ns}	-0.410^{ns}	0.410^{*}
Zn	0.195^{ns}	0.450^{ns}	-0.450^{ns}

Control

Element	vigor	germination potential	damage
Cd	-0.216^{ns}	-0.453^{ns}	0.453^{ns}
Pb	0.000^{ns}	-0.116^{ns}	0.116^{ns}
Zn	0.195^{ns}	0.000^{ns}	0.000^{ns}

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$, $n = 7$.

A correlation analysis (Table 3.19) showed that an impairment of the seed's quality was significantly related to a reduction of the 1000-seed weight.

Table 3.19: Correlation coefficients between the 1000-seed weight and the Tetrazolium quality parameters

variable	vigor	germination potential	damage
1000-seed weight	$\mathbf{0.438}^{*}$	$\mathbf{0.486}^{*}$	$\mathbf{-0.486}^{*}$

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$.

To sum up, the Tetrazolium Test showed an impairment of the seed's quality at the Smelter site. The Pb concentrations detected in the seeds were found to contribute to the impairment, which resulted in a lower 1000-seed weight.

3.3.3 Soybean Contamination

Soybean Ionic Impulsion and Pollution Index

Figure 3.12 shows that the combined effect of Cd, Pb and Zn (II_{soy}) affected especially the soybean seeds. The effect on the other organs was in a delimited range (0.009–0.017, except outliers). Significant differences between the sites could be found for the stipes and the seeds ($p = 0.001$) (Table 3.20).

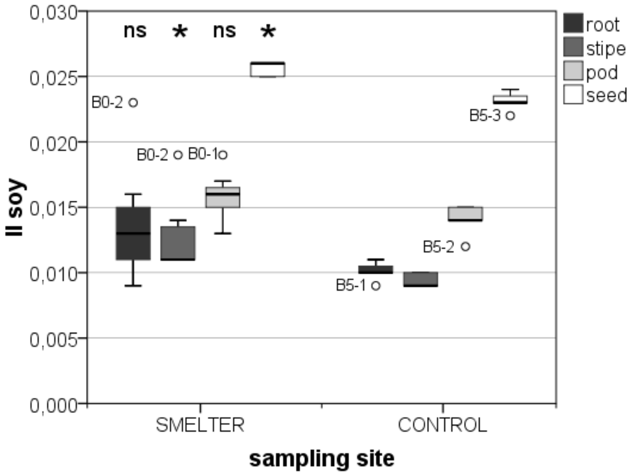

Figure 3.12: Box-and-Whisker Plots for the Soybean Ionic Impulsion

Labeling of the Figure follows the description in Figure 3.1

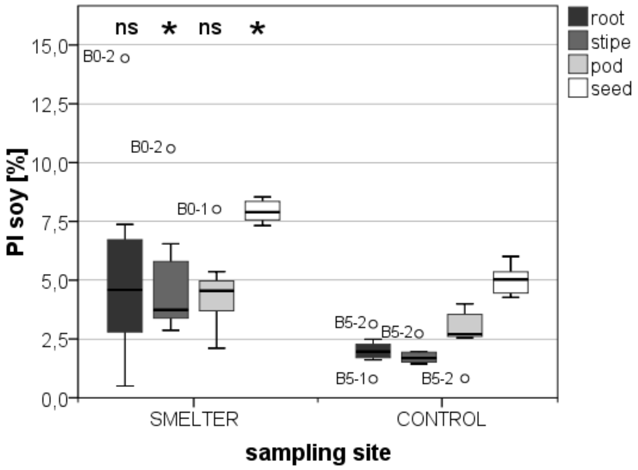

Figure 3.13: Box-and-Whisker Plots of the Soybean Pollution Index

Labeling of the Figure follows the description in Figure 3.1

With the Soybean Pollution Index (PI_{soy}) (Figure 3.13) the contamination of soybean organs resulting from the combined effect of HMs could be estimated. The detected concentrations at the REFERENCE site were set as unpolluted levels (II_{ref}). In reference to Romero et al. [1987], 4 mg/kg Cd, 150 mg/kg Pb and 400 mg/kg Zn were set as toxic levels in the calculations.

Figure 3.13 shows that the combined pollution effect of Cd, Pb and Zn, with respect to the applied toxic limits, was small across all organs and sites ($PI_{soy} < 4\,\%$). The highest index could be observed for the roots and pods. The indices for the Smelter site were in general higher than those for the Control sites. This difference was significant for the stipes and seeds ($p = 0.001$). The negative scores indicate that the pollution is even lower than on the Reference site. In contrast to the II_{soy}, the seeds are the least affected organs.

To sum up, the combined metal pollution impact was significantly higher at the Smelter site. The comparison with the Reference site revealed that the soybean organs of this site were also polluted. Therefore, this site can not provide background values for a comparison with the sampling sites.

Table 3.20: Results of the Mann-Whitney U-test, calculated for data on the Soybean Ionic Impulsion and Pollution Index [%]

Soybean Ionic Impulsion

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	9.5	10.5	−1.834	0.073	0.013^a
	C	7	5.5				0.011^a
stipe	S	7	11	0	−3.216	0.001	0.011^a
	C	7	4				0.009^b
pod	S	7	9.714	9	−2.037	0.053	0.016^a
	C	7	5.286				0.014^a
seed	S	7	11	0	−3.220	0.001	0.026^a
	C	7	4				0.023^b

Soybean Pollution Index

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
root	S	7	9.43	11	−1.725	0.097	0.82^a
	C	7	5.57				-1.92^a
stipe	S	7	11.00	0	−3.130	0.001	-1.30^a
	C	7	4.00				-3.34^b
pod	S	7	9.71	9	−1-981	0.053	1.2^a
	C	7	5.28				-0.71^a
seed	S	7	11.00	0	−3.130	0.001	-0.80^a
	C	7	4.00				-3.92^b

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

Grading of Contamination Levels

Thresholds for foodstuff should prevent health hazards caused by contaminated food. If the thresholds are exceeded, a potential risk of adverse health effects exists.

The mean **Cd** concentrations in the seeds from both sites surpassed the threshold of the *Chinese Food Hygiene Standard* (CFHS) for crops by a factor of 4 and the threshold of the *CODEX STAN 193* of the FAO for legume vegetables by a factor of 2.

Table 3.21: Threshold values for Cd, Pb and Zn concentrations in pulses, soybean and soybean products [mg/kg]

CR EC EU

Element/Foodstuff	Cd	Pb	Zn
cereals, legumes & pulses	—	0.2	—
fats & oils	—	0.1	—
soybean	0.2	—	—

CAA

Element/Foodstuff	Cd	Pb	Zn
foodstuff in general	—	2	100
oils, fats & refined emulsions	—	0.1	—

CGS FAO

Element/Foodstuff	Cd	Pb	Zn
crude & edible vegetable oil	—	0.1	—
legume vegetables	0.1	0.2	—

CFHS

Element/Foodstuff	Cd	Pb	Zn
crops	0.05	0.1	20

with

CR EC EU : Commission Regulation (EC) No 1881/2006, European Union
CAA : Código Alimentario Argentino, Capitulo III , Artículo 156, (Res 1546, 17.9.85)
CGS FAO : Codex General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995)
CFHS : Chinese Food Hygiene Standard [Hao et al., 2011]

Table 3.22: Concentration ranges of heavy metals in the soybean seeds [mg/kg, DW]

Smelter

Element	n	Range	Mean±SD
Cd	3	0.18–0.21	0.20±0.01
Pb	7	1.52–1.75	1.66±0.08
Zn	7	28.88–32.40	30.71±1.30

Control

Element	n	Range	Mean±SD
Cd	7	0.19–0.23	0.21±0.01
Pb	7	1.20–1.41	1.32±0.07
Zn	7	22.42–26.75	24.17±1.67

The **Pb** concentrations in the seeds surpassed all given limits about 13-16 times at both sites. The exceedance was less for the seeds from the Control sites.

Concerning **Zn**, only the threshold value of the CFHS was passed at both sites.

To sum up, the HM concentrations in the seeds – especially Pb – exceeded the applied thresholds. This fact concerned the Smelter site as well as the Control sites.

Health Risks of Heavy Metal Contaminated Soybean Seeds

If the risk indices of the non-cancer Hazard Quotient (ncHQ) and Hazard Index (ncHI) exceed unity, harms to human health through non-cancer effects are possible. The higher the score exceeds unity, the greater is the level of concern. Indices were calculated separately for adults and children because children are seen to be more sensitive to environmental pollution by HMs [Lăcătuşu et al., 1996; US EPA, 2000; Rieuwerts et al., 2000].

Table 3.23: Results of the Mann-Whitney U-test, calculated for data on the non-cancer Hazard Quotient and Hazard Index

non-cancer Hazard Quotient

Element	Group	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	child	S	7	6	14	−1.395	0.209	0.37^a
		C	7	9				0.39^a
	adult	S	7	6	14	−1.395	0.209	0.16^a
		C	7	9				0.17^a
Pb	child	S	7	11	0	−3.148	0.001	0.78^a
		C	7	4				0.61^b
	adult	S	7	11	0	−3.151	0.001	0.33^a
		C	7	4				0.26^b
Zn	child	S	7	11	0	−3.173	0.001	0.19^a
		C	7	4				0.15^b
	adult	S	7	11	0	−3.435	0.001	0.08^a
		C	7	4				0.06^b

non-cancer Hazard Index

Element	Group	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd+Pb+Zn	child	S	7	11	0	−3.130	0.001	1.33^a
		C	7	4				1.13^b
Cd+Pb+Zn	adult	S	7	11	0	−3.148	0.001	0.57^a
		C	7	4				0.48^b

Different letters denote significant differences ($p \leq 0.05$) between the Smelter and the Control sites.

Figure 3.14 shows that the ncHQ for children was in general much higher than for adults. The risk posed by Pb and Zn was significantly higher at the Smelter site than at the Control sites (Table 3.23). The ncHQ did not exceed unity for none of the HMs. The highest quotients could be found for Pb (0.24–0.8) and the lowest ones for Zn (0.06–0.2).

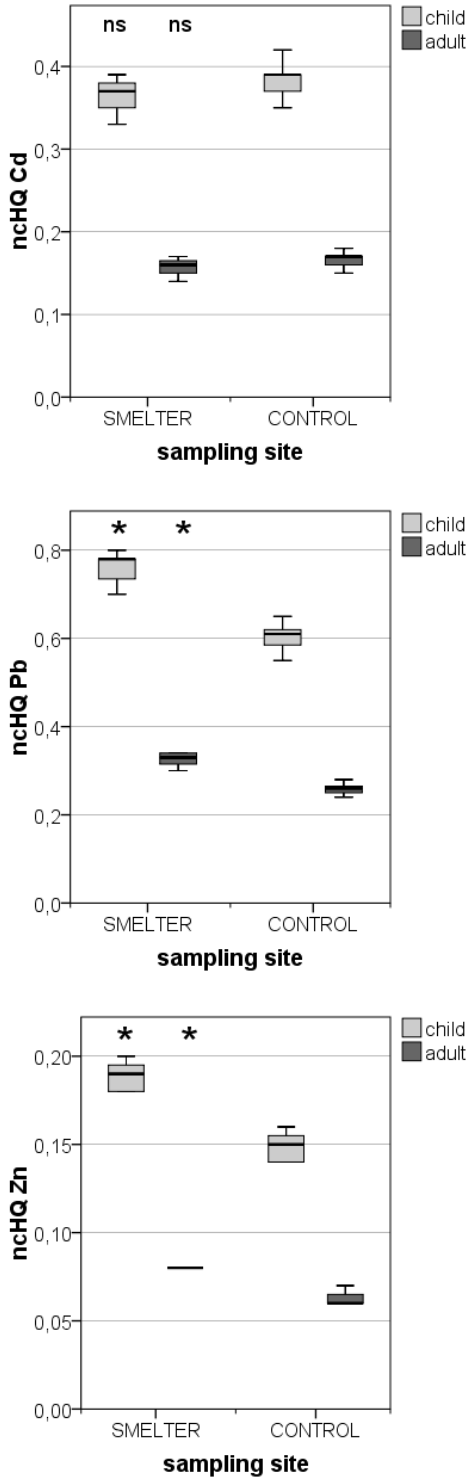

Figure 3.14: Box-and-Whisker Plots of the non-cancer Hazard Quotients of Cd, Pb and Zn for children and adults

Labeling of the Figure follows the description in Figure 3.1

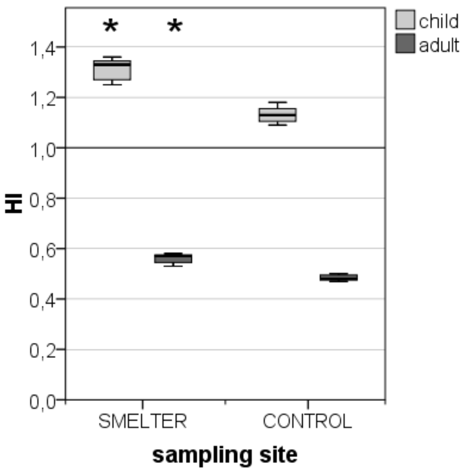

Figure 3.15: Box-and-Whisker Plots of the non-cancer Hazard Index for Cd, Pb and Zn

Labeling of the Figure follows the description in Figure 3.1

The summed risk of all HMs is displayed in Figure 3.15. The ncHI exceeds unity for children at both sites. This risk was significantly lower at the CONTROL sites ($p = 0.001$).

To sum up, a non-cancer health risk is given for children at both sites. This result refers to an estimated daily intake of 55 g contaminated seeds and the combined effect of Cd, Pb and Zn.

3.4 Transfer of Heavy Metals from Soil into Soybean Organs

3.4.1 Curve Model Estimation

The Tables B.5 – B.8 in the appendix show approaches to describe the transfer of HMs from soil to soybean organs with mathematical functions.

The results show that R^2 was < 0.1 for most variable pairs.

Concerning the SMELTER site, the R^2 of Cd needs to be regarded with care because of the small sample size of only three.

A power model seemed best to describe the relationship between Pb in soil and the concentrations found in the roots, stipes and pods. A linear model fitted better for the available fraction in the seeds, but a logarithmic or power model fitted better for the pseudo-total fraction ($R^2_{av} = 0.318$ and $R^2_{pt} = 0.227$).

The relationship between the pseudo-total concentrations of Zn in the soil and those detected in the roots and stipes could be described best with an inverse model. Whereas linear or exponential models fitted best for the relationship of the concentrations in the seeds.

Concerning the Control sites, significant model estimations were found for Pb in the available fraction with the concentrations in the roots ($R^2 = 0.613^*$ (power), $R^2 = 0.707^*$ (exponential)).

Furthermore, good adaptions were achieved for the available fraction of Zn with the concentration in the pods and seeds with an inverse model ($R^2 = 0.466$ and $R^2 = 0.554$, respectively).

The pseudo-total fractions of Cd and Zn are linearly related with their concentration in the seeds ($R^2 = 0.398$ and $R^2 = 0.374$, respectively).

A correlation analysis (Table 3.24) for the same variables confirmed the results.

