EVALUATION OF IRRIGATION WATER QUALITY GUIDELINES FOR ARSENIC AND LEAD, WITH IMPLICATIONS FOR FOOD AND FEED SAFETY

by

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DECLARATION

I, Candice McGladdery, declare that this dissertation, which I hereby submit for the degree MSc. Soil Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ....

DATE: 30/08/2019

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ABSTRACT

As mining and industry continue to impact agricultural lands and waterways, and as competition for access to fresh water increases, the agricultural industry must adapt to grow crops in increasingly polluted lands using ever more contaminated water sources. As a result, the likelihood that crops grown under such conditions could pose a food safety risk is set to rise. This research assesses the extent to which potentially hazardous trace elements, As and Pb, present in irrigation water at concentrations deemed acceptable by the Irrigation Water Quality Guidelines, impact the food (and feed) safety of crops. Four crops are investigated under two glasshouse trials. The first assesses foliar absorption of As and Pb under irrigation to the aboveground biomass and the second assesses root uptake of As and Pb via the effects of medium- to long-term irrigation programs.

Results indicate that under such trace element loaded conditions, some crop parts exceed food (or feed) safety thresholds, with concentrations ranging from 0.01 $mg.kg^{-1}$ to 33.38 $mg.kg^{-1}$ As, and 0.01 $mg.kg^{-1}$ to 62.41 $mg.kg^{-1}$ Pb, on a dry mass basis. Leafy vegetables present the highest food safety risk. Therefore, if international food safety standards for fresh produce are to be adhered to, the Irrigation Water Quality Guidelines for As and Pb should be critically reviewed so as to negate all possible future contamination of fresh produce as a result of irrigation inputs.

A food (and feed) safety consequence matrix is proposed as a means of modelling the effect of irrigating according to the Irrigation Water Quality Guidelines on food (and feed) safety.

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CHAPTER 1: INTRODUCTION AND PURPOSE STATEMENT

1.1 Introduction

As competition for land and water resources, as well as the need to feed a growing population increases, the agricultural industry will be progressively driven to utilise evermore polluted lands and irrigation sources to produce food and animal feeds (Thiam et al. 2015). Consequently, the probability of producing crops that pose a risk to human (or animal) health due to elevated levels of potentially hazardous trace elements are likely to increase. Such elements in foods have been shown to have serious health implications, including increased rates of cancer, birth defects and irreversible neurological and behavioural disorders (WHO 2016, EFSA 2010).

Within the South African context, local water sources have been shown to contain potentially hazardous trace elements in excess of the South African Irrigation Water Quality Guideline (1996) limits. International research has demonstrated that the edible parts of crops grown in soils, or irrigated with water, containing elevated levels of potentially hazardous trace elements have repeatedly exceeded international food safety guidelines (Warren et al. 2003, Alexander et al. 2006, Nayek et al. 2010, Baig and Kazi 2012, Schreck et al. 2014, Yañez et al. 2019). Therefore, irrigating crops with waters containing the maximum allowable concentration of potentially hazardous trace elements in irrigation water could potentially pose a health risk to those who consume the produce.

1.2 Purpose Statement

The purpose of this dissertation is to evaluate the impact of irrigation water quality guidelines for selected potentially hazardous trace elements on food (and feed) safety. As far as the author is aware, the impact of irrigation water quality guideline limits for trace elements on food (and feed) safety has yet to be directly investigated.

In order to achieve the research objectives, two glasshouse trials were conducted: The foliar uptake trial was designed to determine the short-term impact of trace element loaded irrigation water making contact with the aboveground biomass and its effects on food (and feed) safety. The root uptake trial was developed to evaluate the food (and feed) safety impacts of the medium- to long-term effects of growing crops in soils that have been irrigated with As or Pb loaded soils until one year before the land would be deemed unusable for agricultural purposes. A selection of winter season crops was grown to investigate the treatment effects on a variety of crop growth forms, namely: grains (barley), leafy greens (Swiss chard and beetroot), legumes (garden pea) and roots (beetroot).

1.3 Chapter Sequence

Following this introductory chapter: Chapter 2 presents an extensive literature review which covers the multi-disciplinary aspects of food safety, irrigation water quality, soil chemistry and plant physiology. The methodology section is presented in Chapter 3 and includes the hypotheses. The results of the glasshouse foliar absorption trial are presented in Chapter 4 and that of the glasshouse root uptake trial in Chapter 5. Chapter 6 is a synthesises of the results of the previous chapters and presents food and feed safety consequence matrices, to provide real-world insights and future applications of the data. Finally, Chapter 7 summarises the conclusions, along with an evaluation of the hypotheses and provides recommendations for future research.

1.4 Definition of Key Terms

Absorption: (*botany, chemistry*) the condition in which any substance (atoms, ions or molecules) is taken by, or absorbed in bulk by, another substance which can be solid or liquid.

Adsorption: (*chemistry*) the surface phenomenon whereby substances like gases, liquids or dissolved solids, loosely interact with the surface of substrate via intermolecular forces.

Bioavailability: (*soil science*) the amount of an element or compound that is available for uptake by plant roots or soil organisms.

Complexation: (*chemistry*) the union of simpler substances to form an ion or molecule, held together by chemical, rather than physical, forces.

Desorption: (*chemistry*) the phenomenon whereby a substance is released from or through the surface of a substrate.

Food safety standards: (*food science*) regulations relating to the maximum levels of potential risk factors, including potentially hazardous trace elements, in foodstuffs.

"Maximum acceptable concentration": (of trace elements in irrigation water) is the maximum concentration of a trace element in water that may be utilised for irrigation purposes. A field may only be irrigated with this water for maximum of 20 years.

Oxidation: (*chemistry*) an oxidation state where the loss of an electron has occurred under oxidising conditions (well aerated soils).

Phloem: (*botany*) the vascular tissue in plants which conducts sugars and other metabolic products downwards from the leaves.

Precipitate (*n.*): (*chemistry*) an insoluble solid that emerges from a liquid solution.

Precipitation: (*chemistry*) the process through which a substance is deposited in solid form from a solution.

Reduction: (*chemistry*) an oxidation state where the gain of an electron has occurred under reducing conditions (waterlogged soils).

Solubility: (*chemistry*) the ability of a solid, liquid, or gaseous chemical substance (the solute) to dissolve in a solvent (typically a liquid).

"Target water quality": (of trace elements in irrigation water) is the maximum concentration of a trace element in water that may be utilised for irrigation purposes in a field for up to 100 years.

Target Water Quality Range: also known as the “No Effect Range”, this is the range of concentrations at which a particular constituent would have no known of anticipated adverse effect on the fitness for use (as irrigation water) (DAFF 1996). It is the water quality range that is acceptable (DAFF 1996). In this dissertation, the lower limit of this range is referred to as the “target water quality” and the upper limit of this range is referred to as the “maximum acceptable concentration”.

Translocation: (*botany*) the movement of elements and molecules from one plant part to another.

Xylem: (*botany*) the vascular tissue in plants which conducts water and dissolved nutrients upwards from the root (also forms the woody element of the stem).