Table 3.24: Correlation coefficients between the heavy metal concentrations in the soil fractions and soybean organs

Smelter

variable	root	stipe	pod	seed
Cd_{av}	∅	-0.333^{ns}	-0.333^{ns}	-0.333^{ns}
Cd_{pt}	∅	-0.333^{ns}	-0.333^{ns}	-0.333^{ns}
Pb_{av}	$\mathbf{0.619}^{(*)}$	0.333^{ns}	$\mathbf{0.523}^{(*)}$	-0.487^{ns}
Pb_{pt}	$\mathbf{0.714}^{*}$	0.428^{ns}	0.428^{ns}	-0.396^{ns}
Zn_{av}	-0.142^{ns}	-0.333^{ns}	0.238^{ns}	-0.333^{ns}
Zn_{pt}	-0.142^{ns}	-0.333^{ns}	0.238^{ns}	-0.333^{ns}

Control

variable	root	stipe	pod	seed
Cd_{av}	0.050^{ns}	0.050^{ns}	0.102^{ns}	0.052^{ns}
Cd_{pt}	-0.150^{ns}	-0.150^{ns}	$-\mathbf{0.617}^{(*)}$	0.582^{ns}
Pb_{av}	-0.487^{ns}	-0.195^{ns}	0.047^{ns}	-0.142^{ns}
Pb_{pt}	-0.390^{ns}	-0.487^{ns}	0.142^{ns}	-0.047^{ns}
Zn_{av}	0.142^{ns}	0.390^{ns}	$-\mathbf{0.523}^{(*)}$	-0.333^{ns}
Zn_{pt}	0.097^{ns}	-0.150^{ns}	-0.195^{ns}	-0.390^{ns}

Kendall's Tau Correlation, $** = p \leq 0.01$, $* = p \leq 0.05$, $(*) = p \leq 0.1$, $ns = p > 0.1$
$n = 7$, except for Cd at the Smelter site, there $n = 3$; ∅ = no value.

To sum up, the transfer of HMs from contaminated soils to the organs of soybean crops did, in general, not follow simple linear relationships. Although only seven samples per site were applied, correlations were recognizable, especially concerning the Pb concentrations detected at the Smelter site.

3.4.2 Bioconcentration Factor

The calculated Bioconcentration Factors (BCF), which are shown in Figure 3.16 can help to evaluate the incorporation of HMs by plants. Higher quotients reflect a greater potential of the soybean plants to absorb a particular HM, whereas lower quotients suggest a stronger adsorption in soil.

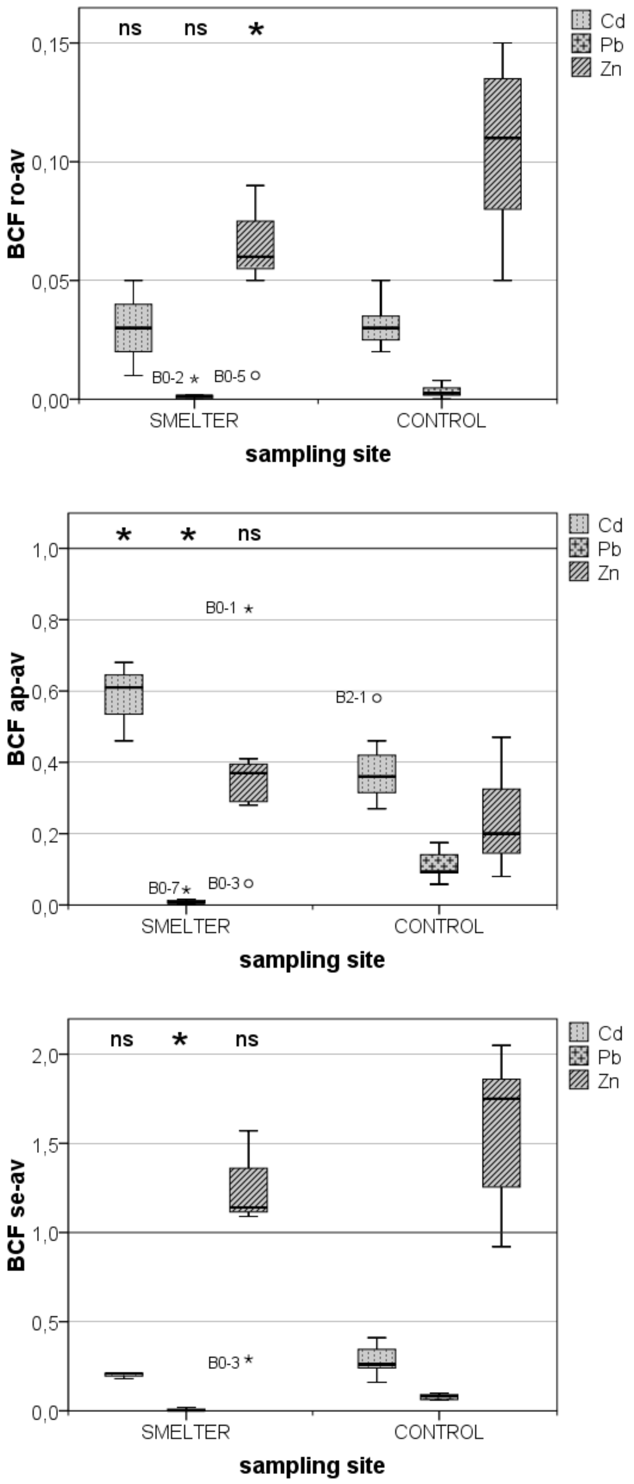

Figure 3.16: Box-and-Whisker Plots for the Bioconcentration Factors for Cd, Pb and Zn

Labeling of the Figure follows the description in Figure 3.1

Table 3.25: Results of the Mann-Whitney U-test, calculated for data on the Bioconcentration Factors of heavy metals

BCF roots – available HM fraction

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	3	5.33	10	−0.118	1.000	0.03^a
	C	7	5.57				0.03^a
Pb	S	7	6.14	15	−1.214	0.259	0.0013^a
	C	7	8.86				0.0025^a
Zn	S	7	5.21	8.5	−2.056	0.038	0.06^a
	C	7	9.79				0.11^b

BCF aerial parts – available HM fraction

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	3	8.50	1.5	−2.058	0.033	0.61^a
	C	7	4.21				0.36^b
Pb	S	7	4.00	0	−3.130	0.001	0.007^a
	C	7	11.00				0.093^b
Zn	S	7	8.71	16	−1.086	0.318	0.37^a
	C	7	6.29				0.20^a

BCF seeds – available HM fraction

Element	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	3	3.00	3	−1.720	0.117	0.21^a
	C	7	6.57				0.26^a
Pb	S	7	4.00	0	−3.130	0.001	0.0017^a
	C	7	11.00				0.082^b
Zn	S	7	5.86	13	−1.473	0.165	1.14^a
	C	7	9.14				1.75^a

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

Cd was mainly accumulated in the aerial parts (stipes and pods) with a median BCF of 0.61 at the SMELTER site. This BCF there was significantly higher than at the CONTROL sites (Table 3.25). In the roots and seeds, only small amounts of Cd were accumulated. The following accumulation sequence is proposed: aerial parts > seeds > roots.

Pb showed the least BCFs across all organs (range: 0.00–0.17). The highest score could be found in the aerial parts at the CONTROL sites (median: 0.093), which differed significantly from the SMELTER site. All considered BCFs were higher at the CONTROL sites, although not significantly for each organ. The following accumulation sequence is proposed: aerial parts > seeds ≈ roots.

A strong accumulation in the seeds with BCF scores > 1 could have been observed for **Zn**. Significant differences were only found for the roots, where the BCF was significantly higher at the CONTROL sites ($p = 0.038$).The following accumulation sequence is proposed: seeds > aerial parts ≥ roots.

To sum up, for each element, the lowest BCFs were obtained for the roots. Lower HM concentrations in the soil caused higher scores of BCF in the roots and seeds. In relation to the detected concentrations in soil, Cd accumulated mainly in the aerial parts, whereas Pb seemed to be strongly retained in soil. Zn was the only HM which showed a potential accumulation in the seeds.

3.4.3 Transfer Factor

The calculated Transfer Factors (TF) in Figure 3.17 show the relative translocation and quantitative accumulation of HMs within the soybean plant for the considered pathway from root to stipes, to pods, to seeds. TF scores above unity suggest a more easy translocation, whereas TF values below unity suggest an accumulation in roots, stipes or pods respectively.

Table 3.26: Results of the Mann-Whitney U-test calculated for data on the Transfer Factors of heavy metals

TF stipes–roots

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	7	4.57	10	-1.855	0.073	6.89^{a}
	C	7	5.43				5.40^{a}
Pb	S	6	3.38	2	-2.562	0.009	2.09^{a}
	C	6	9.17				7.17^{b}
Zn	S	7	10.71	2	-2.875	0.002	1.73^{a}
	C	7	4.29				0.64^{b}

TF pods–stipes

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	7	6.36	16.5	-1.023	0.318	3.73^{a}
	C	7	8.34				4.21^{a}
Pb	S	6	4.57	4	-2.619	0.007	3.59^{a}
	C	6	10.53				6.04^{b}
Zn	S	7	8.29	19	-0.703	0.535	6.15^{a}
	C	7	6.71				5.77^{a}

TF seeds–pods

Organ	Site	n	Mean Rank	M-W U	Z	Exact Sig.	Median
Cd	S	7	4.50	3.50	-2.695	0.004	0.24^{a}
	C	7	10.50				0.66^{b}
Pb	S	6	6.14	15	-1.217	0.259	0.27^{a}
	C	6	8.86				0.58^{a}
Zn	S	7	5.29	9	-1.981	0.053	2.15^{a}
	C	7	9.71				5.14^{a}

Different letters denote significant differences ($p \leq 0.05$) between the SMELTER and the CONTROL sites.

The translocation of **Cd** was highest from the roots into the stipes. The element accumulated in the stipes and pods. From the stipes to the seeds, decreasing TFs were detected.

The TFs for **Pb** differed significantly across the sites for the concentration ratios stipes/roots and pods/stipes ($p = 0.009$ and $p = 0.007$, respectively)

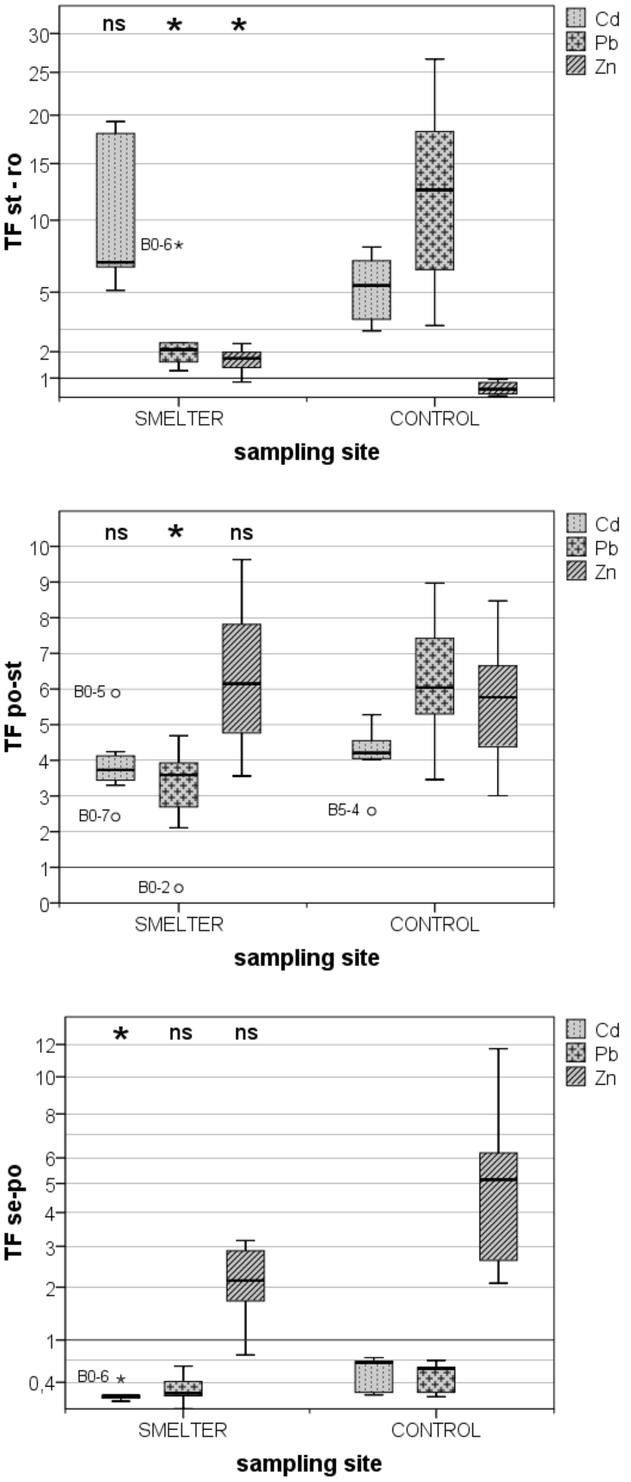

Figure 3.17: Box-and-Whisker Plots of the Transfer Factors for Cd, Pb and Zn

Labeling of the Figure follows the description in Figure 3.1

and were higher at the Control sites. Pb accumulated in the stipes and pods. At both sites, the translocation from the pods into the seeds was below unity. Although not significant, this translocation was higher at the Control sites.

Zn was transferred and accumulated increasingly within the considered pathway from the roots to the seeds, with an extreme enrichment in the latter ones (mean $TF > 2$). This enrichment would have been also significantly higher at the Control sites to a significance level of $\alpha = 6\,\%$ ($p = 0.053$).

To sum up, a transfer from the roots via stipes and pods and into the seeds could be observed for all HMs in question. But only Zn was observed to be easily translocated, resulting in a strong accumulation in the seeds.

Chapter 4

Discussion

The uptake of HMs by plants follows two common pathways: from contaminated soil via the roots and from atmospheric deposition via the foliage. The transfer from the roots into the leaves [Verkleij et al., 2009], as well as the pathway via foliage, followed by a translocation within the plant [Li et al., 2006], are stated to be considerable. In the present study, only the pathway soil → soybean crop → human was considered. The following chapter will discuss the obtained results and relate them with respect to this pathway.

4.1 Heavy Metals in Soil

The results in the Sections 3.2.1 and 3.2.3 revealed that the soils of both sites were enriched in HMs, but this enrichment higher at the SMELTER site.

The EF_{loess} showed a higher enrichment in HMs in comparison to the EF_{crust}. This indicated that mainly the soil – the upper layer of the earth's crust – is directly affected by HM contamination.

Several analyses (see Figures 3.1, 3.4, 3.5 and 3.6) revealed a wide data range at the SMELTER site. According to Salazar [(personal communication), 2011], the concentrations of HMs in the soil at this sampling site already differ a lot within distances below of 2 m. This could be caused by the irregular distribution of slags in the soils and might be a possible explanation for the wide range of the scores.

The PI_{soil} (Figure 3.6) showed that the pollution of the CONTROL sites was even lower than of the REFERENCE site. This difference can be explained by the different geochemical backgrounds (see Section 2.3.1). It should also be considered that the REFERENCE site was represented by one single pool only. Hence, the obtained data was compared with HM concentrations in agricultural loessic soils in Argentina found in Lavado [2006] and Lavado et al. [2007]. The comparison (Table 4.1) shows that only the Pb concentrations at the SMELTER

Table 4.1: Comparison of the heavy metal concentrations of the present study to those of agricultural soils of similar type in Argentina, as reported in the literature [mg/kg, DW]

	present study		Lavado (2006)		Lavado et al. (2007)	
	contaminated	control	contaminated	control	contaminated	control
Cd	0.31±0.11	0.24±0.03	1.25	0.75	1.25± 0.4	0.73±0.23
Pb	294.17±355.64	8.61±1.15	19.06	25	31.77±3.43	19.25±2.68
Zn	35.2±11.62	26.23±2.21	40.33	44.63	54.22±8.87	30.69±11.73

With the following differences of analyzed soil fractions:

Present study: <63 µm fraction; entic Haplustoll; culture: soybean; extraction: aqua regia; contaminated smelter site.

Lavado (2006): <2 mm fraction; typic/vertic Argiustolls and entic Haplustolls; culture: soybean; extraction: aqua regia; contaminated by road traffic, city and farm exhausts.

Lavado et al. (2007): <2 mm fraction; typic Argiustolls; culture: wheat; extraction: aqua regia; contaminated with 30 t biosolids.

site were of exception, whereas the other contamination levels of contaminated and control sites were comparable.