1.5 List of Abbreviations

ANOVA: Analysis of variance

$BMDL_{0.5}$: Benchmark dose lower confidence limits for a 0.5 % increased incidence of certain associated aliments

EFSA: European Food Safety Authority

FAO: Food and Agriculture Organization of the United Nations

FDA: United States Food and Drug Administration

ICP-MS: Inductively coupled plasma mass spectrometry

ICP-OES: Inductively coupled plasma optical emission spectrometry

LD_{50}: Concentration of toxicant sufficient to kill 50 % of the population (Lethal Dose 50 %)

LOAEL: Lowest-observed-adverse-effect levels

ORD: Oral reference dose

WHO: World Health Organisation

US EPA: United States Environmental Protection Agency

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

As mining and industry continue to impact agricultural lands and waterways (Thiam et al. 2015) and the need to feed a population of 10 billion people by 2050 looms (Ranganathan et al. 2018), the agricultural industry must prepare to adapt to growing crops within increasingly polluted lands and water sources. As a result, the likelihood of producing contaminated crops that may pose a human health risk is set to rise.

In order to ensure that foods are safe for human consumption, food safety regulators such as the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) are continuously updating their guidelines to provide thresholds for potentially hazardous substances in foods. There have been multiple food safety studies that have investigated the effects of growing crops in impacted agricultural systems: particularly with reference to the accumulation of microorganisms, such as *E. coli*, or potentially hazardous trace elements, such as arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg) or uranium (U) in the edible parts of crops (Mireles et al. 2004, Rattan et al. 2005, Arora et al. 2008, Charry et al. 2008, Avici and Deveci 2013, Allende and Monaghan 2015, Cherfi et al. 2015, Raja et al. 2015, Zhang et al. 2015, Balkhair and Ashraf 2016).

This study has been designed to evaluate the effect two potentially hazardous trace elements present in irrigation water on food safety. While similar studies have been conducted, for the first time as far as the author is aware, this study also investigates the direct impact of applying the irrigation water quality guidelines to food (and feed) safety of fresh produce.

2.2 Food Safety and Irrigation Water Quality

The consumption of staple foods grown in historically contaminated locations is now recognized as a tangible route of human exposure to potentially hazardous trace elements (Allende and Monaghan 2015, Punshon et al. 2016, Rehman et al. 2017). In this dissertation, the link between irrigation water quality guidelines and food safety will be investigated and in doing so, add to the knowledge regarding the relationship between irrigation water quality, soil contamination and food safety.

2.2.1 Proposed "phytotoxic-zootoxic" approach to trace element risk assessments in irrigation water

Many trace elements are essential for metabolic activities of plants and animals (Cu, Zn and Mn). Others are toxic to both plants and animals (F and Cr), while some are highly toxic to animals, but not plants (Pb) (Chen et al. 2016, Rehman et al. 2017). In a review of "Trace elements from Soil to Human", the authors set out to determine the effect of various trace elements on plants and humans (Kabatha-Pendias and Mukherjee 2007). Based on the findings of Kabatha-Pendias and Mukherjee (2007), a "phytotoxic-zootoxic" approach is proposed here to evaluate the toxicity potential of elements in irrigation water. The aim being to assess whether phytotoxic symptoms in crop plants will occur before the food safety threshold is reached. While a variety of factors affect the level of toxicity of trace elements in water-soil-crop systems (pH, salinity, ionic composition, chemical speciation, organic matter content, crop type and cultivar, clay mineralogy, iron oxides, redox status, root-microbes and agronomic practices), this approach aims to simplify the evaluation by comparing the concentration ranges that exhibit phytotoxicity in a variety of crops with zootoxicity in humans when edible parts are consumed. Assuming biophysicochemical conditions that are relatively common in agronomic systems (oxidising conditions, neutral soils with the potential to acidify, low to moderate salinity), the proposed "phytotoxic-zootoxic" approach to trace element risk assessments in irrigation water is illustrated in Figure 2.1. The choice of trace elements investigated for this dissertation was largely determined by the "phytotoxic-zootoxic" approach.

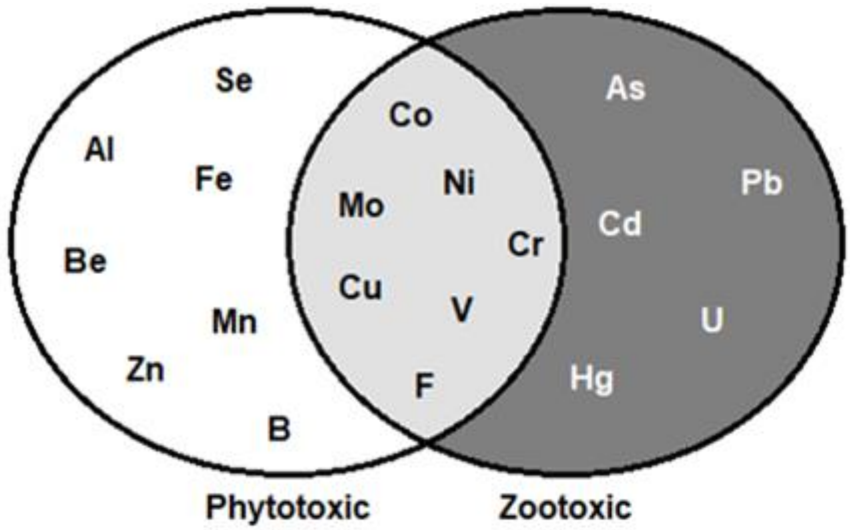

FIG 2.1: Proposed phytotoxic-zootoxic approach to trace element risk assessment in irrigation water

Building on the work of Kabatha-Pendias and Mukherjee (2007), and with reference to Figure 2.1, the following terms have been developed by the author of this dissertation to describe trace element risk. The elements in white have been termed: "plant-limited trace elements", the elements in dark-grey: "food safety-limited trace elements" and the elements in light-grey: "dual-risk trace elements".

"Plant-limited trace elements" describe trace elements that are far more likely to exhibit phytotoxic symptoms before the elements accumulate to the extent that their presence is a health concern. For example, aluminium (Al) is phytotoxic at a pH below 4.6, which is possible in poorly managed agricultural soils, is non-carcinogenic and has no known oral reference dose (ORD). Note that aluminium toxicity symptoms will present long before iron (Fe) toxicity becomes apparent. Beryllium (Be) is phytotoxic between 0.5 and 5 $mg.kg^{-1}$ soil but is non-toxic to humans and animals when ingested (Kabatha-Pendias and Mukherjee 2007). Boron (B), selenium (Se) and zinc (Zn) all show a trend similar to that of Be: low phytotoxic concentrations with high, or non-existent, zootoxic concentrations when consumed (Kabatha-Pendias and Mukherjee 2007).

“Food safety-limited trace elements”, illustrated in dark-grey in Figure 2.1, are more likely to accumulate in plant tissues to the extent that they become a zootoxic risk before phytotoxic symptoms are exhibited. Note that potentially hazardous accumulation more often refers to trace element concentrations that may lead to chronic exposure, instead of acute toxicity symptoms. According to Kabatha-Pendias and Mukherjee (2007), Hg becomes zootoxic before phytotoxic, as does Pb, As, Cd and the radionuclides. For example, plants grown in historically contaminated soils with up to 30 000 $mg.kg^{-1}$ Pb were found to accumulate up to 117.35 $mg.kg^{-1}$ Pb (dry mass) with no phytotoxicity symptoms (McGladdery et al. 2018).