The results in Section 3.2.3 showed that applying different threshold values leads to different results. Nevertheless, the comparison with thresholds of different countries made an official grading of the detected contamination levels possible. The Cd, Pb and Zn concentrations at both sites exceeded the German check values of the *BBodSchV*. This indicated that, with regard to the quality of crops, impairments could be expected by all HMs in question. The concentrations of Pb detected at the Smelter site exceeded most of the considered thresholds. This implies that not only the natural soil functions might be adversely effected, but also a potential health risk for human is given.

Considering individual metals, no significant differences across the sites could be detected for **Cd**. The low enrichment is in accordance with Adriano [1986]. On the base of various investigations, he concluded that soils which have been contaminated by smelting activities show usually Cd concentrations close to background values.

In the available fraction at the Control sites, the Cd concentration was surprisingly higher than in the pseudo-total fraction (see Figure 3.1). First of all, it should be considered that only the soil samples of the Control sites were processed for the present study, whereas other soil data was directly taken over (see Section 2.4). Moreover, the processing of the pseudo-total fraction involved an ashing before the extraction procedure. The soil material was muffled at about 450°C. According to Wahle [1930], the lowest volatilizing temperature for Cd is 429°C. Thus, a partial loss of the metal by volatilizing during the muffling process could have been possible. Another assumption is that Cd could have been introduced by fertilizers or sewage sludge application at the Control sites. This assumption became more feasible with a look at the Enrichment Factors: EF*crust* showed a higher enrichment of Cd at the Control sites than at the Smelter site. Although no reliable information could be obtained about the agricultural practices at the sampling sites, fertilizer application would be an explanation, as it is well known that fertilizers definitely contain Cd.

Pb was highly significant enriched in the soils at the SMELTER site (see Figures 3.1 and 3.4) (range_{av}: 18.23–640.71 mg/kg, range_{pt}: 23.49–1 050.72 mg/kg). This finding was in accordance with other studies, which report even much higher Pb concentrations in the soil around lead smelters: 908–37 300 mg/kg in the Czech Republic [Rieuwerts and Farago, 1996], 34–8 714 mg/kg in Argentina [Fernandez-Turiel et al., 2001], 171–5 906 mg/kg in Spain [Cala and Kunimine, 2003], 37–5 200 mg/kg in New Mexico [Brandvold et al., 1996]. The extremely high EF_{crust} for Pb at the SMELTER site (786.57 ± 827.66) was in coincidence with the study of Bermudez [2011]. He observed an EF_{crust} of 467.73 ± 478.63 for Pb in *Yocsina*, a municipality in the Province of Córdoba, where cement industries are in operation.

Figure 3.1 showed that the concentrations of **Zn** differed most between the fractions. Especially the pseudo-total content was significantly higher at the SMELTER site. This reflects the natural distribution of Zn in soil: A small exchangeable part is bound to soil particles and organic ligands. This fraction is readily available to plants, especially under slightly acid conditions, which were confirmed for the investigated soils (see Section 3.2.2). The larger content is occluded in clay minerals, metal oxides or hydroxides and therefore hardly soluble, but may become mobile over time, upon further acidification [Kabata-Pendias, 2011; Merian et al., 2004].

4.2 Heavy Metals in Soybean Plants

According to Adriano [1986], soybeans are "very sensitive species to the presence of heavy metals in soil", meaning that they potentially absorb higher amounts of HMs than other plants. This was stated by several studies which investigated soybeans amongst other crops, like maize and wheat [Lavado et al., 2001; Lavado, 2006], rice [Li et al., 2008] and cowpea and pepper [Hao et al., 2011].

A comparison with data from other investigations on the uptake of HMs by soybean plants was conducted. Table 4.2 shows, that also Lavado et al. [2001]; Lavado [2006] and De Souza Silva [2006] found considerable concentrations of Cd, Pb and Zn in soybean tissues at contaminated and control sites, especially in the seeds.

Concerning individual organs, the HM concentrations in the **roots** were low in comparison to the other organs (see Figure 3.7). This was in contradiction to the literature, reviewed by Alloway [1999] and Adriano [1986]. They stated that the largest accumulation of HMs in plants takes place in the roots. The stage of growth, when samples were taken, may have had an influence on the low concentrations. It can be assumed as well, that shortly before harvest, the absorption by roots is least and that the majority of substances was already

Table 4.2: Comparison of HM concentrations in different soybean organs reported in the literature and in the present study [mg/kg, DW]

Cd

	present study		Lavado (2006)		De Souza Silva (2006)
organ	contaminated	control	contaminated	control	contaminated
root	0.07±0.04	0.06±0.03	—	—	—
stipe	0.28±0.07	0.23±0.02	0.20	0.18	—
leaves	—	—			0.03–1.50
pods	0.36±0.05	0.39±0.01	—	—	0.95
seeds	0.20±0.01	0.21±0.01	0.11	0.17	0.27–0.89

Pb

	present study		Lavado (2006)		De Souza Silva (2006)
organ	contaminated	control	contaminated	control	contaminated
root	7.06±12.51	0.26±0.32	—	—	—
stipe	4.89±6.91	1.08±0.19	3.44	4.05	—
leaves	—	—			3.14–9.46
pods	2.82±1.32	2.73±0.20	—	—	0.73–9.06
seeds	1.66±0.08	1.32±0.07	0.45	0.2	6.13–19.58

Zn

	present study		Lavado (2006)		De Souza Silva (2006)
organ	contaminated	control	contaminated	control	contaminated
root	4.97±0.75	4.86±0.74	—	—	—
stipe	3.28±0.75	2.15±0.27	24.37	12.89	—
leaves	—	—			55.2–599.58
pods	7.39±3.22	4.91±1.09	—	—	17.82–339.19
seeds	30.71±1.30	24.17±1.67	30.70	33.87	30.02–171.39

With the following differences in the analyses:

Present study: entic Haplustoll; extraction: 1:5 HNO_3:HCl; contaminated smelter site

Lavado (2006): typic/vertic Argiustolls and entic Haplustolls; extraction: perchloric and nitric acid; soil contaminated by road traffic, city and farm exhausts, concentration range see Table 4.1

De Souza Silva (2007): clayic Oxisol; extraction: wet digestion; contaminated with metallic depositions,range of total contents: 20-27 mg/kg Cd, 173-332 mg/kg Pb and 101-553 mg/kg Zn

translocated to the aerial parts. An exception to this observation was found for Pb at the Smelter site, where the concentration indeed was highest in the roots. In reference to Shute and Macfie [2006], it might be, that the high Pb contents in soil affected the root membranes which led to their damage, followed by an uncontrolled absorption of Pb.

The concentrations in the **stipes** were always between the concentrations of roots and pods. Because no exceptional observation was made, the concentrations in this organ will not be discussed.

Figure 3.7 showed that only in **pods** no significant concentration differences across sites could be observed. Furthermore, in most cases, the highest concentrations were detected in this organ. Thus, it was taken into account that also atmospheric deposition can cause considerable HM loads in the aerial parts of plants [Verkleij et al., 2009]. In several articles by the working group around Pignata [Carreras and Pignata, 2002; Carreras et al., 2009; Pignata et al., 2004;

Rodriguez et al., 2011] it is stated that the urban and suburban atmosphere of Córdoba City is polluted with HMs. The hairy surface of the pods could even have enhanced the adsorption of particle matter. Therefore, it was assumed that the absorption of HMs via the pod surface rather contributed to the concentrations in the tissue than the HM concentrations in the soil.

The **seeds** of both sites contained considerable concentrations of HMs (see Figure 3.7). The highest concentrations were found for Zn, which is essential for plants. Lucas (1967, in [Adriano, 1986]) reported a sufficiency range of 20–50 ppm for soybeans and Kloke et al. [1984], a general toxicity limit for plants of 150 ppm. The detected concentrations were in the sufficiency range and therefore not detrimental. Also, the Cd concentrations seemed not to affect the quality of the soybean plants. But the high Pb concentrations in the seeds of the SMELTER site may indeed have been a cause for phytotoxic effects. A stunned growth was visible to the eye (see Figure 3.8). The reduction of the biomass and consequently lower yield (see Tables 3.12 – 3.14) is assumed to have resulted from the inhibition of cell elongation and division by Pb [Verkleij et al., 2009]. The impairment of growth and a reduction in yield of soybean crops caused by Pb, have also be observed by Békésiová et al. [2008] and Hao et al. [2011].

It must be underlined that the results of the Germination Test (see Section 3.3.2) do not reflect the germination process under field conditions [Casini et al., 1997]. Also, the results of the Tetrazolium Test (see Section 3.3.2) can be influenced by parameters, like specie-specific responses to climate or genetic differences, factors which can not be determined by this test [De França Neto et al., 1998]. Nevertheless, both tests stated that the quality of the seeds was significantly affected at the SMELTER site, most likely by the concentrations of Pb in the seeds.

A risk for human health, due to the consumption of HM contaminated edible parts of crops, is already given at much lower concentrations than those that can cause phytotoxic effects [Zheljazkov et al., 2008]. Therefore, the detected HM concentrations in the seeds were compared with threshold values for soybean foodstuff, defined by different countries (see Section 3.3.3). The legislation stands are very general and need to take into account further criteria [Locatelli, 2007]. They do not distinguish well between legumes, pulses and cereals and only a few legislation established thresholds for Cd and Zn. Furthermore, thresholds concerning soybean products in particular do not exist in any of them. Only the *Commission Regulation (EC) No. 1881* of the European Union gives a Cd-threshold for soybean, but does not define, which products are meant. This makes a precise evaluation difficult. Nevertheless, a surpassing of the thresholds was observed for all HMs in question at the SMELTER and the CONTROL sites. Whereas the exceedance for Cd and Zn was only marginal, the concentrations of Pb in the soybean seeds from both sites exceeded all limits by a factor of 13–16.

Based on these results, the non-cancer Hazard Quotients (ncHQ) were calculated (see Section 3.3.3). They revealed that each single HM does not pose a risk of adverse health effects, neither to children nor to adults. Only the combined effect of the HMs may cause a harm to children's health (see Figure 3.15). This risk was significantly higher on the SMELTER site. But it has to be taken into account that the calculations were made on the basis of a chronic daily intake of 55 g pulses [Tripathi et al., 1997]. This estimation might be too high for the diet of a child. Furthermore, "pulses" can also include other legume fruits, like peas and beans. Depending on country, age and habits, the daily intake of pulses can be very different. Additionally, soybean seeds are not consumed in a raw state, but in processed forms, like oil or tofu. The processing could alter the concentrations of HMs in the resulting foodstuff. Thus, an exact evaluation would require detailed information about a specific setting.

4.3 Transfer of Heavy Metals from Soil into Soybean Organs

It was clear from the results in Section 3.4 that an uptake of HMs from soil took place, followed by a translocation within the soybean plant and an accumulation in the different organs. But this transfer could not be satisfactorily described by mathematical functions. The reason was that the concentrations of HMs in the soil fractions were insufficiently correlated with the HM concentrations in the soybean organs (see Section 3.4.1).

In reference to Dudka et al. [1996], relationships usually follow linear, plateau, Freundlich-type or Langmuir sorption models. Moreover, the total HM content of soils is not a good predictor for the uptake of HMs by plants [McBride et al., 1997] and is consequently not a good predictor in risk assessment [Gupta et al., 1996; Chen et al., 2009; Datta and Young, 2005]. The assessment of the "real" phytoavailable HM fraction is difficult, as well. A lot of intents with various extraction solutions were made and applied to different soil-plant-systems, for instance by Chen et al. [1996]; François et al. [2004]; Chojnacka et al. [2005] andChen et al. [2009]. A general agreement about which extractant is the most suitable one, does not exist [Abollino et al., 2006]. Obviously, the properties of soil and plants are very type and species-specific and depend on environmental factors, as well (see Section 1.1). Due to this, a single extraction solution can not reflect all the parameters that influence the site specific availability of HMs. Thus, the "available" fraction is always just an approximation. Furthermore, the soil of the rhizosphere is most important for evaluating the availability of HMs for plants [Youssef, 1997]. But this is normally investigated by pot experiments. The field conditions made it only possible to sample the bulk soil, within a radius of 15 cm, around the main root. Additionally, only small sample sizes were considered and they even contained several outliers (see Table B.3). Apart from the applied extraction solution of 0.5 M HCl for the available fraction, these could be reasons for the poor relationship between the HM concentrations in the soil and the soybean organs.

Still, some of the regressions described the HM uptake by soybean plants. The best fittings were, in general, obtained for Cd and Pb at the SMELTER site and for Pb and Zn at the CONTROL sites. Adriano [1986] suggested that up to a concentration of 4 ppm Cd, a linear correlation can be expected. This was also the case in the present study. De Vries et al. [2007] reported that the often assumed linear relationships do either not exist or that the relationships for toxic metals follow different models. This was evidently for Pb, whose relationship between soil and plant content could be best described by a power model.

"In fact, the relation of PTE [potentially toxic elements, including HMs] between their concentration in soil and their absorption content in plants under field conditions has not been clearly eludicated" [Lavado et al., 2007]. According to this statement, it was observed that, although significantly higher concentrations of Pb and Zn in the soil of the SMELTER site led to significantly higher concentrations of these HMs in the soybean tissue, the calculated Bioconcentration Factors (BCF) indicated a higher accumulation in the roots and seeds for the CONTROL sites (see Figure 3.16). A similar observation was made by Hao et al. [2011], who found higher BCFs for Cd, Pb and Zn in a slightly contaminated soil than in a heavily contaminated soil. Also [Wang et al., 2006] reported of decreasing BCFs with increasing total or DTPA-extractable concentrations of Cd or Pb, and assumed that the accumulation does not solely depend on the soil metal content. These findings suggest a higher availability of HMs in the soils of the CONTROL sites, which may be related to different bio-geochemical properties.

Referring to the results on the Activity Ratio (AR), Pb was the most available HM at the SMELTER site (see Figure 3.2). This indicated that most of the Pb was present in 0.5 M HCl soluble forms. As the correlation analysis in Table 3.2 showed, the high pseudo-total content had an influence on the concentration in the available fraction. This result is in contradiction to what is reported about forms of Pb in soils. There is a common agreement that Pb is the least mobile and available HM in soils [Kabata-Pendias, 2011; Alloway, 1999]. According to Abrahams [2002] and Rieuwerts and Farago [1996], most of the Pb at smelter sites is present as hardly soluble sulfides and phosphates. Additionally, Lavado et al. [2007] stated that Pb is strongly bound in loessic soils when the pH value is > 5.5 – conditions which met the circumstances of the present study (see Section 3.2.2). Nevertheless, Ettler et al. [2005] also found a considerable pool of > 40 % Pb in the available fraction (1 M $MgCl_2$) of heavily contaminated soil at a smelter site . He reported that Pb, originating from smelter fall-out, can be present in forms of chlorides, which are highly soluble. Furthermore, Ettler et al. [2009] also investigated the weathering products of slags and found significant contents of highly soluble lead-copper complexes, like lammerite and olivenite. Ma and Uren [1998] stated that the transformation of anthropogenically introduced HMs into stable forms might take a long time. Therefore, it can be assumed that only a small amount of Pb has undergone a transformation into highly stable forms, since the closure of the smelter. It can be assumed as well, that the steadily leaching of the slags contributes to the large pool of available Pb at the SMELTER site.

Referring to the presented box plots in Figures 3.1 and 3.7, a similar box pattern across the sites – with the exception of Pb in soybean organs – indicated that the principle metal behavior had not been influenced by chemical and biological site-specific soil properties.

Although the physico-chemical soil parameters had not the reported strong influence on the HMs availability (see Section 1.1), the concentrations of Pb were correlated with the content of the OM and the Zn concentrations with the pH value. According to this, the lower pH value and the lower content of OM at the SMELTER site could have forced the availability of HMs. But beside pH value and OM, there are various other parameters which can influence the availability and uptake of HMs (see Section 1.1). It is therefore assumed that either soybean plants may have mechanisms to defend or block an accumulation of HMs, if cultivated in highly contaminated soils, or that other not-determined site specific parameters had a stronger influence on the availability of HMs than the HM concentrations in the soil.

Cd is known for its high mobility in soil [Kabata-Pendias, 2011; Alloway, 1999; Adriano, 1986]. This could be confirmed in this study by its AR > 60 % (see Figure 3.2). It is assumed that the often reported interaction with Zn ([Lambert et al., 2007; Shute and Macfie, 2006]) had an influence on its high availability, which in turn might have caused the considerable accumulation in the roots. Cd and Zn were found to act synergistically (see Figure 3.3). This means that enhanced concentrations of Zn in soil led to a desorption of Cd from the binding sites, which in turn raised the concentration of available Cd in the soil solution and forced the competition of both metal ions at the common root membrane carrier. Because this carrier is recognized to have a higher affinity for Cd, this might have caused the higher uptake [Christensen, 1984; Shute and Macfie, 2006]. The main transfer of Cd took place from the roots to the stipes, where also the highest accumulation was detected. According to Kuboi et al. [1986] legumes are weak accumulators of Cd, but considerable concentrations of Cd in the stipes of soybean plants in relation to the concentrations in soil were also found by Shute and Macfie [2006]; Lavado [2006] and Arao et al. [2003].

Although no high accumulation in any tissue could be observed for **Pb**, a considerable translocation took place from the roots into the stipes and pods ($TF_{st-ro} > 1$ and $TFpo - st > 1$). The barrier of the Casparian strip seemed to not have blocked this transfer. It is notable that this translocation was significantly higher at the CONTROL sites. $TF_{st-ro} > 1$ for Pb and Zn were also found by Rodriguez [2010], who investigated into the uptake of elements by soybeans planted in fly-ash contaminated soil. Masaharu and Ae [2009] found a TF_{st-ro} of 5.6 % for the Enrei Soybean and of 10.1 % for the Suzuyutake Soybean. Thus, the detected TFs in the present study were in a comparable range.

Although the AR of **Zn** was the lowest, this element was easily taken up by the plant and translocated within the tissues.

With regard to potential adverse health effects, which may result from the intake of HM contaminated foodstuff, the BCFs for the seeds are the most important ones to consider. Independent of the investigated site, the strongest accumulation in the seeds was observed for Zn. Similar findings were made by De Souza Silva [2006], who reported extreme Zn accumulation in soybean seeds and concluded that the allocation of Zn is not restricted by the roots of soybean plants. According to the Figures 3.16 and 3.17, neither for Cd nor for Pb, a high transfer from the pods into the seeds or an accumulation in the seeds could be observed. Nevertheless, considerable contents, especially of Pb, could be detected in the seeds.

Finally, the concept of the "soil-plant-barrier", which was introduced by Chaney [1980], seems not to withstand for soybean plants. In this concept, Pb belongs to the group of elements which can be absorbed by the roots and transferred to the stipes, but not in sufficient quantities to cause a risk of transfer to the food chain. The findings of former studies and of the present one showed that Pb *can* be transferred in considerable quantities into the seeds of soybean crops and therefore a risk of transfer into the food chain is given.

Chapter 5

Summary

The detected **Cd** concentrations did not differ significantly across the sites, neither in the soils nor in the soybean organs. The concentrations in soil passed the German check value of the *BBodSchV*, but an adverse effect to the plant's quality could not be detected. A considerable accumulation of Cd could be observed in the aerial parts and the translocation from there into the seeds caused concentrations which slightly passed the thresholds for soybean foodstuff.

The detected concentrations of **Pb** at the Smelter site were exceptional in all the analyses. A significantly higher contamination of soil and soybean organs with Pb for this site was found. The thresholds of most legislations were exceeded several times. In consequence, the natural soil functions might be impaired. Additionally, phytotoxic effects were even visible to the eye and led to a significant decrease in yield. No considerable accumulation took place in any tissue and the main translocation was observed from the roots into the stipes and pods. Nonetheless, Pb was translocated into the seeds, too. At the Smelter site, as well as on the Control sites, these contents surpassed the thresholds for soybean foodstuff several times. Although the concentrations of Pb in the soil and the soybean organs were significantly less at the Control sites, a significantly higher accumulation in all organs was observed. Furthermore, an allocation of considerable amounts into the seeds was even observed at the Control sites. These findings led to two assumptions: First, that soybean plants might possess a blocking mechanism for Pb, if exposed to high concentrations of this metal in soil. Second, the uptake of Pb can not be blocked completely. This remarkable finding was also made by other authors.

For **Zn**, only the pseudo-total content in soil was significantly higher at the Smelter site; amongst the soybean organs this affected the stipes and the seeds. Independent of the site, Zn was found to be readily taken up by the soybean plants and easily translocated within the tissues. This resulted in a strong accumulation in the seeds. With regard to the plant's quality, significant detrimental effects could not be observed. With regard to human health, the thresholds for soybean foodstuff were only passed slightly.

5.1 Conclusion

The present study was based on the hypothesis:

> "High concentrations of heavy metals in soil are transferred and translocated to soybean crops and ultimately accumulate in the seeds with consequences for plant quality and food safety."

It could be fully verified for Pb at the SMELTER site and partly for Cd, Pb and Zn at both sites.

The concentrations of HMs in soil were generally within the world range for unpolluted soil, with the exception of those of Pb at the SMELTER site, where this element was extremely high enriched. A transfer from soil to soybean crops and a translocation within the plant took place for all HMs in question. Although a definite accumulation in the seeds could only be confirmed for Zn, also the concentrations of Cd, and especially Pb, were elevated in the seeds. Only in the case of Pb at the SMELTER site, this caused significant adverse effects on the plant's quality. In terms of food safety, the thresholds for soybean foodstuff were only slightly surpassed by Cd and Zn, but several times exceeded by the Pb concentrations in the seeds. Especially by the combined effect of the HMs, a potential health risk (primarily for children) is given. Surprisingly, this is not only true for the SMELTER site, but also for the CONTROL sites.

The detected concentrations of Cd, Pb and Zn in soil and soybean organs in the municipality of Bouwer, Argentina are seen to result from the overall environmental pollution of this area. The significant contamination with Pb of the SMELTER site is seen to be caused by the former smelter actions and the nowadays remains. The serious affection of this site leads to detrimental effects for all receptors.

5.2 Outlook

This study provided a first overview about the contamination levels of soil and soybean plants in the municipality of Bouwer. Because this contamination was found to pose a significant health risk, especially in the vicinity of the former lead smelter, a good and overall risk assessment will only be achieved by further investigations. A study about the usage of soybeans for the remediation of HMs in soil is already in progress [Salazar, 2011 (personal communication)].

With respect to the detected poor relationships between the HM concentrations in soil and soybean organs, further investigations should cover a higher sample size and could apply other extraction solutions.

The uptake of HMs via foliage has also been identified as an important pathway of contamination [Prasad and Hagemeyer, 1999]. Thus, further studies in this direction are suggested in order to estimate the contribution of atmospheric pollution to the HM concentrations in the plant's organs, especially in the aerial parts.

An analysis of nutrients and enzymatic activity in the plants tissue would add information about the plant's quality.

As the contaminated smelter area is located close to the village of Bouwer, also the direct pathways of HM intake by human via soil particles or air should be investigated.

Finally, an analysis of other HMs like As, Cr, Cu, Hg, and Ni, which could as well be present in high concentrations, due to the former smelting activities [Rieuwerts and Farago, 1996; Stafilov et al., 2010], would also be of interest.

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Appendix A

Laboratory Material and Instruments

Aqua regia	Mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in a volume ratio of 1:3.
Balance	OHAUS, Adventurer AR2140, USA.
Blank	Analytic solution, resulting from the same proceedings and treatments as applied to corresponding sample material, with the difference that no sample material is added.
EC-Meter	*OAKTON*, Economy Conductivity Meter, CON 500 Series.
FAAS	*PerkinElmer*, Model AA3110.
Filter paper	From *Munktell & Filtrak*, Baerenstein, Germany; Grade: 391, Diameter: 110 mm, 84 g/m^2, Lot. 07-168, Art. 3.104.110.
Freeze drier	*RIFICOR*, Liofilizador L-05.
Grinder	Recco, MOC01, Falbella, Argentina.
HCl (20 %)	Hydrogen chloride solution of 20 %, made from HCl (37 %).
HCl (37 %)	Hydrogen chloride solution of 69 %, of quality, from *Panreac Quimica Sau*, Castellar de Vallés, Spain; PA-ACS-ISO, 131020.1612.
HNO_3 (65 %)	Nitric acid solution of 65 %, of quality, from *Merck KgaA*, Darmstadt, Germany; K41875456, 1.00456.2500.
Milli-Q-water	Ultra-pure water of "Type 1" (ISO 3696) [DIN ISO 3696, 1991]. The electrical conductivity of the used Milli-Q-water was 0.0054 S/m.
pH-Meter	*ALTRONIX*, TPX II Microcomputer; pH, mV & °C Meter.
Tetrazolium	Salt of 2,3,5-triphenyl tetrazolium chloride (TCT).
Ultrasonic bath	Lavador Ultrasónico, Testlab; Neocientífica s.a., Córdoba, Argentina.

Appendix B

Tables

Table B.1: Levene's Test for data on the Smelter and the Control sites

Parameters	n per site		Levene's Test d	
Variable	S	C	Sig. based on mean	Sig. based on median
soil organic matter [%]	7	7	0.315	0.341
soil pH value	7	7	0.918	0.719
soil electrical conductivity [μS/cm]	7	7	0.074	0.392
soy dry matter stipe [%]	7	7	0.498	0.473
soy dry matter husk [%]	7	7	0.124	0.202
soy dry matter seed [%]	7	7	0.124	0.202
soy husks/plant [n]	7	7	0.321	0.601
soy seeds/plant [n]	7	7	0.504	0.820
soy seeds/husk [n]	7	7	0.060	0.230
soy 1000 seed weight [g]	7	7	0.267	0.308
soy germination [%]	7	7	0.077	0.194
soy tetrazolium vigor [%]	7	7	0.728	0.885
soy tetrazolium germination potential [%]	7	7	0.011	0.193
soy tetrazolium damage [%]	7	7	0.014	0.207
soil Pb bioavailable fraction [ppm]	7	7	0.010	0.016
soil Pb pseudo-total fraction [ppm]	7	7	0.032	0.060
soy Pb in root [ppm]	7	7	0.040	0.173
soy Pb in stipe [ppm]	7	7	0.044	0.231
soy Pb in husk [ppm]	7	7	0.049	0.128
soy Pb in seed [ppm]	7	7	0.516	0.746
soil Cd bioavailable fraction [ppm]	0	7	—	—
soil Cd pseudo-total fraction [ppm]	0	7	—	—
soy Cd in root [ppm]	7	7	0.189	0.219
soy Cd in stipe [ppm]	7	7	0.034	0.040
soy Cd in husk [ppm]	7	7	0.007	0.112
soy Cd in seed [ppm]	7	7	1.000	1.000
soil Zn bioavailable fraction [ppm]	7	7	0.132	0.460
soil Zn pseudo-total fraction [ppm]	7	7	0.013	0.086
soy Zn in root [ppm]	7	7	0.482	0.524
soy Zn in stipe [ppm]	7	7	0.014	0.020
soy Zn in husk [ppm]	7	7	0.061	0.110
soy Zn in seed [ppm]	7	7	0.460	0.504

Table B.2: Descriptive Parameters: Smelter–Control

Parameter	n		Range (Min–Max)		Mean±SD		Std. error Mean		Median		MAD		Variance	
Variable	S	C	S	C	S	C	S	C	S	C	S	C	S	C
soil organic matter [%]	7	7	4.75–5.98	4.36–6.70	5.28±0.41	5.66±0.75	0.16	0.28	5.19	5.74	0.44	0.55	0.17	0.56
soil pH value	7	7	6.36–6.92	6.51–7.08	6.59±0.19	6.82±0.20	0.07	0.07	6.52	6.84	0.23	0.19	0.04	0.04
soil electrical conductivity [μS/cm]	7	7	46.60–404.67	72.70–117.45	114.38±128.52	89.90±17.36	48.58	6.56	68.50	83.15	11.10	13.65	16517.82	301.43
soy dry matter root [%]	7	7	7.98–14.47	6.42–9.82	11.24±2.56	7.52±1.38	0.96	0.52	11.16	6.69	3.18	2.29	6.57	7.91
soy dry matter stipe [%]	7	7	24.69–44.36	18.90–31.81	34.07±7.87	23.15±4.33	2.97	1.63	37.39	21.78	11.47	4.14	62.07	18.81
soy dry matter pod [%]	7	7	12.19–19.32	14.31–21.16	16.17±2.74	18.23±2.09	1.03	0.79	16.70	18.27	1.97	1.05	7.53	4.38
soy dry matter seed [%]	7	7	29.47–48.74	44.81–55.41	38.50±7.79	51.08±3.69	2.94	1.39	34.22	52.98	12.57	6.19	60.72	13.63
soy pods/plant [n]	7	7	9.17–32.65	22.50–64.82	20.24±9.52	39.58±14.51	3.60	5.49	18.95	36.04	10.44	13.54	90.62	210.62
soy seeds/plant [n]	7	7	21.17–77.00	49.71–143.53	43.09±21.40	84.38±30.69	8.09	11.60	42.32	75.21	21.22	25.50	458.06	941.88
soy seeds/husk [n]	7	7	1.63–2.36	1.96–2.24	2.13±0.26	2.14±0.10	0.10	0.04	2.23	2.14	0.09	0.09	0.07	0.01
soy 1000 seed weight [g]	7	7	80.72–136.38	122.63–153.15	112.21±18.42	139.61±10.83	6.96	4.09	109.76	140.58	26.62	12.04	339.45	117.29
soy germination [%]	7	7	40.00–95.00	70.00–100.00	73.57±21.35	86.43±11.07	8.07	4.19	80.00	85.00	10.00	10.00	455.95	122.62
soy tetrazolium vigor [%]	7	7	40.00–100.00	50.00–100.00	60.86±21.07	83.00±18.06	7.96	6.83	50.00	90.00	28.00	12.00	443.81	326.33
soy tetrazolium germination potential [%]	7	7	60.00–100.00	78.00–100.00	82.14±16.80	94.00±8.49	6.35	3.21	90.00	100.00	10.00	10.00	282.14	72.00
soy tetrazolium damage [%]	7	7	0.00–40.00	0.00–23.00	17.86±16.80	6.14±8.80	6.35	3.33	10.00	0.00	10.00	10.00	282.14	77.48
soil Cd water-extractable fraction [ppm]	0	6	—	0.00–0.00	—	0.00±0.00	—	0.00	—	0.00	—	0.00	—	0.00
soil Cd available fraction [ppm]	3	7	0.19–0.22	0.20–0.31	0.20±0.02	0.26±0.04	0.01	0.02	0.20	0.24	0.09	0.05	0.00	0.00
soil Cd pseudo-total fraction [ppm]	3	7	0.24–0.44	0.19–0.28	0.31±0.11	0.24±0.03	0.07	0.01	0.25	0.24	0.03	0.03	0.01	0.00
soy Cd root [ppm]	7	7	0.03–0.15	0.00–0.10	0.07±0.04	0.06±0.03	0.01	0.01	0.07	0.07	0.02	0.02	0.00	0.00
soy Cd stipe [ppm]	7	7	0.19–0.39	0.21–0.26	0.28±0.07	0.23±0.02	0.03	0.01	0.28	0.23	0.05	0.02	0.00	0.00
soy Cd pod [ppm]	7	7	0.27–0.41	0.38–0.42	0.36±0.05	0.39±0.01	0.02	0.01	0.39	0.39	0.01	0.01	0.00	0.00
soy Cd seed [ppm]	7	7	0.18–0.21	0.19–0.23	0.20±0.01	0.21±0.01	0.00	0.00	0.20	0.21	0.01	0.01	0.00	0.00
soil Pb water-extractable fraction [ppm]	0	6	—	0.00–0.00	—	0.00±0.00	—	0.00	—	0.00	—	0.00	—	0.00
soil Pb available fraction [ppm]	7	7	18.23–640.71	4.01–9.07	220.20±214.98	5.85±1.54	81.26	0.58	193.34	5.64	187.58	3.43	46217.67	2.38
soil Pb pseudo-total fraction [ppm]	7	7	23.49–1050.72	6.85–9.93	294.17±355.64	8.61±1.15	134.42	0.44	235.85	8.63	225.92	1.78	126477.40	1.33
soy Pb root [ppm]	7	7	0.00–34.97	0.01–1.00	7.06±12.51	0.26±0.32	4.73	0.11	2.03	0.15	1.88	0.16	156.56	0.10
soy Pb stipe [ppm]	7	7	1.12–20.33	0.86–1.42	4.89±6.91	1.08±0.19	2.61	0.07	1.90	1.03	0.86	0.39	47.80	0.03
soy Pb pod [ppm]	7	7	1.50–5.54	2.51–3.06	2.82±1.32	2.73±0.20	0.50	0.08	2.35	2.74	0.40	0.32	1.75	0.04
soy Pb seed [ppm]	7	7	1.52–1.75	1.20–1.41	1.66±0.08	1.32±0.07	0.03	0.03	1.70	1.33	0.29	0.19	0.01	0.00
soil Zn water-extractable fraction [ppm]	0	6	—	0.00–0.02	—	0.08±0.01	—	0.00	—	0.01	—	0.01	—	0.00
soil Zn available fraction [ppm]	7	7	4.40–26.30	3.50–8.80	8.50±7.91	5.61±1.97	2.99	0.74	5.20	5.00	1.30	1.40	62.54	3.88
soil Zn pseudo-total fraction [ppm]	7	7	20.00–51.50	23.90–29.20	35.20±11.62	26.23±2.21	4.39	0.84	30.55	26.60	3.95	2.70	135.06	4.89
soy Zn root [ppm]	7	7	4.04–5.96	4.17–6.38	4.97±0.75	4.86±0.74	0.28	0.28	5.10	4.80	0.74	0.55	0.57	0.55
soy Zn stipe [ppm]	7	7	2.35–4.36	1.83–2.53	3.28±0.75	2.15±0.27	0.28	0.10	3.22	2.10	0.87	0.43	0.56	0.07
soy Zn pod [ppm]	7	7	4.47–13.55	2.85–6.40	7.39±3.22	4.91±1.09	1.22	0.41	6.84	4.86	1.98	0.58	10.34	1.19
soy Zn seed [ppm]	7	7	28.88–32.40	22.42–26.75	30.71±1.30	24.17±1.67	0.49	0.63	30.81	23.99	4.06	4.89	1.70	2.80
soil Fe available fraction [ppm]	7	7	389.20–897.60	165.90–284.80	629.83±182.00	203.46±43.16	68.79	16.31	596.20	201.10	311.40	188.10	33123.71	1862.51
soil Fe pseudo-total fraction [ppm]	7	7	8725.80–18795.80	0.00–2392.10	11178.04±3615.51	1466.99±662.18	1366.53	234.12	9368.30	1565.50	7734.20	1565.50	13071917.64	438487.24