“Dual-risk trace elements” is the term put forward to describe elements that have the potential to be both phytotoxic and zootoxic, or are elements with zootoxic potential, without a set ORD. Chromium (Cr) is a border-line trace element, because phytotoxicity symptoms may appear within a wide range of Cr concentrations in the soil (50 - 5 000 $mg.kg^{-1}$) (Nicholson and Chambers 2008). Furthermore, chronic zootoxicity symptoms of Cr appear in humans that consume between 70 - 100 mg of Cr per kilogram of body mass per day (Kabatha-Pendias and Mukherjee 2007). Therefore, the zootoxic risk is improbable, but possible in cultivars which are tolerant to Cr and subsequently grown in the upper end of the phytotoxicity range and thereby accumulate elevated levels of Cr in their biomass. Like chromium, vanadium (V) exhibits both phytotoxic and zootoxic affects (Nicholson and Chambers 2008). In plants, V toxicity manifests like a phosphate deficiency. The zootoxic potential of cobalt (Co) and molybdenum (Mo) have yet to be determined; however, both have exhibited carcinogenic potential when consumed at low concentrations (Nicholson and Chambers 2008). Fluoride (F) is hazardous to humans when consumed at 20-30 $mg.day^{-1}$, however, no phytotoxic symptoms are shown when the element is present in soil under certain conditions, but F is phytotoxic when absorbed via foliar uptake (Kabatha-Pendias and Mukherjee 2007). In alkaline soil high in calcium (Ca) (calcareous soils), F will precipitate and not be plant available. However, in acid soils low in Ca, phytotoxicity symptoms may occur. Therefore, in the case of F, irrigation method as well as soil chemistry may contribute to the extent of phytotoxic-zootoxic risk.

2.2.2 Selection criteria and choice of potentially hazardous trace elements for this study

Based on the phytotoxic-zootoxic approach to trace element risk assessment, two food-safety-limited elements were chosen to evaluate the potential health risks associated with consuming crops grown in environments enriched with such elements via irrigation at concentrations allowed by the South African Irrigation Water Quality Guidelines.

Initially, all 19 trace elements from within the South African Irrigation Water Quality Guidelines were considered for this study. Of those 19 trace elements, the 7 "plant-limited trace elements" and 7 "dual-risk trace elements" were excluded based on the high probability of phytotoxicity symptoms and low food safety risk. However, it is recommended that certain "dual-risk trace elements", such as Cr, Co, V and Mo, should be considered in future investigations.

From the remaining five "food safety-limited trace elements" (As, Pb, Cd, Ur and Hg), cadmium was excluded, as the method detection limit of the inductively coupled plasma optical emission spectrometry (ICP-OES) would be too high to gather meaningful results. Mercury was also eliminated, because it was not on the list of potentially hazardous trace elements in the South African Irrigation Water Quality Guidelines at the commencement of the trial. Finally, uranium was excluded as it was deemed too challenging to source for research purposes. As a result, As and Pb were chosen.

These two elements are well suited for research for the following reasons: The United States Environmental Protection Agency (US EPA) has listed As and Pb as the first and second most hazardous substances to human health (ATSDR 2015). While both elements pose a high risk to human health, they denote vastly different chemical properties and therefore are of interest for comparative purposes: As is an anion, representative of the metalloids or semi-metals, while Pb is a typical type B metal cation with high atomic mass and low oxidation state (Stumm and Morgan 1996). Because the elements are chemically distinct from one another, their interactions within the soil-water-plant continuum are likely to infer differences in plant uptake and translocation

(Kabatha-Pendias and Mukherjee 2007). Finally, mining and industrial activities have been shown to cause elevated As and Pb in South African waterways, which will be discussed in detail later in this chapter. Therefore, investigating As and Pb in irrigation water has practical applications within the local context.

2.2.3 Results from previous studies of crops grown in trace element rich environments

A multitude of studies have investigated the impacts of producing crops in trace element contaminated environments on food safety (Mireles et al. 2004, Rattan et al. 2005, Arora et al. 2008, Charry et al. 2008, Avici and Deveci 2013, Allende and Monaghan 2015, Cherfi et al. 2015, Raja et al. 2015, Zhang et al. 2015, Balkhair and Ashraf 2016).

The findings of some of the studies which considered As or Pb are summarised in Table 2.1. These studies assessed the bioaccumulation of As and/or Pb in the edible parts of crops when grown in either artificially dosed, naturally occurring or pre-existing levels of contamination in soil, irrigation water, or both. So far as the author is aware, no publication has yet to focus directly on assessing the food safety of crops impacted by As or Pb as a result of irrigation inputs at water quality threshold concentrations stipulated by irrigation water quality guidelines.

Most publications report results on a dry mass basis, as is the agronomic standard. Other publications, which tend to demonstrate a strong food safety focus report results on a fresh mass basis, as people tend to consume fruits and vegetables fresh. Therefore, food safety guidelines also tend to be determined on fresh mass. The pros and cons of each approach are discussed in Section 2.4.2.

TABLE 2.1: Results of previous studies on As or Pb accumulation in crops grown in contaminated soils and/or irrigated with As or Pb loaded water

Element	Total Element Load Soil ($mg.kg^{-1}$)	Irrigation ($mg.L^{-1}$)	Crop	Element in edible parts ($mg.kg^{-1}$)	Mass basis (FM^{Δ} /DM∞)	Reference
As	748	-	Lettuce	17.81	DM	(Warren et al. 2003)
			Radish leaf	14.1	DM	
			Radish tuber	8.39	DM	
			Radish peel	21.3	DM	
	65	-	Lettuce	6.77	DM	(Warren et al. 2003)
	57.3	0.098	Pea	0.99	FM	(Baig and Kazi 2012)
			Peppermint	0.99	FM	
			Spinach	0.98	FM	
	57.3	0.008	Spinach	0.93	FM	(Baig and Kazi 2012)
			Peppermint	0.92	FM	
			Pea	0.87	FM	
	49	-	Lettuce	10.1	DM	(Yañez et al. 2019)
			Broadbean	0.42	DM	
	49	1.44	Broadbean	1.28	DM	(Yañez et al. 2019)
	46	-	Tomato	0.16	FM	(Paltseva et al. 2018)
			Lettuce	0.07	FM	
			Carrot	0.02	FM	
	15.7	0.52	Potato	0.73	DM	(Das et al. 2004)
			Rice	0.14	DM	
Pb	2000	-	Lettuce	35.6	DM	(Schreck et al. 2014)
	25.5	Smelter[AD]	Lettuce	171.5	DM	
	752	0.37	Bean	8.30	DM	(Nayek et al. 2010)
			Pea	6.03	DM	
	744.5	-	Lettuce	19.68	DM	(Alexander et al. 2006)
			Onion	8.75	DM	
			Pea	1.40	DM	
	52.7	-	Cabbage	34	DM	(Singh et al. 2012)
			Pea	19	DM	
	34.65	-	Tea leaves	3.84	DM	(Wen et al. 2018)
	21.95	0.09	Cauliflower	17.9	DM	(Singh et al. 2010)
			Radish	13.2	DM	
			Cabbage	9.4	DM	
	17.1	-	Cabbage	5.8	DM	(Ter Haar 1970)
			Lettuce	3.2	DM	
			Carrot	2.1	DM	
	15.38	0.43	Spinach	2.90	DM	(Mahmood and Malik 2014)
			Cabbage	2.86	DM	
			Coriander	2.04	DM	
			Carrot	1.97	DM	
			Beetroot	0.64	DM	
			Potato	0.49	DM	
	0.867	0.18	Brinjal	14.2	DM	(Amin et al. 2013)
			Tomato	12.7	DM	
			Onion	11.2	DM	
			Garlic	8.3	DM	

$^{\Delta}$ fresh mass basis. ∞ dry mass basis.
[AD] atmospheric deposition for 6 weeks.

Baig and Kazi (2012) demonstrated that pea, peppermint and spinach grown in soil containing 57.3 $mg.kg^{-1}$ total As and irrigated with approximately 0.1 $mg.L^{-1}$ As will exceed modern food safety standards (Clever and Jie 2014). Note the maximum allowable level of As in irrigation water is 2 $mg.L^{-1}$ which accumulates to 177.8 $mg.kg^{-1}$ after 20 years at a rate of 1 000 mm per year, more than double the level of As in the Baig and Kazi (2012) trial. Similarly, Paltseva et al. (2018) demonstrated that crops grown in Pb loaded soil at 220 $mg.kg^{-1}$ will exceed food safety guidelines, with carrot showing Pb contamination of an order of magnitude greater than the food safety threshold of 0.2 $mg.kg^{-1}$. The results of Yañez et al. (2019) demonstrated the effect of As loaded irrigation water on broadbean seeds (inside the pod): broadbean seeds grown in soils containing 49 $mg.kg^{-1}$ As and irrigated with distilled water accumulated 0.42 $mg.kg^{-1}$ As (dry mass), but when grown in the same soil and irrigated with river water containing 1.44 $mg.L^{-1}$ As, the seeds accumulated 1.28 $mg.kg^{-1}$ As (dry mass). Therefore, the additional As inputs through irrigation water had a significant effect on the food safety of broadbeans, even though the seeds were not in direct contact with the irrigation water, which suggests translocation of As from leaves, stems and/or pods to seeds.

Since food safety guidelines are based on fresh mass data, the food safety of produce reported in all dry mass (DM) trials could not be determined with absolute certainly, as the water status of each crop at harvest was unknown. However, if one applies a conservative dry mass to fresh mass conversion factor of 0.10 (assuming a moisture content of 90 %), all DM results above 5 $mg.kg^{-1}$ probably exceeded the modern food safety guideline limit for As or Pb (Clever and Jie 2014). Therefore, of all the publications reporting As and Pb on a dry mass basis listed in Table 2.1, over half of the edible parts would have exceeded the food safety guideline thresholds and thereby would not be fit for human consumption.

The results of the studies listed in Table 2.1 have been plotted in Figure 2.2. Regression lines were drawn to fit the highest coefficient of determination (R^2), with Pb generating a linear regression line and As producing a logarithmic regression line. While conclusions on the relationship between soil As or Pb concentration and crop uptake could not be drawn by amalgamating the results of previous studies (due to the inherent variability of crop species, soil type, soil redox status and environmental conditions), general trends may be observed. In terms of As uptake, certain crops tend to accumulate a large quantity of As at low soil concentrations and then plateau as soil As load approaches 1 000 mg.kg^{-1}. However, Pb concentrations in plant tissues seem to be directly proportional to Pb concentrations in the soil, with crop Pb concentrations increasing as soil Pb is increased.

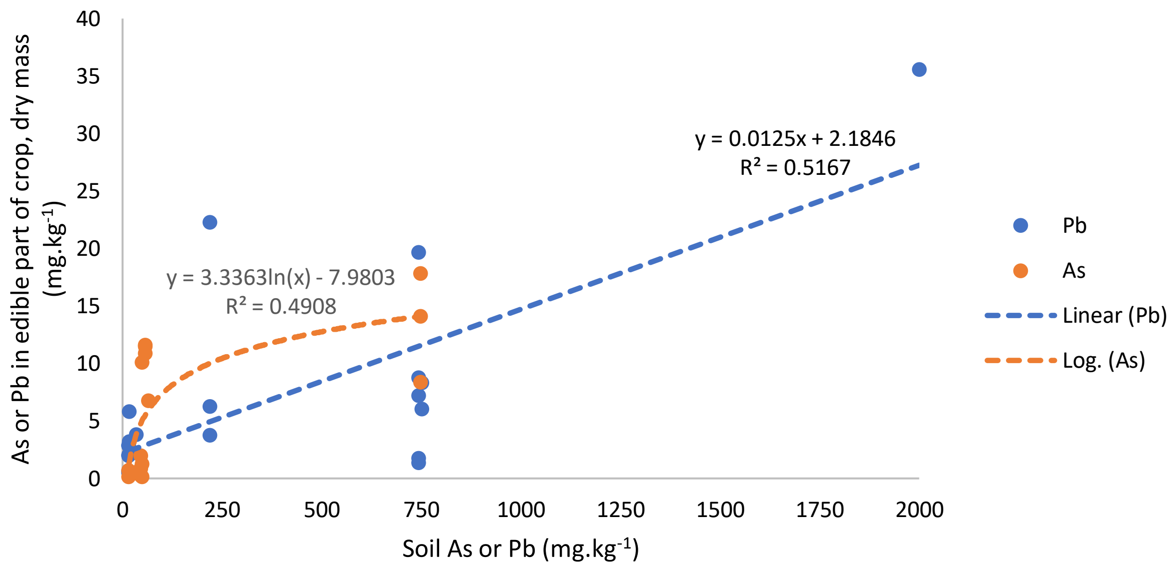

FIG 2.2: Amalgamation of results of previous studies on As and Pb accumulation in the edible parts of crops grown in contaminated soils

To limit the effect of crop species on As and Pb uptake versus soil concentration, a case-study of lettuce from the literature presented in Table 2.1 is shown in Figure 2.3. Lettuce was chosen because it was the most common crop presented in previous studies that is grown in oxidising soil environments, similar to the redox environment of crops grown for the purpose of this dissertation. While soil type and environmental factors could not be controlled, the amalgamation of data from the literature on lettuce presented a similar trend to that of Figure 2.3, except with far greater coefficients of determination, suggesting a stronger link between the regression lines and data points.

Therefore, in the case of lettuce, the leaves seem to exhibit a finite absorption capacity for As, but not for Pb (up to 2 000 $mg.kg^{-1}$ of Pb in soil). Furthermore, at low soil concentrations of As or Pb, lettuce leaves will tend to accumulate proportionally higher concentrations of As; however, at high soil concentrations of As or Pb, lettuce leaves will accumulate proportionally higher concentrations of Pb. The uptake and translocation mechanisms of As and Pb in crops are discussed in Section 2.9.