Table B.3: Standardized values (z-scores) to detect outliers

Pool	B0-1	B0-2	B0-3	B0-4	B0-5	B0-6	B0-7	B2-1	B2-2	B2-3	B5-1	B5-2	B5-3	B5-4	REF
soil organic matter [%]	−0.35	−0.15	0.10	−0.32	−0.66	0.78	0.33	1.62	0.05	−1.12	0.53	0.50	0.11	1.17	−2.61
soil pH value	−1.64	−0.94	0.96	0.22	−0.80	−0.94	−0.90	0.17	−0.15	−0.94	0.69	0.59	1.71	1.48	0.50
soil electrical conductivity [μS/cm]	−0.62	−0.48	3.54	−0.18	−0.37	−0.39	−0.34	−0.32	−0.31	−0.24	−0.04	−0.20	0.07	0.20	−0.31
soy dry matter stipe [%]	0.50	0.62	0.72	0.43	−1.68	0.97	0.86	−0.14	0.19	0.87	−1.21	0.28	−2.28	−0.60	0.48
soy dry matter husk [%]	−0.95	0.34	−0.14	−0.63	−2.72	0.46	0.77	0.31	1.10	1.26	0.08	0.13	0.72	0.20	−0.92
soy dry matter seed [%]	−0.95	0.34	−0.14	−0.63	−2.72	0.46	0.77	0.31	1.10	1.26	0.08	0.13	0.72	0.20	−0.92
soy pods/plant [n]	−0.82	0.20	−0.71	−1.30	0.21	−0.57	−1.36	0.37	−0.01	−0.47	1.58	2.35	0.44	0.43	−0.34
soy seeds/plant [n]	−1.08	0.02	−0.64	−1.28	0.44	−0.47	−1.29	0.38	0.09	−0.41	1.28	2.50	0.39	0.44	−0.37
soy seeds/husk [n]	−2.77	−1.00	0.55	0.16	1.27	0.77	0.99	0.05	0.60	0.44	−0.95	0.44	−0.23	0.05	−0.34
soy 1000 seed weight [g]	−2.31	0.47	−0.39	−0.86	0.10	−1.29	−0.88	0.08	0.69	−0.21	1.32	0.81	1.13	0.65	0.70
soy germination [%]	−1.78	−2.36	0.86	−0.02	0.57	0.57	−0.61	0.27	0.86	−0.61	1.15	0.86	0.27	−0.31	0.27
soy tetrazolium vigor [%]	−1.15	−0.61	0.19	−1.06	−1.50	1.17	−1.06	−0.17	1.17	−1.06	1.17	0.77	0.28	0.72	1.17
soy tetrazolium germination potential [%]	−1.70	−2.06	0.08	0.08	0.79	0.79	−1.35	0.08	0.79	−0.78	0.79	0.79	0.79	0.08	0.79
soy tetrazolium damage [%]	1.69	2.05	−0.09	−0.09	−0.80	−0.80	1.34	−0.09	−0.80	0.84	−0.80	−0.80	−0.80	−0.09	−0.80
soil Cd available fraction [ppm]	−0.49	−0.14	—	—	−0.67	—	—	−0.49	0.21	1.08	−0.14	0.03	1.43	1.25	−2.06
soil Cd pseudo-total fraction [ppm]	−0.15	2.77	—	—	−0.31	—	—	−1.08	−0.46	0.31	0.00	−0.77	−0.31	−0.15	0.15
soy Cd root [ppm]	0.69	2.45	−0.79	0.39	−1.08	−0.20	0.10	0.10	−0.49	0.10	−0.49	0.98	−0.20	0.39	−1.96
soy Cd stipe [ppm]	−1.16	0.52	1.27	0.71	−0.79	−0.04	2.58	−0.41	−0.79	−0.41	−0.79	0.15	−0.60	−0.23	—
soy Cd pod [ppm]	−2.70	0.78	0.28	0.53	0.53	−1.46	−0.71	0.53	1.03	0.04	0.04	0.28	0.53	0.28	—
soy Cd seed [ppm]	−0.97	0.63	−0.97	−1.77	−0.17	−0.17	0.63	−0.17	−0.97	2.23	0.63	0.63	−0.17	0.63	—
soil Pb available fraction [ppm]	0.49	0.95	2.99	−0.10	1.03	−0.38	−0.49	−0.57	−0.56	−0.56	−0.54	−0.56	−0.56	−0.56	−0.57
soil Pb pseudo-total fraction [ppm]	0.34	0.75	3.30	−0.19	0.47	−0.37	−0.43	−0.49	−0.48	−0.48	−0.48	−0.48	−0.49	−0.49	−0.48
soy Pb root [ppm]	0.04	3.54	−0.16	−0.20	0.36	−0.34	−0.38	−0.37	−0.37	−0.35	−0.38	−0.38	−0.37	−0.36	−0.27
soy Pb stipe [ppm]	−0.04	3.40	−0.22	−0.37	0.35	−0.21	−0.29	−0.36	−0.42	−0.38	−0.40	−0.31	−0.38	−0.35	—
soy Pb pod [ppm]	−0.52	0.08	0.51	−1.40	3.03	−0.87	−0.47	−0.27	0.16	−0.29	−0.04	−0.03	0.31	−0.18	—
soy Pb seed [ppm]	1.37	1.11	0.48	0.79	0.17	1.16	1.16	−1.13	−1.02	−0.82	−0.76	−1.49	−0.61	−0.40	—
soil Zn available fraction [ppm]	−0.34	−0.24	3.48	0.05	−0.43	−0.06	−0.36	−0.57	−0.59	−0.34	0.35	0.12	−0.15	−0.32	−0.59
soil Zn pseudo-total fraction [ppm]	0.33	2.04	2.16	−0.26	−1.22	−0.05	−0.13	−0.78	−0.78	−0.23	−0.80	−0.44	−0.32	−0.51	0.99
soy Zn root [ppm]	0.86	0.25	−0.78	0.89	1.45	−0.92	−1.22	−0.11	−0.49	−1.04	−0.93	2.03	−0.17	0.17	—
soy Zn stipe [ppm]	1.58	0.63	0.12	1.18	2.06	−0.46	−0.15	−1.11	−0.28	−1.04	−0.76	−0.23	−0.77	−0.77	—
soy Zn pod [ppm]	2.80	0.26	−0.39	1.27	−0.42	0.41	−0.64	0.09	−0.27	−0.49	−0.53	−1.25	−0.27	−0.57	—
soy Zn seed [ppm]	0.59	0.39	0.91	1.35	0.69	1.28	1.00	−0.19	−0.42	−1.27	−1.26	−0.78	−1.36	−0.93	—

|value| ≥ 3.29 > |value| ≥ 2.58 > |value| ≥ 1.96

Table B.4: Mann-Whitney U-test for data on the Control sites B2 and B5

Variable	Site	n	Mean Rank	M-W U	Z	Exact Sig.
soil organic matter [%]	B2	3	3.33	4.00	−0.71	0.629
	B5	4	4.50			
soil pH value	B2	3	2.00	0.00	−2.12	0.057
	B5	4	5.50			
soil electrical conductivity [μS/cm]	B2	3	2.00	0.00	−2.12	0.057
	B5	4	5.50			
soy dry matter stipe [%]	B2	3	5.33	2.00	−1.41	0.229
	B5	4	3.00			
soy dry matter husk [%]	B2	3	5.67	1.00	−1.77	0.114
	B5	4	2.75			
soy dry matter seed [%]	B2	3	5.67	1.00	−1.77	0.114
	B5	4	2.75			
soy husks/plant [n]	B2	3	2.00	0.00	−2.12	0.057
	B5	4	5.50			
soy seeds/plant [n]	B2	3	2.00	0.00	−2.12	0.057
	B5	4	5.50			
soy seeds/husk [n]	B2	3	5.33	2.00	−1.44	0.229
	B5	4	3.00			
soy 1000 seed weight [g]	B2	3	2.33	1.00	−1.77	0.114
	B5	4	5.25			
soy germination [%]	B2	3	3.33	4.00	−0.72	0.629
	B5	4	4.50			
soy tetrazolium vigor [%]	B2	3	3.17	3.50	−0.89	0.400
	B5	4	4.62			
soy tetrazolium germination potential [%]	B2	3	3.00	3.00	−1.18	0.400
	B5	4	4.75			
soy tetrazolium damage [%]	B2	3	5.00	3.00	−1.18	0.400
	B5	4	3.25			
soil Cd bioavailable fraction [ppm]	B2	3	3.33	4.00	−0.71	0.629
	B5	4	4.50			
soil Cd pseudo-total fraction [ppm]	B2	3	3.67	5.00	−0.35	0.857
	B5	4	4.25			
soy Cd in root [ppm]	B2	3	3.50	4.50	−0.54	0.629
	B5	4	4.38			
soy Cd in stipe [ppm]	B2	3	3.50	4.50	−0.54	0.629
	B5	4	4.38			
soy Cd in husk [ppm]	B2	3	4.67	4.00	−0.73	0.629
	B5	4	3.50			
soy Cd in seed [ppm]	B2	3	3.50	4.50	−0.56	0.629
	B5	4	4.38			
soil Pb bioavailable fraction [ppm]	B2	3	3.33	4.00	−0.71	0.629
	B5	4	4.50			
soil Pb pseudo-total fraction [ppm]	B2	3	4.00	6.00	0.00	1.000
	B5	4	4.00			
soy Pb in root [ppm]	B2	3	4.83	3.50	−0.89	0.400
	B5	4	3.38			
soy Pb in stipe [ppm]	B2	3	3.17	3.50	−0.89	0.400
	B5	4	4.62			
soy Pb in husk [ppm]	B2	3	3.00	3.00	−1.06	0.400
	B5	4	4.75			
soy Pb in seed [ppm]	B2	3	3.00	3.00	−1.06	0.400
	B5	4	4.75			
soil Zn bioavailable fraction [ppm]	B2	3	2.00	0.00	−2.12	0.057
	B5	4	5.50			
soy Zn in root [ppm]	B2	3	4.00	3.00	−1.06	0.400
	B5	4	4.00			
soy Zn in stipe [ppm]	B2	3	3.00	3.00	−1.07	0.400
	B5	4	4.75			
soy Zn in husk [ppm]	B2	3	3.00	1.00	−1.77	0.114
	B5	4	4.75			
soy Zn in seed [ppm]	B2	3	5.67	3.00	−1.06	0.400
	B5	4	2.75			

Table B.5: Curve estimations for the available fraction of heavy metals in soil at the Smelter site

Dep.		Root			Stipe			Pod			Seed	
Param.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.
Cd												
Lin	**0.96**	27.00	0.12	**0.73**	2.70	0.35	0.06	0.07	0.84	0.43	0.75	0.55
Log	**0.97**	33.28	0.11	**0.71**	2.49	0.36	0.05	0.06	0.85	0.41	0.70	0.56
Inv	**0.98**	42.19	0.10	0.70	2.29	0.37	0.05	0.05	0.86	0.39	0.64	0.57
Pow	0.86	6.29	0.24	0.68	2.11	0.38	0.05	0.05	0.86	0.40	0.66	0.57
Exp	0.85	5.66	0.25	0.70	2.28	0.37	0.06	0.06	0.85	0.41	0.71	0.55
Pb												
Lin	0.01	0.03	0.88	0.02	0.12	0.74	0.22	1.42	0.29	0.32	2.33	0.19
Log	0.09	0.38	0.57	0.12	0.65	0.46	0.27	1.82	0.24	0.27	1.89	0.23
Inv	0.13	0.60	0.48	0.11	0.63	0.46	0.13	0.76	0.42	0.17	1.04	0.35
Pow	0.38	2.42	0.20	0.21	1.29	0.31	0.31	2.21	0.20	0.28	1.91	0.23
Exp	0.08	0.36	0.58	0.05	0.29	0.61	0.31	2.21	0.20	0.32	2.32	0.19
Zn												
Lin	0.14	0.81	0.41	0.10	0.56	0.49	0.08	0.44	0.54	0.01	0.07	0.80
Log	0.15	0.89	0.39	0.14	0.79	0.41	0.06	0.30	0.61	0.05	0.25	0.64
Inv	0.16	0.98	0.37	0.19	1.18	0.33	0.03	0.13	0.73	0.12	0.66	0.45
Pow	0.14	0.81	0.41	0.12	0.66	0.45	0.05	0.26	0.63	0.05	0.26	0.63
Exp	0.13	0.75	0.43	0.09	0.47	0.52	0.08	0.45	0.53	0.02	0.08	0.79