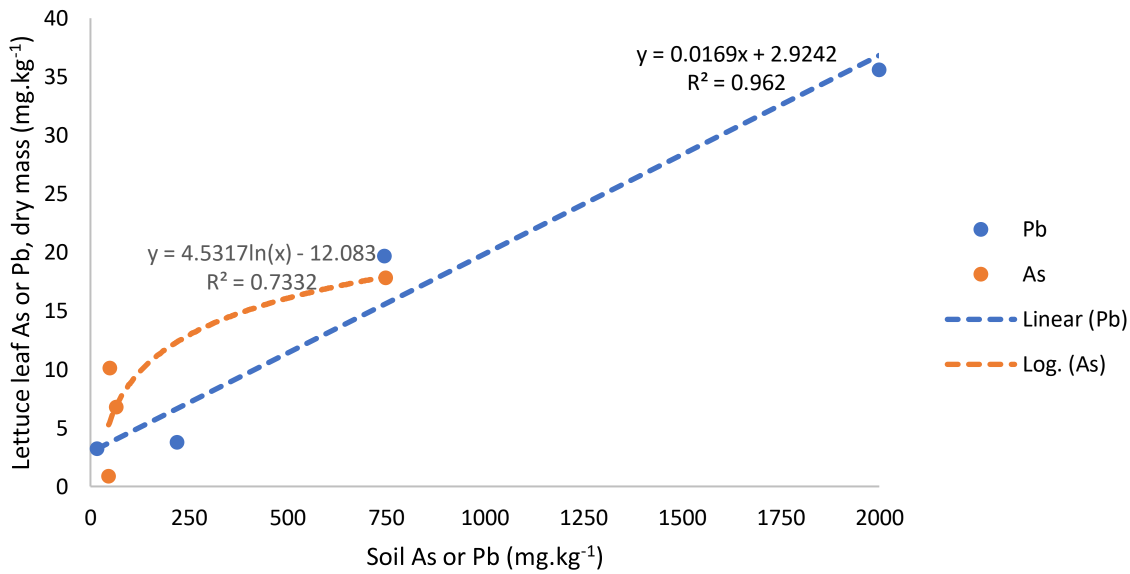

FIG 2.3: Results of previous studies on As and Pb accumulation in lettuce leaves grown in contaminated soils

2.3 Human Health Implications

As demonstrated in Section 2.2, crops accumulate significant quantities of As and Pb when grown in trace element impacted environments. The health implications of being exposed to As or Pb through the consumption of contaminated foods are discussed in the following sections.

2.3.1 Human exposure to dietary arsenic and associated health risks

Arsenic is ranked first on the priority list of hazardous substances complied by the US Environmental Protection Agency (US EPA) (ATSDR 2015). Arsenic is a ubiquitous element, found in relative abundance within the earth's crust as elemental arsenic (As) and two oxyanions, arsenite (AsIII) and arsenate (AsV), collectively termed inorganic arsenic (iAs) (Kabata-Pendias and Mukherjee 2007). Inorganic arsenic has been shown to accumulate in soils, with soils exhibiting As-enrichment compared to their parent materials (Mandal and Suzuki 2002). Consumption of As contaminated foods, particularly foods with high iAs, has been linked to numerous chronic health conditions, collectively termed "Arsenicosis", including: developmental neurotoxicity in children, blackfoot disease, cirrhosis, peripheral neuropathy, diabetes, heart failure and cancer (Sambu and Wilson 2008, Abdul et al. 2015, Alamolhodaei et al. 2015, Tsuji et al. 2015). Additionally, chronic As exposure has been associated with elevated risk of miscarriages and still-births in some developing African nations (Amadi et al. 2017). Inorganic arsenic is now seen as the critical species of concern when evaluating the human health risks of dietary As, since most organic As is considered either non-toxic or potentially-toxic to humans (Cubadda et al. 2017).

According to Cubadda et al. (2017), the primary source of iAs exposure to humans is through the consumption of rice and rice-derived products, non-rice-based cereals and cereal products (typically wheat), certain vegetables, fruits and fruit juices, shellfish, seaweeds and alcohol. Only populations that are chronically exposed to high As levels in drinking water (>50 µg/L), show drinking water as the primary source of iAs intake (EFSA 2009, Podgorski et al. 2017). Many crops exhibit the potential to accumulate substantial quantities of iAs in their edible parts (Das et al. 2004, Paltseva et al. 2018), while animals metabolise and/or excrete most of the iAs they consume, resulting in minimal iAs in meat and meat-based products (Feldmann and Krupp 2011, Lei et al. 2013). This is likely the reason why the allowable concentration of As in animal feed is 10 times higher than that for human consumption. Terrestrial mammals, including humans, typically metabolise and excrete As in the form of monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA), while in marine environments, As is incorporated into non-toxic organic structures (e.g. arsenosugars and arsenolipids) and accumulated (Feldmann and Krupp 2011). DMA, and traces of MMA, are present in various plant-based foods (Cubadda et al. 2017). *In vivo* studies have shown DMA and

MMA to have adverse effects on the kidneys, bladder and thyroid (FDA 2016). While DMA has demonstrated carcinogenic potential in animals, but MMA has not (IARC 2012). Accordingly, both DMA and MMA have been classified as "potentially toxic" from a food safety perspective (Feldmann and Krupp 2011). Arsenobetaine, a form of organic As abundant in shellfish, see Table 2.2, is largely assumed to be of no toxicological concern (FAO/WHO 2011). Current food safety guidelines have set maximum thresholds of total As in food that range between 0.1 $mg.kg^{-1}$ and 0.3 $mg.kg^{-1}$ fresh mass (DAFF 2011, Clever and Jie 2014). However, the new train of thought calls for iAs, and the ratio of iAs to total As, to be used as a more reliable means of identifying hazardous As exposure (EU 2015, Cubadda et al. 2017), highlighting the realisation that a better understanding of As chemistry is needed. Table 2.2 illustrates the general trends regarding As speciation in food groups that are important contributors to dietary As intake.

TABLE 2.2: Estimated distribution of As species within food groups of significance in terms of human dietary exposure (Cubadda et al. 2017)

Food group	Inorganic As (%)	Organic As		Total (%)
		DMA and MMA (%)	Arsenosugars and arsenolipids (%)	
Rice	60	39	1	100
Non-rice cereals	95	5	0	100
Vegetables	90	9	1	100
Fruit	80	20	0	100
Shellfish	45	5	50	100
Seaweeds	10	89	1	100
Beverages	95	5	0	100
Water	100	0	0	100

Non-rice cereals (such as wheat), vegetables, fruit and beverages (such as beer) that exhibit high quantities of As are of serious concern to the general public, since at least 80% of the total As contained therein is inorganic and extremely hazardous to human health. Shellfish and seaweeds are less so, as the majority of As contained therein is of the organic form. Due to the semiaquatic anaerobic conditions of rice paddy fields that reduce arsenite to the more mobile arsenate, and the ability of rice roots to adsorb and translocate significant quantities of As into the grain, rice grains tend to contain extraordinarily high iAs concentrations (Cubadda et al. 2017, Davis et al. 2017), with the mean iAs concentration in published data being reported at 0.13 $mg.kg^{-1}$ (Lynch et al. 2014). All other terrestrial food groups only exhibit elevated levels of iAs when grown in highly As contaminated environments, or cooked and/or processed in As contaminated water (Lynch et al. 2014). Human populations that consume largely cereal- and vegetable-based diets are most at risk of chronic As exposure, particularly those in geographical regions that exhibit elevated As levels in rock, soil and groundwater (Kabata-Pendias and Mukherjee 2007). The highest rates of arsenicosis have been reported in some parts of China, Mongolia, South-East Asia, Eastern Europe, East Africa, Chile, Argentina and Western United States (Kabata-Pendias and Mukherjee 2007).

2.3.2 Human exposure to dietary lead and associated health risks

Lead is ranked second on the priority list of hazardous substances complied by the US EPA (ATSDR 2015). According to the World Health Organisation (2016), no known level of Pb exposure is considered safe. Human exposure to Pb is primarily through food and water, and, in the case of toddlers and small children, the consumption of soil (Guerin et al. 2017). Children are most at risk of Pb toxicity, as their gastrointestinal tracts absorb 4 to 5 times more Pb from food than their adult counterparts (WHO 2016). Therefore, the Pb content of milk and cereal-based products are closely monitored by international organisations, as these foods are typically the first to be introduced to the diets of infants (EFSA 2010).

Lead is a cumulative toxicant known to affect multiple body systems (Guerin et al. 2017). Once consumed, it is distributed to the brain, kidney, liver and bones (Guerin et al. 2017). Lead accumulated in teeth and bones have been shown to persist for up to 30 years (Guerin et al. 2017). Furthermore, Pb in the bones of pregnant mothers has been shown to remobilise into the blood stream and become a source of exposure to the developing foetus, resulting in an increased risk of birth defects (Kim et al. 2016). Lead exposure in children has been linked to developmental neurotoxicity, often manifesting as reduced intelligence quotient (IQ), attention deficit disorder (ADD), antisocial behaviour or limited educational attainment (WHO 2016). Tragically, these neurological and behavioural effects are believed to be irreversible (WHO 2016, EFSA 2010). Other effects of chronic Pb toxicity include renal impairment, hypertension, immunotoxicity, anaemia and toxicity to the reproductive organs (WHO 2016). In adults, Pb exposure has been linked to nephrotoxicity, cancer and cardiovascular effects (EFSA 2010, Vazquez et al. 2017).

According to the European Food Safety Authority (2012), the highest contributors of Pb to the European diet are cereals and grains (14.3 - 16.3%), seafood (18%), fruit (14.4%), milk and dairy products (10.6%), vegetables (8.4 - 14.3%), water (7 - 11%), and alcoholic beverages (6.7 - 14%). Due to the fact that higher plants do not possess specific Pb translocation mechanisms, root and tuber crops typically exhibit considerably higher Pb concentrations in their edible parts compared to other crops, often exceeding food safety limits of 0.1 to 0.3 $mg.kg^{-1}$ fresh mass when grown in lead-rich environments (Alexander et al. 2006, Khan et al. 2008, Ding et al. 2013, Amin et al. 2013). Lead in fresh produce is largely present in inorganic form with +2 oxidation state (Vazquez et al. 2015). However, some organic forms of Pb have been identified in certain foods and beverages, such as trimethyl Pb in wines from southern France (Vazquez et al. 2015). Due to limited speciation of Pb in food products, total Pb concentration is considered a reliable means of identifying hazardous Pb exposure (Vazquez et al. 2015).

The Institute for Health Metrics and Evaluation (IHME) estimated that chronic Pb exposure accounted for 853 000 deaths in 2013, most of which occurred in developing nations (WHO 2016). Further estimations from the IHME indicated that globally 9.3% of idiopathic intellectual disability, 4% of ischaemic heart disease and 6.6% of strokes, were attributed to the long-term effects of Pb exposure (WHO 2016). According to the WHO (2016), Pb toxicity is entirely preventable. While great advances have been made to limit the use of Pb in fuel, paint and piping, more needs to be done to address Pb contamination in fresh produce, processed foods and the environment (EFSA 2010).

2.4 Food Safety Guidelines for Trace Elements

Historically, the primary focus of food safety has been the prevention of microbial contamination and spoilage (McLaughlin et al. 1999, Zaccheo et al. 2017). It was only as recently as 1997 that trace element contamination was first discussed at the Joint FAO/WHO Expert Committee on Food Additives (JEFCA 1997). In 1998, the first general limit for Pb in foods was set at 2 $mg.kg^{-1}$ (JECFA 2002). The following year, a general limit of 1 mg/kg^{-1} for cadmium and mercury and “none” for As was proposed (JECFA 2002). It wasn’t until the SCOOP-task 3.2.11 of 2004 that the various sources of human dietary exposure to As, Cd, Pb and Hg were investigated, leading the way towards the development of source-specific guidelines (EU 2004). In 2006, the first food source-specific guidelines were established for Pb, Cd, Hg and Sn, which saw a marked decrease from the original general limit of 2 $mg.kg^{-1}$ for Pb and 1 $mg.kg^{-1}$ for Cd and Hg (EC 2006). Today, the CODEX STAN Alimentarius serves as the benchmark for international food standards, providing limits for As, Cd, Pb, Hg, methylmercury, Sn and radionuclides in various food products (CODEX STAN 2015).

Countries vary greatly in the number of trace elements and variety of food products monitored. China holds the most comprehensive food safety guidelines: monitoring 6 elements from a wide variety of fresh and processed foods, including limits that are specific to fruits, vegetables and grains (Clever and Jie 2014). Ireland also allocates food safety standards according to fruit and vegetable sub-groups but is limited to Cd and Pb (FSAI 2009). Today, South Africa makes use of The Commission of the European Communities’ food safety guidelines (DAFF 2011). The aforementioned food safety guidelines are summarised in Table 2.3. The food safety guidelines for Canada, USA and FAO/WHO were also investigated, but have been omitted from Table 2.3 because they either did not list enough elements or did not specify food safety standards for fruits, vegetables and grains (AFFI 2015, CODEX STAN 2015, GOC 2018, US FDA 2018). One noteworthy observation is that food safety guidelines are generally consistent from nation to nation. The methods by which food safety guidelines were determined are discussed in the following section.

TABLE 2.3: Summary of food safety guidelines for fresh produce in China, the EU (South Africa) and Ireland

Potentially hazardous trace element	Regulatory body / Country	Acceptable range in foods ($mg.kg^{-1}$ FM)	Specific to agronomic produce	Threshold value ($mg.kg^{-1}$ FM)
Arsenic (As)	European Union (& South Africa)	0.1 – 0.3	Rice (polished)	0.2
	Canada	0.1 – 3.5	N/A	
	Australia / New Zealand	1.0 – 2.0	Cereals	1.0
	China	0.2 – 0.5	Grains (excl. rice) and fresh vegetables	0.5
			Rice	0.2
Cadmium (Cd)	European Union (& South Africa)	0.05 – 0.2	Wheat, rice, soybean, leaf vegetables, fresh herbs, leafy brassica, celery, parsnips, salsify, horseradish and fungi	0.2
			Other cereals, potatoes, root, tuber and stem vegetables	0.1
			Fruit and all other vegetables not previously mentioned	0.05
	Australia / New Zealand	0.1 – 0.5	Peanuts	0.5
			Grains, root and tuber vegetables, leafy vegetables	0.1
	China	0.05 – 0.5	Nuts, peanuts and seeds	0.5
			Rice, beans, celery, leaf vegetables	0.2
			Grains (excl. rice), legumes, root, tuber and stem vegetables	0.1
			Fruit and all other vegetables not previously mentioned	0.05
Chromium (Cr)	China	0.5 – 1.0	Grains and beans	1.0
			Fresh vegetables	0.5
Mercury (Hg)	European Union (& South Africa)	0.1 – 1.0	N/A	
	Canada	0.5 – 1.0	N/A	
	Australia / New Zealand	0.5 – 1.0	N/A	
	China	0.01 – 1.0	Grains	0.02
			Fresh vegetables	0.01
Lead (Pb)	European Union (& South Africa)	0.1 – 0.3	Brassica vegetables, leaf vegetables, fungi	0.3
			Cereals, legumes, pulses, berries and small fruit	0.2
			All other fruit and vegetables not previously mentioned	0.1
	Canada	0.08 – 10.0	N/A	
	Australia / New Zealand	0.1 – 0.3	Brassica vegetables	0.3
			Cereals, pulses and legumes	0.2
			Fruit and all other vegetables not previously mentioned	0.1
	China	0.1 – 0.3	Brassica vegetables, leaf vegetables	0.3
			Cereals, legumes and potatoes	0.2
			Fruit and all other vegetables not previously mentioned	0.1

$^{\Delta}$Tin was excluded from guidelines as it only applies to canned food products.