Table B.6: Curve estimations for the pseudo-total fraction of heavy metals in soil at the Smelter site

Dep.		Root			Stipe			Pod			Seed	
Param.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.
Cd												
Lin	**0.79**	3.70	0.31	**0.94**	14.41	0.16	0.27	0.36	0.65	**0.71**	2.46	0.36
Log	**0.80**	4.01	0.29	**0.93**	12.71	0.17	0.25	0.34	0.66	**0.70**	2.29	0.37
Inv	**0.82**	4.42	0.28	**0.92**	11.01	0.19	0.24	0.31	0.68	0.68	2.11	0.38
Pow	0.61	1.59	0.43	0.91	9.65	0.20	0.24	0.32	0.67	0.68	2.15	0.38
Exp	0.60	1.49	0.44	0.92	10.78	0.19	0.26	0.35	0.66	**0.70**	2.31	0.37
Pb												
Lin	0.00	0.00	0.97	0.01	0.03	0.87	0.10	0.56	0.49	0.22	1.39	0.29
Log	0.08	0.35	0.59	0.11	0.63	0.46	0.21	1.36	0.30	0.23	1.46	0.28
Inv	0.14	0.65	0.46	0.13	0.73	0.43	0.15	0.88	0.39	0.17	1.04	0.35
Pow	0.31	1.84	0.25	0.18	1.13	0.34	0.27	1.88	0.23	0.23	1.47	0.28
Exp	0.03	0.10	0.76	0.01	0.07	0.80	0.17	1.05	0.35	0.21	1.37	0.30
Zn												
Lin	0.14	0.79	0.41	0.15	0.92	0.38	0.00	0.02	0.91	0.13	0.77	0.42
Log	0.18	1.10	0.34	0.21	1.30	0.31	0.00	0.00	0.95	0.09	0.49	0.52
Inv	0.23	1.49	0.28	0.27	1.81	0.24	0.01	0.07	0.80	0.04	0.22	0.66
Pow	0.15	0.89	0.39	0.16	0.92	0.38	0.00	0.02	0.91	0.09	0.50	0.51
Exp	0.11	0.63	0.46	0.11	0.63	0.46	0.00	0.00	0.95	0.14	0.79	0.42

Table B.7: Curve estimations for the available fraction of heavy metals in soil at the CONTROL sites

Dep.		Root			Stipe			Pod			Seed	
Param.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.
						Cd						
Lin	0.00	0.01	0.94	0.00	0.01	0.94	0.02	0.08	0.78	0.10	0.53	0.50
Log	0.00	0.01	0.94	0.00	0.01	0.94	0.02	0.08	0.79	0.10	0.56	0.49
Inv	0.00	0.01	0.94	0.00	0.01	0.94	0.02	0.08	0.79	0.10	0.57	0.48
Pow	0.01	0.04	0.86	0.00	0.01	0.91	0.02	0.08	0.79	0.10	0.53	0.50
Exp	0.01	0.04	0.85	0.00	0.01	0.92	0.02	0.08	0.79	0.09	0.51	0.51
						Pb						
Lin	0.29	2.06	0.21	0.12	0.65	0.46	0.03	0.14	0.72	0.03	0.14	0.72
Log	0.24	1.59	0.26	0.10	0.57	0.49	0.05	0.25	0.64	0.03	0.16	0.70
Inv	0.18	1.08	0.35	0.08	0.46	0.53	0.08	0.41	0.55	0.04	0.19	0.68
Pow	**0.61**	7.92	**0.04**	0.12	0.69	0.44	0.06	0.30	0.61	0.03	0.17	0.70
Exp	**0.71**	12.04	**0.02**	0.13	0.77	0.42	0.03	0.17	0.70	0.03	0.15	0.72
						Zn						
Lin	0.05	0.26	0.63	0.05	0.26	0.63	0.40	3.35	0.13	0.37	2.99	0.14
Log	0.06	0.33	0.59	0.04	0.20	0.67	0.44	4.01	0.10	0.47	4.35	0.09
Inv	0.07	0.36	0.58	0.03	0.13	0.73	0.47	4.37	0.09	0.55	6.20	0.06
Pow	0.04	0.23	0.65	0.05	0.26	0.63	0.40	3.32	0.13	0.46	4.21	0.10
Exp	0.03	0.17	0.69	0.06	0.32	0.60	0.36	2.86	0.15	0.37	2.91	0.15

Table B.8: Curve estimations for the pseudo-total fraction of heavy metals in soil at the CONTROL sites

Dep.		Root			Stipe			Pod			Seed	
Param.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.	R^2	F	Sig.
						Cd						
Lin	0.10	0.56	0.49	0.10	0.56	0.49	0.27	1.81	0.24	0.40	3.31	0.13
Log	0.11	0.59	0.48	0.11	0.59	0.48	0.24	1.57	0.27	0.36	2.75	0.16
Inv	0.11	0.61	0.47	0.11	0.61	0.47	0.21	1.34	0.30	0.31	2.29	0.19
Pow	0.10	0.53	0.50	0.10	0.57	0.48	0.25	1.65	0.26	0.34	2.63	0.17
Exp	0.09	0.49	0.52	0.10	0.54	0.50	0.28	1.91	0.23	0.39	3.15	0.14
						Pb						
Lin	0.06	0.33	0.59	0.26	1.72	0.25	0.06	0.35	0.58	0.00	0.02	0.90
Log	0.06	0.30	0.61	0.23	1.50	0.27	0.08	0.42	0.55	0.00	0.01	0.91
Inv	0.05	0.28	0.62	0.21	1.30	0.31	0.09	0.49	0.51	0.00	0.01	0.92
Pow	0.20	1.25	0.31	0.28	1.94	0.22	0.08	0.45	0.53	0.00	0.01	0.92
Exp	0.21	1.36	0.30	0.31	2.20	0.20	0.07	0.38	0.57	0.00	0.01	0.92
						Zn						
Lin	0.03	0.14	0.73	0.02	0.08	0.79	0.13	0.75	0.42	0.37	2.99	0.14
Log	0.03	0.17	0.70	0.01	0.06	0.81	0.14	0.81	0.41	0.37	2.91	0.15
Inv	0.04	0.21	0.67	0.01	0.05	0.83	0.15	0.86	0.40	0.36	2.83	0.15
Pow	0.03	0.14	0.72	0.01	0.06	0.82	0.12	0.67	0.45	0.36	2.86	0.15
Exp	0.02	0.11	0.75	0.01	0.07	0.80	0.11	0.62	0.47	0.37	2.94	0.15

Table B.9: Raw Data: Soil Cd

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
				available					
B2-1	a	7.012	1	25	0.06	0.20	0.20	0.01	6.43
	b	7.028	1	25	0.05	0.19			
	c	7.001	1	25	0.06	0.21			
B2-2	a	7.046	1	25	0.07	0.25	0.24	0.01	2.99
	b	7.036	1	25	0.07	0.24			
	c	7.027	1	25	0.07	0.23			
B2-3	a	7.021	1	25	0.09	0.31	0.29	0.02	8.62
	b	7.002	1	25	0.08	0.29			
	c	7.004	1	25	0.07	0.26			
B5-2	a	7.005	1	25	0.04	0.13	0.23	0.09	37.73
	b	6.997	1	25	0.07	0.25			
	c	7.037	1	25	0.08	0.30			
B5-3	a	7.007	1	25	0.09	0.30	0.31	0.01	2.07
	b	7.040	1	25	0.09	0.32			
	c	6.996	1	25	0.09	0.31			
B5-4	a	6.999	1	25	0.08	0.29	0.30	0.02	7.02
	b	6.995	1	25	0.08	0.28			
	c	7.032	1	25	0.09	0.32			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	0.00
				pseudo-total					
B2-1	a	5.003	1	50	0.02	0.20	0.19	0.01	6.31
	b	5.027	1	50	0.02	0.18			
	c	4.999	1	50	0.02	0.18			
B2-2	a	4.999	1	50	0.02	0.16	0.23	0.06	28.42
	b	5.005	1	50	0.02	0.23			
	c	5.028	1	50	0.03	0.29			
B2-3	a	4.991	1	50	0.03	0.28	0.28	0.02	5.48
	b	5.007	1	50	0.03	0.26			
	c	5.020	1	50.05	0.03	0.29			
B5-2	a	5.029	1	50	0.02	0.20	0.21	0.01	6.93
	b	4.996	1	50	0.02	0.22			
	c	5.010	1	50	0.02	0.20			
B5-3	a	5.002	1	50	0.03	0.25	0.24	0.02	9.64
	b	4.995	1	50	0.02	0.21			
	c	5.012	1	50	0.03	0.25			
B5-4	a	4.996	1	50	0.03	0.25	0.25	0.01	3.86
	b	5.010	1	50.05	0.02	0.24			
	c	5.019	1	50	0.03	0.26			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	—
				water-extractable					
B2-1	a	7.001	1	25	0.00	0.00	0.00	0.00	0.00
	b	7.030	1	25	0.00	0.00			
B2-2	a	7.027	1	25	0.00	0.00	0.00	0.00	0.00
	b	7.029	1	25	0.00	0.00			
B2-3	a	7.010	1	25	0.00	0.00	0.00	0.00	0.00
	b	7.010	1	25	0.00	0.00			
B5-2	a	7.053	1	25	0.00	0.00	0.00	0.00	0.00
	b	7.003	1	25	0.00	0.00			
B5-3	a	7.003	1	25	0.00	0.00	0.00	0.00	0.00
	b	7.008	1	25	0.00	0.00			
B5-4	a	7.022	1	25	0.00	0.00	0.00	0.00	0.00
	b	6.998	1	25	0.00	0.00			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	0.00
				certified material					
GBW 07405	a	0.458	1	10	—	—	—	0.00	—

Table B.10: Raw Data: Soil Pb

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
				available					
B2-1	a	7.012	1	25	1.13	4.03	4.01	0.03	0.66
	b	7.028	1	25	1.12	3.98			
	c	7.001	1	25	1.12	4.01			
B2-2	a	7.046	1	25	1.70	6.02	5.64	0.54	9.51
	b	7.036	1	25	1.48	5.26			
	c	7.027	1	25	1.63	5.80			
B2-3	a	7.021	1	25	1.66	5.89	5.67	0.23	4.00
	b	7.002	1	25	1.59	5.66			
	c	7.004	1	25	1.52	5.44			
B5-2	a	7.005	1	25	1.60	5.72	5.76	0.21	3.60
	b	6.997	1	25	1.56	5.58			
	c	7.037	1	25	1.69	5.99			
B5-3	a	7.007	1	25	1.61	5.75	5.41	0.30	5.55
	b	7.040	1	25	1.48	5.27			
	c	6.996	1	25	1.46	5.21			
B5-4	a	6.999	1	25	1.41	5.04	5.36	0.31	5.85
	b	6.995	1	25	1.50	5.35			
	c	7.032	1	25	1.59	5.67			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	—
				pseudo-total					
B2-1	a	5.003	1	50	0.67	6.65	6.85	0.38	5.57
	b	5.027	1	50	0.67	6.62			
	c	4.999	1	50	0.73	7.29			
B2-2	a	4.999	1	50	0.95	9.48	9.67	0.45	4.71
	b	5.005	1	50	0.93	9.33			
	c	5.028	1	50	1.02	10.18			
B2-3	a	4.991	1	50	0.92	9.21	9.47	0.25	2.59
	b	5.007	1	50	0.95	9.52			
	c	5.020	1	50.05	0.97	9.69			
B5-2	a	5.029	1	50	0.91	9.03	8.63	0.65	7.57
	b	4.996	1	50	0.91	9.09			
	c	5.010	1	50	0.82	8.16			
B5-3	a	5.002	1	50	0.93	9.32	8.17	1.00	12.24
	b	4.995	1	50	0.76	7.65			
	c	5.012	1	50	0.76	7.53			
B5-4	a	4.996	1	50	0.83	8.29	7.56	0.64	8.46
	b	5.010	1	50.05	0.71	7.08			
	c	5.019	1	50	0.73	7.31			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	0.00
				water-extractable					
B2-1	a	7.001	1	25	0.00	0.00	0.00	0.00	—
	b	7.030	1	25	0.00	0.00			
B2-2	a	7.027	1	25	0.00	0.00	0.00	0.00	—
	b	7.029	1	25	0.00	0.00			
B2-3	a	7.010	1	25	0.00	0.00	0.00	0.00	—
	b	7.010	1	25	0.00	0.00			
B5-2	a	7.053	1	25	0.00	0.00	0.00	0.00	—
	b	7.003	1	25	0.00	0.00			
B5-3	a	7.003	1	25	0.00	0.00	0.00	0.00	—
	b	7.008	1	25	0.00	0.00			
B5-4	a	7.022	1	25	0.00	0.00	0.00	0.00	—
	b	6.998	1	25	0.00	0.00			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	—
				certified material					
GBW 07405	a	0.458	1	10	14.15	308.68	308.68	0.00	—

Table B.11: Raw Data: Soil Zn

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
				available					
B2-1	a	7.012	4	25	0.27	3.91	3.62	0.37	10.27
	b	7.028	4	25	0.23	3.20			
	c	7.001	4	25	0.26	3.76			
B2-2	a	7.046	4	25	0.26	3.66	3.51	0.21	5.89
	b	7.036	4	25	0.24	3.37			
	c	7.027	4	25	0.23	3.33			
B2-3	a	7.021	4	25	0.34	4.80	4.91	0.20	4.03
	b	7.002	4	25	0.36	5.14			
	c	7.004	4	25	0.34	4.80			
B5-2	a	7.005	4	25	0.55	7.82	7.50	0.37	4.98
	b	6.997	4	25	0.50	7.09			
	c	7.037	4	25	0.53	7.58			
B5-3	a	7.007	4	25	0.43	6.09	5.99	0.09	1.48
	b	7.040	4	25	0.42	5.95			
	c	6.996	4	25	0.41	5.93			
B5-4	a	6.999	4	25	0.38	5.42	5.05	0.78	15.45
	b	6.995	4	25	0.39	5.58			
	c	7.032	4	25	0.29	4.15			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	—
				pseudo-total					
B2-1	a	5.003	6	50	0.41	24.46	24.07	0.42	1.74
	b	5.027	6	50	0.40	23.63			
	c	4.999	6	50	0.40	24.12			
B2-2	a	4.999	6	50	0.43	25.63	24.09	1.38	5.73
	b	5.005	6	50	0.38	22.95			
	c	5.028	6	50	0.40	23.69			
B2-3	a	4.991	6	50	0.48	28.85	29.20	1.58	5.41
	b	5.007	6	50	0.52	30.92			
	c	5.020	6	50.05	0.47	27.82			
B5-2	a	5.029	6	50	0.48	28.57	27.32	2.13	7.80
	b	4.996	6	50	0.48	28.82			
	c	5.010	6	50	0.43	25.81			
B5-3	a	5.002	6	50	0.46	27.83	28.42	0.53	1.86
	b	4.995	6	50	0.48	28.59			
	c	5.012	6	50	0.48	28.85			
B5-4	a	4.996	6	50	0.44	26.24	26.64	0.74	2.77
	b	5.010	6	50.05	0.44	26.20			
	c	5.019	6	50	0.46	27.49			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	0.00
				water-extractable					
B2-1	a	7.001	1	25	0.00	0.00	0.016	0.023	143.75
	b	7.030	1	25	0.01	0.03			
B2-2	a	7.027	1	25	0.00	0.00	0.000	0.000	—
	b	7.029	1	25	0.00	0.00			
B2-3	a	7.010	1	25	0.00	0.00	0.007	0.010	142.00
	b	7.010	1	25	0.00	0.01			
B5-2	a	7.053	1	25	0.01	0.02	0.011	0.015	136.36
	b	7.003	1	25	0.00	0.00			
B5-3	a	7.003	1	25	0.00	0.01	0.007	0.010	142.85
	b	7.008	1	25	0.00	0.00			
B5-4	a	7.022	1	25	0.00	0.00	0.000	0.000	—
	b	6.998	1	25	0.00	0.00			
Blank	a	0.000	1	25	0.00	0.00	0.00	0.00	—
				certified material					
GBW 07405	a	0.458	10	10	0.89	194.59	194.59	0.00	—