2.4.1 Determination of food safety guidelines

Clinical and epidemiological cohort studies of large population groups from various countries, as well as biochemical and toxicological laboratory-based animal studies, are performed to determine human food safety guidelines (FAO JECFA 2011). The cohort studies originally aimed to quantify chronic dietary exposure of potentially hazardous elements, such as Pb, As, Hg and Cd, through food intake. However, later meetings resolved to include the impact of drinking and cooking water on daily exposure (EFSA 2014). After quantifying average daily intake per age class (infants and toddlers, children, adolescents, adults, elderly and very elderly) to the 95th percentile, benchmark dose lower confidence limits for a 0.5 % increased incidence of certain associated aliments ($BMDL_{0.5}$), such as specific cancers, are determined from sample subsets (EFSA 2014). Thereafter, the $BMDL_{0.5}$ values are worked back per contribution of each food group to potentially hazardous chronic exposure and a preliminary food safety guideline is tabled.

Acute toxicological studies on humans and animals are also included in the development of food safety guidelines (EFSA 2014). In human studies, lowest-observed-adverse-effect levels (LOAELs) are determined on a per body weight basis. In animal studies, both LOAELs and LD_{50}, the concentration of toxicant sufficient to kill 50 % of the population, are reported. While LOAELs and LD_{50} are valuable, food safety guidelines for trace elements are based on the associated $BMDL_{0.5}$ values, as the aim of the guidelines is to mitigate health risks associated with chronic exposure to elements through dietary intake (FAO JECFA 2011, EFSA 2014, CODEX STAN 2015).

Taking arsenic as an example: the European Food Safety Authority (2014) investigated a data set of 107 646 analytical results collected in 21 European countries to estimate dietary exposure to inorganic arsenic (iAs), the most detrimental form of the element. A total of 101 020 food samples were analysed for As content, of which, 2 753 reported iAs. The remainder were converted from total As to iAs through corroborated conversion factors (EFSA 2014). A total of 24 884 drinking water samples were also analysed, where total As was equal to iAs. Using dietary surveys completed by 53 728 individuals, and assuming a 60 kg body mass per adult subject, the mean dietary exposure to iAs among all surveys in the adult population ranged from 0.09 to 0.38 $\mu g.kg^{-1}$ body weight per day and from 0.14 to 0.64 $\mu g.kg^{-1}$ body weight per day at the 95th percentile (EFSA 2014). Thereafter, data from human clinical/epidemiological studies as well as biochemical and toxicological animal studies were analysed to determine $BMDL_{0.5}$ limits via dose response modelling (BMDS version 2.1.1). For example, a prospective cohort epidemiological study of 8 086 subjects aged 40 years and older, monitored over 12 years, was one of 4 cohort studies used to evaluate $BMDL_{0.5}$ limits for urinary cancer among East Asian populations (FAO JECFA 2011). Arsenic in their drinking water was used as the key determinate for As exposure, while external factors such as smoking habits were recorded and analysed separately (FAO JECFA 2011).

While a $BMDL_{0.5}$ may be a conservative figure from which to draw food safety guidelines, the total number of analytical results employed in these studies bear testament to the reliability of the findings. Guidelines continue to be updated and improved upon with increasingly detailed population surveys to limit the influence of external variables when determining the impacts of individual food groups, such as fruits, vegetables and grains, on chronic daily elemental intake (EFSA 2014). Unlike irrigation water quality guidelines, where over two thirds of modern guidelines can be traced back to a single water quality criteria report of the National Technical Advisory Committee to the Secretary of the Interior, USA, in 1968 (USNSA 1972), food safety guidelines are continually being revised at quarterly meetings of the Joint FAO/WHO Expert Committee on Food Additives.

To date, the FAO/WHO has yet to publish an absolute food safety guideline for As, citing the need for more detailed dietary surveys, the inclusion of drinking and cooking water to daily exposure models, as well as additional cohort studies from under-represented regions (FAO/WHO 2016). However, guidelines for lead and Cd have been available for some time (CODEX STAN 2015). Unconstrained by the need to collate data from globally representative population groups, countries such as China and the

European Union are paving the way by introducing new elements, such as As and chromium (Cr), into food safety guidelines.

2.4.2 Limitations of modern food safety guidelines for trace elements

Unlike China and the EU, most countries, like South Africa, have not collated enough epidemiological data to develop food safety guidelines specific to the eating habits of their own populations or to contribute to the refinement of the FAO/WHO guidelines. Only populations from Europe, western and eastern Asian countries, and to a certain extent, the United States and Canada, are well represented in the development of global food safety guidelines which countries like South Africa utilise (JECFA 2011). Therefore, a distinct Anglo-Asian bias exists in modern food safety guidelines which should be acknowledged by South American and African policy makers.

Another limitation to current guidelines is the number of potentially hazardous elements, such as radionucleotides, nickel, chromium and mercury that have yet to be included. While the CODEX STAN (2015) did include most potentially hazardous elements, the findings were not readily adopted. While the development of food safety guidelines for trace elements is relatively new, the slow incorporation of new elements needs to be addressed to mitigate the associated human health risks. Some nations, such as the United States, are so seemingly averse to setting food safety guidelines that to date the FDA has not set a general food safety guideline for any element (FDA 2018). Regulations have only been set for a few highly specific cases, such as As in infant rice cereal (FDA 2018). Similarly, the limited number of food groups that are included in most food safety guidelines needs to be expanded to include fresh produce, as most focus on processed goods (DAFF 2011, GOC 2018). This is particularly important for developing nations, like South Africa, where fresh produce and staple grains make up a large portion of the local diet (Labadarios et al. 2007).

All food safety guidelines for trace elements are expressed on a fresh mass basis. The advantage of such is that people consume most produce either fresh or cooked, not dried. Therefore, quantifying daily intake of certain foods is simplified when determined on a fresh mass basis and does not necessitate the use of conversion factors, which are a potential source of error (Nielsen 2010). However, moisture content of fresh produce can vary tremendously depending on the crop's water status at harvest, storage duration and conditions after harvest, cooking practices and other factors (Rickman et al. 2007, Nielsen 2010). Agronomists, and biologists alike, almost always present plant analysis data on a dry mass basis (SERAS 1994). Dry mass is considered a more reliable measure of mass than fresh mass, as it excludes effects of fluctuating water contents in biological materials. Dry mass thereby normalises results to a substantially uniform standard, allowing for direct comparison between datasets (SERAS 1994).

Finally, it is a methodological standard that all root and tuber vegetables be peeled before analysing for trace elements (CODEX STAN 2015). While this practice is done to prevent residual soil from contaminating the samples, peels have been shown to contain 5 to 70 times more As than the internal tissues (Muñoz et al. 2002). Since food scientists expresses mass on a fresh basis to cater for the form in which foods are consumed, it appears inconsistent to omit peels from trace element determination, when many consume root and tuber vegetables in their entirety. Therefore, a large source of dietary trace element intake may be omitted from guideline development due to this methodological standard requirement.