Table B.12: Raw Data: Soybean Cd

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
					root				
B2-1	a	2.991	1	10	0.02	0.07	0.07	0.01	12.74
	b	3.025	1	10	0.02	0.06			
	c	3.006	1	10	0.02	0.08			
B2-2	a	2.994	1	10	0.02	0.05	0.05	0.00	0.30
	b	3.006	1	10	0.02	0.05			
	c	2.996	1	10	0.03	0.09			
B2-3	a	2.994	1	10	0.03	0.09	0.07	0.02	33.33
	b	3.036	1	10	0.01	0.05			
	c	2.997	1	10	0.02	0.08			
B5-1	a	3.027	1	10	0.02	0.06	0.05	0.01	30.91
	b	3.031	1	10	0.01	0.04			
	c	3.004	1	10	0.01	0.04			
B5-2	a	3.001	1	10	0.03	0.11	0.10	0.01	7.00
	b	3.009	1	10	0.03	0.09			
	c	3.003	1	10	0.03	0.10			
B5-3	a	2.994	1	10	0.01	0.03	0.06	0.02	39.86
	b	2.991	1	10	0.02	0.07			
	c	3.006	1	10	0.02	0.07			
B5-4	a	3.025	1	10	0.01	0.05	0.08	0.03	36.28
	b	2.992	1	10	0.02	0.08			
	c	2.997	1	10	0.03	0.11			
B0-1	a	3.003	1	10	0.03	0.11	0.09	0.01	14.54
	b	3.000	1	10	0.03	0.09			
	c	3.000	1	10	0.02	0.08			
B0-2	a	2.992	1	10	0.05	0.16	0.15	0.02	11.94
	b	3.009	1	10	0.04	0.13			
	c	3.035	1	10	0.04	0.15			
B0-3	a	3.029	1	10	0.01	0.03	0.04	0.01	13.71
	b	3.009	1	10	0.01	0.04			
	c	3.012	1	10	0.01	0.04			
B0-4	a	2.992	1	10	0.02	0.08	0.08	0.01	10.43
	b	2.994	1	10	0.02	0.07			
	c	3.013	1	10	0.03	0.09			
B0-5	a	3.000	1	10	0.01	0.04	0.03	0.01	37.88
	b	3.030	1	10	0.01	0.03			
	c	3.007	1	10	0.01	0.02			
B0-6	a	3.005	1	10	0.01	0.04	0.06	0.03	58.01
	b	3.001	1	10	0.01	0.04			
	c	2.990	1	10	0.03	0.10			
B0-7	a	2.995	1	10	0.02	0.07	0.07	0.01	15.95
	b	2.999	1	10	0.02	0.08			
	c	3.021	1	10	0.02	0.06			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					stipe				
B0-1	a	1.495	1	10	0.03	0.17	0.19	0.05	28.18
	b	1.510	1	10	0.02	0.15			
	c	1.508	1	10	0.04	0.24			
B0-2	a	1.495	1	10	0.04	0.29	0.28	0.01	4.97
	b	1.515	1	10	0.04	0.28			
	c	1.497	1	10	0.04	0.26			
B0-3	a	1.499	1	10	0.05	0.33	0.32	0.01	4.30
	b	1.506	1	10	0.05	0.33			
	c	1.503	1	10	0.05	0.31			
B0-4	a	1.496	1	10	0.05	0.31	0.29	0.02	8.57
	b	1.499	1	10	0.04	0.28			
	c	1.501	1	10	0.04	0.27			
B0-5	a	1.528	1	10	0.03	0.22	0.21	0.01	6.40
	b	1.481	1	10	0.03	0.20			
	c	1.492	1	10	0.03	0.21			
B0-6	a	2.992	1	10	0.08	0.26	0.25	0.01	3.40

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Table B.12 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	2.991	1	10	0.07	0.24			
	c	3.004	1	10	0.08	0.26			
B0-7	a	3.001	1	10	0.08	0.27	0.39	0.21	52.92
	b	3.074	1	10	0.08	0.27			
	c	3.002	1	10	0.19	0.63			
B2-1	a	2.964	1	10	0.07	0.22	0.23	0.01	3.90
	b	2.994	1	10	0.07	0.24			
	c	3.025	1	10	0.07	0.23			
B2-2	a	3.001	1	10	0.07	0.23	0.21	0.02	8.88
	b	3.001	1	10	0.06	0.20			
	c	3.048	1	10	0.06	0.19			
B2-3	a	2.995	1	10	0.07	0.25	0.23	0.02	8.31
	b	2.999	1	10	0.06	0.21			
	c	3.055	1	10	0.07	0.22			
B5-1	a	3.003	1	10	0.06	0.20	0.21	0.01	5.55
	b	3.002	1	10	0.07	0.22			
	c	3.004	1	10	0.06	0.21			
B5-2	a	2.988	1	10	0.08	0.27	0.26	0.01	2.14
	b	2.948	1	10	0.08	0.26			
	c	2.982	1	10	0.08	0.26			
B5-3	a	2.992	1	10	0.07	0.22	0.22	0.00	0.94
	b	2.961	1	10	0.07	0.22			
	c	2.997	1	10	0.07	0.22			
B5-4	a	2.939	1	10	0.07	0.23	0.24	0.00	0.98
	b	2.966	1	10	0.07	0.24			
	c	2.952	1	10	0.07	0.24			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					pod				
B0-1	a	1.506	1	10	0.03	0.19	0.27	0.08	29.05
	b	1.494	1	10	0.05	0.34			
	c	1.492	1	10	0.04	0.29			
B0-2	a	1.508	1	10	0.06	0.40	0.41	0.01	2.20
	b	1.516	1	10	0.06	0.40			
	c	1.504	1	10	0.06	0.42			
B0-3	a	1.507	1	10	0.06	0.41	0.39	0.01	3.21
	b	1.510	1	10	0.06	0.38			
	c	1.492	1	10	0.06	0.38			
B0-4	a	1.508	1	10	0.06	0.39	0.40	0.01	3.37
	b	1.506	1	10	0.06	0.39			
	c	1.510	1	10	0.06	0.41			
B0-5	a	1.481	1	10	0.06	0.41	0.40	0.01	1.65
	b	1.505	1	10	0.06	0.39			
	c	1.484	1	10	0.06	0.40			
B0-6	a	2.999	1	10	0.11	0.36	0.32	0.06	19.84
	b	2.997	1	10	0.07	0.25			
	c	2.996	1	10	0.11	0.36			
B0-7	a	2.997	1	10	0.10	0.34	0.35	0.01	2.72
	b	3.043	1	10	0.11	0.36			
	c	3.028	1	10	0.10	0.35			
B2-1	a	3.018	1	10	0.12	0.39	0.40	0.01	2.08
	b	3.032	1	10	0.12	0.41			
	c	3.020	1	10	0.12	0.41			
B2-2	a	3.045	1	10	0.13	0.42	0.42	0.01	2.59
	b	3.019	1	10	0.13	0.43			
	c	3.006	1	10	0.12	0.41			
B2-3	a	3.011	1	10	0.12	0.41	0.38	0.03	9.04
	b	3.032	1	10	0.10	0.34			
	c	2.952	1	10	0.12	0.39			
B5-1	a	2.995	1	10	0.12	0.41	0.38	0.04	10.71
	b	3.005	1	10	0.12	0.41			
	c	3.055	1	10	0.10	0.34			
B5-2	a	3.012	1	10	0.11	0.38	0.39	0.01	2.51

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Table B.12 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	3.041	1	10	0.12	0.40			
	c	3.055	1	10	0.12	0.39			
B5-3	a	3.050	1	10	0.12	0.41	0.40	0.01	3.04
	b	2.965	1	10	0.12	0.39			
	c	2.969	1	10	0.12	0.41			
B5-4	a	2.953	1	10	0.12	0.39	0.39	0.01	2.28
	b	3.079	1	10	0.12	0.40			
	c	3.030	1	10	0.12	0.38			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					seed				
B0-1	a	1.508	1	10	0.02	0.15	0.19	0.04	19.54
	b	1.513	1	10	0.03	0.20			
	c	1.501	1	10	0.03	0.21			
B0-2	a	1.491	1	10	0.03	0.21	0.21	0.01	4.44
	b	1.513	1	10	0.03	0.22			
	c	1.503	1	10	0.03	0.20			
B0-3	a	1.517	1	10	0.03	0.18	0.19	0.03	14.61
	b	1.495	1	10	0.03	0.23			
	c	1.513	1	10	0.03	0.18			
B0-4	a	1.499	1	10	0.03	0.18	0.18	0.01	4.06
	b	1.498	1	10	0.03	0.19			
	c	1.492	1	10	0.03	0.18			
B0-5	a	1.579	1	10	0.03	0.21	0.20	0.02	8.82
	b	1.518	1	10	0.03	0.18			
	c	1.504	1	10	—	—			
B0-6	a	3.007	1	10	0.06	0.20	0.20	0.00	0.19
	b	2.996	1	10	0.06	0.20			
	c	3.049	1	10	0.06	0.20			
B0-7	a	2.997	1	10	0.06	0.21	0.21	0.00	0.88
	b	3.011	1	10	0.06	0.21			
	c	2.999	1	10	0.06	0.20			
B2-1	a	3.058	1	10	0.06	0.19	0.20	0.01	5.99
	b	2.968	1	10	0.06	0.20			
	c	3.002	1	10	0.06	0.21			
B2-2	a	3.012	1	10	0.06	0.21	0.19	0.01	6.60
	b	2.984	1	10	0.06	0.18			
	c	2.995	1	10	0.06	0.18			
B2-3	a	3.039	1	10	0.07	0.22	0.23	0.01	5.16
	b	2.984	1	10	0.07	0.22			
	c	3.047	1	10	0.07	0.24			
B5-1	a	2.991	1	10	0.07	0.22	0.21	0.01	4.94
	b	3.041	1	10	0.06	0.20			
	c	3.000	1	10	0.07	0.22			
B5-2	a	2.936	1	10	—	—	0.21	0.02	9.65
	b	2.963	1	10	0.06	0.20			
	c	3.075	1	10	0.07	0.22			
B5-3	a	2.969	1	10	0.06	0.20	0.20	0.01	6.91
	b	3.037	1	10	0.07	0.22			
	c	3.045	1	10	0.06	0.20			
B5-4	a	3.081	1	10	0.06	0.21	0.21	0.01	5.17
	b	2.989	1	10	0.07	0.22			
	c	3.097	1	10	0.06	0.20			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					certified material				
CTA-OTL-1	a	2.002	1	10	—	—	—	0.00	—

Values with ▨ background shading included mishaps during the processing. Thus, they were excluded from the calculations.

Table B.13: Raw Data: Soybean Pb

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
					root				
B0-1	a	3.003	1	10	1.35	4.49	3.79	0.63	16.51
	b	3.000	1	10	1.08	3.60			
	c	3.000	1	10	0.98	3.28			
B0-2	a	2.992	1	10	11.72	39.17	34.97	3.85	11.00
	b	3.009	1	10	10.26	34.10			
	c	3.035	1	10	9.60	31.63			
B0-3	a	3.029	1	10	0.64	2.12	2.03	0.14	6.79
	b	3.009	1	10	0.56	1.87			
	c	3.012	1	10	0.63	2.10			
B0-4	a	2.992	1	10	0.48	1.60	1.62	0.10	6.30
	b	2.994	1	10	0.52	1.73			
	c	3.013	1	10	0.46	1.53			
B0-5	a	3.000	1	10	2.50	8.34	6.61	1.53	23.14
	b	3.030	1	10	1.83	6.04			
	c	3.007	1	10	1.64	5.44			
B0-6	a	3.005	1	10	0.10	0.34	0.37	0.20	52.83
	b	3.001	1	10	0.06	0.19			
	c	2.990	1	10	0.17	0.58			
B0-7	a	2.995	1	10	0.00	0.00	0.00	0.00	0.00
	b	2.999	1	10	0.00	0.00			
	c	3.021	1	10	0.00	0.00			
B2-1	a	2.991	1	10	0.04	0.15	0.15	0.16	102.44
	b	3.025	1	10	0.00	0.00			
	c	3.006	1	10	0.10	0.32			
B2-2	a	2.994	1	10	0.03	0.11	0.12	0.01	5.67
	b	3.006	1	10	0.04	0.12			
	c	2.996	1	10	0.00	0.00			
B2-3	a	2.994	1	10	0.12	0.39	0.31	0.08	24.18
	b	3.036	1	10	0.09	0.30			
	c	2.997	1	10	0.07	0.24			
B5-1	a	3.027	1	10	0.00	0.00	0.01	0.01	173.21
	b	3.031	1	10	0.00	0.00			
	c	3.004	1	10	0.01	0.02			
B5-2	a	3.001	1	10	0.00	0.00	0.06	0.10	173.21
	b	3.009	1	10	0.05	0.17			
	c	3.003	1	10	0.00	0.00			
B5-3	a	2.994	1	10	0.00	0.00	0.15	0.19	124.40
	b	2.991	1	10	0.03	0.09			
	c	3.006	1	10	0.11	0.36			
B5-4	a	3.025	1	10	0.15	0.49	0.24	0.24	99.47
	b	2.992	1	10	0.00	0.00			
	c	2.997	1	10	0.07	0.25			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	
					stipe				
B0-1	a	1.495	1	10	0.45	3.02	2.76	0.38	13.58
	b	1.510	1	10	0.35	2.33			
	c	1.508	1	10	0.44	2.94			
B0-2	a	1.495	1	10	3.00	20.09	20.33	1.35	6.67
	b	1.515	1	10	3.30	21.78			
	c	1.497	1	10	2.86	19.10			
B0-3	a	1.499	1	10	0.35	2.31	1.87	0.41	22.11
	b	1.506	1	10	0.23	1.49			
	c	1.503	1	10	0.27	1.81			
B0-4	a	1.496	1	10	0.11	0.76	1.12	0.32	28.15
	b	1.499	1	10	0.19	1.27			
	c	1.501	1	10	0.20	1.34			
B0-5	a	1.528	1	10	0.92	6.01	4.76	1.12	23.54
	b	1.481	1	10	0.65	4.39			
	c	1.492	1	10	0.58	3.87			
B0-6	a	2.992	1	10	0.56	1.86	1.90	0.21	11.08

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Table B.13 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	2.991	1	10	0.51	1.72			
	c	3.004	1	10	0.64	2.13			
B0-7	a	3.001	1	10	0.36	1.21	1.49	0.25	16.84
	b	3.074	1	10	0.48	1.56			
	c	3.002	1	10	0.51	1.70			
B2-1	a	2.964	1	10	0.29	0.98	1.13	0.30	26.67
	b	2.994	1	10	0.44	1.48			
	c	3.025	1	10	0.28	0.94			
B2-2	a	3.001	1	10	0.31	1.02	0.86	0.20	22.78
	b	3.001	1	10	0.19	0.64			
	c	3.048	1	10	0.28	0.93			
B2-3	a	2.995	1	10	0.33	1.10	1.03	0.08	7.87
	b	2.999	1	10	0.32	1.05			
	c	3.055	1	10	0.29	0.94			
B5-1	a	3.003	1	10	0.31	1.02	0.93	0.21	22.25
	b	3.002	1	10	0.21	0.70			
	c	3.004	1	10	0.33	1.08			
B5-2	a	2.988	1	10	0.48	1.60	1.42	0.36	25.45
	b	2.948	1	10	0.29	1.00			
	c	2.982	1	10	0.49	1.65			
B5-3	a	2.992	1	10	0.27	0.91	1.03	0.48	46.54
	b	2.961	1	10	0.46	1.55			
	c	2.997	1	10	0.18	0.62			
B5-4	a	2.939	1	10	0.48	1.65	1.19	0.41	34.21
	b	2.966	1	10	0.27	0.89			
	c	2.952	1	10	0.30	1.02			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	
					pod				
B0-1	a	1.506	1	10	0.12	0.78	2.30	1.40	61.08
	b	1.494	1	10	0.39	2.58			
	c	1.492	1	10	0.53	3.54			
B0-2	a	1.508	1	10	0.45	2.98	2.85	0.57	20.11
	b	1.516	1	10	0.34	2.23			
	c	1.504	1	10	0.51	3.36			
B0-3	a	1.507	1	10	0.49	3.23	3.24	0.39	12.12
	b	1.510	1	10	0.55	3.64			
	c	1.492	1	10	0.43	2.86			
B0-4	a	1.508	1	10	0.21	1.43	1.50	0.07	4.48
	b	1.506	1	10	0.23	1.54			
	c	1.510	1	10	0.23	1.54			
B0-5	a	1.481	1	10	0.81	5.48	5.54	0.10	1.74
	b	1.505	1	10	0.82	5.48			
	c	1.484	1	10	0.84	5.65			
B0-6	a	2.999	1	10	0.55	1.84	1.99	0.63	31.75
	b	2.997	1	10	0.43	1.45			
	c	2.996	1	10	0.80	2.68			
B0-7	a	2.997	1	10	0.68	2.25	2.35	0.11	4.68
	b	3.043	1	10	0.71	2.34			
	c	3.028	1	10	0.75	2.47			
B2-1	a	3.018	1	10	0.80	2.64	2.53	0.19	7.47
	b	3.032	1	10	0.70	2.31			
	c	3.020	1	10	0.80	2.64			
B2-2	a	3.045	1	10	0.94	3.07	2.92	0.13	4.51
	b	3.019	1	10	0.86	2.87			
	c	3.006	1	10	0.85	2.83			
B2-3	a	3.011	1	10	0.76	2.51	2.51	0.16	6.40
	b	3.032	1	10	0.71	2.34			
	c	2.952	1	10	0.79	2.66			
B5-1	a	2.995	1	10	0.79	2.64	2.74	0.21	7.54
	b	3.005	1	10	0.78	2.60			
	c	3.055	1	10	0.91	2.98			
B5-2	a	3.012	1	10	0.81	2.70	2.75	0.73	26.53

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Table B.13 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	3.041	1	10	0.62	2.05			
	c	3.055	1	10	1.07	3.51			
B5-3	a	3.050	1	10	1.00	3.27	3.06	0.21	6.71
	b	2.965	1	10	0.90	3.05			
	c	2.969	1	10	0.85	2.86			
B5-4	a	2.953	1	10	0.76	2.58	2.61	0.09	3.28
	b	3.079	1	10	0.83	2.71			
	c	3.030	1	10	0.77	2.54			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	
					seed				
B0-1	a	6.054	1	10	0.99	1.64	1.75	0.15	8.42
	b	6.017	1	10	1.11	1.85			
B0-2	a	6.006	1	10	0.97	1.61	1.70	0.13	7.87
	b	6.006	1	10	1.08	1.80			
B0-3	a	5.998	1	10	0.98	1.64	1.58	0.08	4.83
	b	5.991	1	10	0.92	1.53			
B0-4	a	5.990	1	10	0.97	1.62	1.64	0.03	1.75
	b	6.042	1	10	1.00	1.66			
B0-5	a	6.038	1	10	0.87	1.45	1.52	0.11	6.92
	b	6.077	1	10	0.97	1.60			
B0-6	a	6.000	1	10	1.05	1.75	1.71	0.04	2.60
	b	6.029	1	10	1.01	1.68			
B0-7	a	6.035	1	10	1.02	1.70	1.71	0.02	1.12
	b	5.992	1	10	1.03	1.72			
B2-1	a	6.022	1	10	0.73	1.22	1.27	0.07	5.36
	b	6.084	1	10	0.80	1.31			
B2-2	a	6.005	1.05	10	0.71	1.25	1.29	0.06	4.67
	b	6.004	1	10	0.80	1.34			
B2-3	a	6.048	1	10	0.78	1.29	1.33	0.06	4.31
	b	5.998	1	10	0.82	1.37			
B5-1	a	6.002	1	10	0.82	1.36	1.34	0.03	2.23
	b	6.013	1	10	0.79	1.32			
B5-2	a	5.997	1	10	0.67	1.12	1.20	0.11	9.44
	b	6.076	1	10	0.78	1.28			
B5-3	a	6.021	1	10	0.80	1.32	1.37	0.07	5.39
	b	5.993	1	10	0.85	1.43			
B5-4	a	6.011	1	10	0.96	1.60	1.41	0.26	18.48
	b	6.077	1	10	0.75	1.23			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	
					certified material				
CTA-OTL-1	a	2.002	1	10	1.32	6.61	6.61	0.00	

Values with background shading included mishaps during the processing. Thus, they were excluded from the calculations.

Table B.14: Raw Data: Soybean Zn

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
					root				
B0-1	a	3.003	4	10	0.43	5.77	5.54	0.26	4.75
	b	3.000	4	10	0.42	5.61			
	c	3.000	4	10	0.39	5.25			
B0-2	a	2.992	4	10	0.38	5.08	5.10	0.25	4.86
	b	3.009	4	10	0.40	5.36			
	c	3.035	4	10	0.37	4.86			
B0-3	a	3.029	4	10	0.30	4.03	4.36	0.30	6.95
	b	3.009	4	10	0.33	4.43			
	c	3.012	4	10	0.35	4.62			
B0-4	a	2.992	4	10	0.42	5.63	5.56	0.06	1.16
	b	2.994	4	10	0.41	5.50			
	c	3.013	4	10	0.42	5.54			
B0-5	a	3.000	4	10	0.50	6.65	5.96	0.67	11.29
	b	3.030	4	10	0.45	5.93			
	c	3.007	4	10	0.40	5.31			
B0-6	a	3.005	4	10	0.29	3.83	4.26	0.69	16.29
	b	3.001	4	10	0.29	3.88			
	c	2.990	4	10	0.38	5.06			
B0-7	a	2.995	4	10	0.33	4.46	4.04	0.38	9.47
	b	2.999	4	10	0.30	3.96			
	c	3.021	4	10	0.28	3.71			
B2-1	a	2.991	4	10	0.36	4.79	4.84	0.16	3.23
	b	3.025	4	10	0.38	5.01			
	c	3.006	4	10	0.35	4.71			
B2-2	a	2.994	4	10	0.35	4.66	4.57	0.13	2.78
	b	3.006	4	10	0.34	4.48			
	c	2.996	4	10	0.39	5.25			
B2-3	a	2.994	4	10	0.36	4.78	4.17	0.53	12.72
	b	3.036	4	10	0.29	3.83			
	c	2.997	4	10	0.29	3.90			
B5-1	a	3.027	4	10	0.34	4.49	4.25	0.30	7.17
	b	3.031	4	10	0.30	3.91			
	c	3.004	4	10	0.33	4.34			
B5-2	a	3.001	4	10	0.50	6.64	6.38	0.27	4.24
	b	3.009	4	10	0.48	6.41			
	c	3.003	4	10	0.46	6.10			
B5-3	a	2.994	4	10	0.29	3.88	4.80	0.81	16.81
	b	2.991	4	10	0.39	5.16			
	c	3.006	4	10	0.40	5.36			
B5-4	a	3.025	4	10	0.39	5.10	5.04	0.09	1.74
	b	2.992	4	10	0.38	5.07			
	c	2.997	4	10	0.37	4.94			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					stipe				
B0-1	a	1.495	1	10	0.56	3.73	3.98	0.29	7.38
	b	1.510	1	10	0.59	3.90			
	c	1.508	1	10	0.65	4.30			
B0-2	a	1.495	1	10	0.49	3.30	3.22	0.07	2.23
	b	1.515	1	10	0.48	3.17			
	c	1.497	1	10	0.48	3.18			
B0-3	a	1.499	1	10	0.40	2.69	2.81	0.36	12.95
	b	1.506	1	10	0.48	3.22			
	c	1.503	1	10	0.38	2.52			
B0-4	a	1.496	1	10	0.58	3.91	3.66	0.31	8.36
	b	1.499	1	10	0.50	3.32			
	c	1.501	1	10	0.56	3.76			
B0-5	a	1.528	1	10	0.66	4.34	4.36	0.21	4.80
	b	1.481	1	10	0.62	4.17			
	c	1.492	1	10	0.68	4.58			
B0-6	a	2.992	1	10	0.67	2.24	2.35	0.10	4.43

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Table B.14 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	2.991	1	10	0.70	2.35			
	c	3.004	1	10	0.73	2.45			
B0-7	a	3.001	1	10	0.83	2.75	2.60	0.14	5.22
	b	3.074	1	10	0.77	2.49			
	c	3.002	1	10	0.77	2.56			
B2-1	a	2.964	1	10	0.49	1.66	1.83	0.16	8.94
	b	2.994	1	10	0.59	1.98			
	c	3.025	1	10	0.56	1.86			
B2-2	a	3.001	1	10	0.71	2.38	2.49	0.11	4.35
	b	3.001	1	10	0.78	2.59			
	c	3.048	1	10	0.76	2.49			
B2-3	a	2.995	1	10	0.60	1.99	1.89	0.17	8.73
	b	2.999	1	10	0.60	1.99			
	c	3.055	1	10	0.52	1.70			
B5-1	a	3.003	1	10	0.58	1.92	2.11	0.35	16.40
	b	3.002	1	10	0.57	1.91			
	c	3.004	1	10	0.76	2.51			
B5-2	a	2.988	1	10	0.79	2.65	2.53	0.11	4.17
	b	2.948	1	10	0.73	2.49			
	c	2.982	1	10	0.73	2.44			
B5-3	a	2.992	1	10	0.59	1.98	2.10	0.14	6.66
	b	2.961	1	10	0.61	2.06			
	c	2.997	1	10	0.68	2.25			
B5-4	a	2.939	1	10	0.61	2.06	2.10	0.12	5.55
	b	2.966	1	10	0.66	2.24			
	c	2.952	1	10	0.59	2.01			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					pod				
B0-1	a	1.506	4	10	0.31	8.34	13.55	4.51	33.29
	b	1.494	4	10	0.60	16.12			
	c	1.492	4	10	0.60	16.19			
B0-2	a	1.508	4	10	0.25	6.68	6.84	0.14	2.09
	b	1.516	4	10	0.26	6.86			
	c	1.504	4	10	0.26	6.97			
B0-3	a	1.507	1	10	0.82	5.43	5.11	0.31	6.00
	b	1.510	1	10	0.73	4.81			
	c	1.492	1	10	0.76	5.10			
B0-4	a	1.508	4	10	0.35	9.34	9.51	0.16	1.66
	b	1.506	4	10	0.36	9.54			
	c	1.510	4	10	0.36	9.64			
B0-5	a	1.481	1	10	0.77	5.22	5.03	0.16	3.24
	b	1.505	1	10	0.74	4.95			
	c	1.484	1	10	0.73	4.93			
B0-6	a	2.999	4	10	0.62	8.30	7.23	1.70	23.47
	b	2.997	4	10	0.40	5.27			
	c	2.996	4	10	0.61	8.12			
B0-7	a	2.997	4	10	0.45	6.01	4.47	2.61	58.49
	b	3.043	4	10	0.45	5.94			
	c	3.028	1	10	0.44	1.45			
B2-1	a	3.018	4	10	0.45	5.99	6.40	0.40	6.29
	b	3.032	4	10	0.49	6.42			
	c	3.020	4	10	0.51	6.79			
B2-2	a	3.045	4	10	0.42	5.57	5.44	0.17	3.16
	b	3.019	4	10	0.40	5.25			
	c	3.006	4	10	0.41	5.51			
B2-3	a	3.011	4	10	0.39	5.17	4.86	0.42	8.69
	b	3.032	4	10	0.33	4.38			
	c	2.952	4	10	0.37	5.04			
B5-1	a	2.995	4	10	0.38	5.04	4.74	0.26	5.53
	b	3.005	4	10	0.35	4.63			
	c	3.055	4	10	0.35	4.54			
B5-2	a	3.012	1	10	0.85	2.83	2.85	0.21	7.45

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Table B.14 (continued)

Pool	Rep.	Weighted sample [g]	Dilution	Final volume [ml]	Detected value [ppm]	Calculated value [mg/kg]	Mean	SD	Coefficient of variation [%]
	b	3.041	1	10	0.80	2.64			
	c	3.055	1	10	0.94	3.06			
B5-3	a	3.050	4	10	0.43	5.69	5.43	0.31	5.80
	b	2.965	4	10	0.41	5.50			
	c	2.969	4	10	0.38	5.08			
B5-4	a	2.953	4	10	0.36	4.88	4.65	1.46	31.51
	b	3.079	4	10	0.24	3.08			
	c	3.030	4	10	0.45	5.98			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					seed				
B0-1	a	1.508	7	10	0.64	29.75	29.60	0.67	2.25
	b	1.513	7	10	0.62	28.87			
	c	1.501	7	10	0.65	30.18			
B0-2	a	1.491	7	10	0.60	28.31	28.88	0.54	1.86
	b	1.513	7	10	0.64	29.37			
	c	1.503	7	10	0.62	28.96			
B0-3	a	1.517	7	10	0.67	30.82	30.81	0.46	1.51
	b	1.495	7	10	0.67	31.28			
	c	1.513	7	10	0.66	30.35			
B0-4	a	1.499	7	10	0.68	31.57	32.40	0.88	2.70
	b	1.498	7	10	0.69	32.33			
	c	1.492	7	10	0.71	33.31			
B0-5	a	1.579	7	10	0.69	30.63	30.00	0.89	2.97
	b	1.518	7	10	0.64	29.37			
	c	1.504	7	10	—	—			
B0-6	a	3.007	10	10	0.95	31.76	32.14	0.33	1.02
	b	2.996	10	10	0.97	32.34			
	c	3.049	10	10	0.98	32.31			
B0-7	a	2.997	10	10	0.94	31.40	31.13	0.51	1.64
	b	3.011	10	10	0.95	31.45			
	c	2.999	10	10	0.92	30.54			
B2-1	a	3.058	10	10	0.82	26.85	26.75	0.27	1.00
	b	2.968	10	10	0.80	26.95			
	c	3.002	10	10	0.79	26.45			
B2-2	a	3.012	10	10	0.79	26.23	25.90	0.35	1.34
	b	2.984	10	10	0.76	25.54			
	c	2.995	10	10	0.78	25.94			
B2-3	a	3.039	10	10	0.70	23.00	22.76	0.24	1.07
	b	2.984	10	10	0.68	22.76			
	c	3.047	10	10	0.69	22.51			
B5-1	a	2.991	10	10	0.66	22.16	22.78	0.54	2.36
	b	3.041	10	10	0.70	23.05			
	c	3.000	10	10	0.69	23.13			
B5-2	a	2.936	1	10	—	—	24.56	0.55	2.25
	b	2.963	10	10	0.72	24.17			
	c	3.075	10	10	0.77	24.95			
B5-3	a	2.969	10	10	0.66	22.37	22.42	0.18	0.81
	b	3.037	10	10	0.69	22.62			
	c	3.045	10	10	0.68	22.27			
B5-4	a	3.081	10	10	0.72	23.53	23.99	0.41	1.69
	b	2.989	10	10	0.73	24.29			
	c	3.097	10	10	0.75	24.15			
Blank	a	0.000	1	10	0.00	0.00	0.00	0.00	—
					certified material				
CTA-OTL-1	a	2.002	10	10	0.89	44.46	44.46	0.00	—

Values with ▨ background shading included mishaps during the processing. Thus, they were excluded from the calculations.