2.5 South African Animal Feed Guidelines

While the focus of this dissertation is to investigate the effect of As and Pb accumulation in crops via irrigation inputs on human food safety, animal feed guidelines have been included in the results (Chapters 4 and 5) to determine whether the crop residues (remaining leaves and stems) would be fit for animal consumption. Table 2.4 presents the applicable animal feed regulations for As and Pb from the Farm Feeds General Guidelines (Act No. 36 of 1947) of South Africa. Note that the Pb feed guidelines are significantly higher (10 $mg.kg^{-1}$ or 40 $mg.kg^{-1}$ at 12% moisture) than the As feed guidelines (2 $mg.kg^{-1}$ or 4 $mg.kg^{-1}$ at 12% moisture).

TABLE 2.4: As and Pb regulations for plant derived animal feeds (Act No. 36 of 1947)

Element	Farm feeds	Maximum content ($mg.kg^{-1}$), with a feed moisture content of 120 $g.kg^{-1}$
As	Feed ingredients with the exception of:	2.0
	Hays, straws, lucerne, roughages and bagasse	4.0
Pb	Feed ingredients with the exception of:	10.0
	Green roughages	40.0

The guidelines for Pb refer to “green roughage”, which, while not defined in Act 36, is defined by the dairy industry as “containing moisture from 60 – 90 % and classified into various types, such as: pasture, cultivated fodder crops, tree leaves, roots and crops” (DKP 2019). Green roughages are allocated a much higher feed safety threshold, likely in order to compensate for the high moisture content when consumed by animals.

2.6 Irrigation Water Quality Guidelines for Trace Elements

Irrigation water quality guidelines from around the world typically make use of two threshold values per element of potential concern: a lower, 100-year, threshold, and a higher, 20-year threshold (CCME 1987, DWAF 1964, ANZECC and ARMCANZ 2000, CCME 2008, du Plessis et al. 2017). In a recent update, the South African irrigation water quality guidelines have adopted this approach as well (du Plessis et al. 2017). The lower threshold is considered the “target” trace element concentration and represents the concentration of trace element that can be applied in irrigation water for 100 years before phytotoxicity in the soil is reached (CCME 1987). In contrast, the higher threshold represents the maximum acceptable concentration that can be applied before reaching phytotoxicity in only 20 years. Most guidelines require that certain “forgiving” soil conditions must be met (fine textured, neutral to alkaline pH) before the 20-year element concentration may be utilised (CCME 1987, DWAF 1964, ANZECC and ARMCANZ 2000, CCME 2008, du Plessis et al. 2017).

For example: Assuming 1000 mm of irrigation per hectare per year (DWAF 1996), the 20 year threshold for aluminium has been set at 20.0 $mg.L^{-1}$ and the 100 year threshold has been set at 5.0 mg.L^{-1}. Therefore, after 20 years irrigating at the higher concentration, 0.4 kg of Al would have been deposited per square meter; but, after 100 years of irrigating at the lower concentration, 0.5 kg of Al would have been deposited per square meter. The longer the planned time that a field is to be irrigated, the lower is the permissible concentration of trace element contamination in irrigated water.

For this dissertation, the lower permitted concentration (100-year threshold) for each element was referred to as the “target water quality” and the upper limit (20-year threshold) was referred to as the “maximum acceptable” concentration.

2.6.1 Historical development of irrigation water quality guidelines

The origins of South Africa’s irrigation water quality guidelines for trace element thresholds can be traced back to the Water Quality Criteria (WQC) report of 1972, prepared by the US National Academy of Sciences (NAS-NAE 1973, Westcot and Ayers 1984, CCME 1987, DWAF 1996, ANZECC and ARMCANZ 2000). The US Water Quality Act of 1965 made first provision for states and federal government to establish water quality standards and subsequently the National Technical Advisory Committee to the Secretary of the Interior prepared a WQC report in 1968 (NAS-NAE 1973). Although the WQC report of 1972 drew significantly from its 1968 predecessor, it also presented a synopsis of studies from 1913 to 1972 and critically reviewed all presented data to develop various water quality guidelines, including those for agricultural use. The guidelines presented two recommendations for maximum trace element concentrations in irrigation waters: the first pertains to continuous use on all soil types, while the second allows for 20 years of irrigation on fine textured, neutral to alkaline soils (NAS-NAE 1973). This set the standard for all subsequent guidelines, with minor alterations, such as the move from “continuous use” to use for 100 years. In 1984, the Food and Agriculture Organisation of the United Nations (FAO) adopted all irrigation water quality guidelines set out in the WQC report of 1972, which have remained unchanged to the present day (Westcot and Ayers 1984, FAO 2017). Figure 2.4 graphically summarises the advances in irrigation water quality guidelines from first publication to the present day.

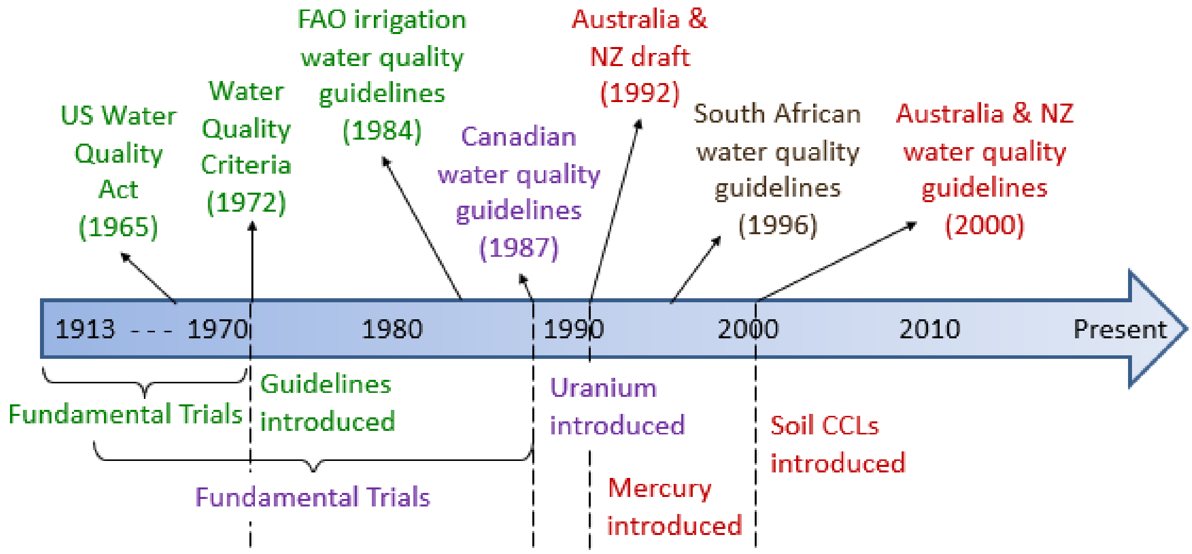

FIG 2.4: Critical publications and milestones in the development of irrigation water quality guidelines

The next advancement was the introduction of the Canadian Water Quality Guidelines in 1987 (CCME 1987). The guidelines reviewed and incorporated research from 1933 to 1987 and presented new guidelines for B, Co, F, Pb and Zn. It is here that uranium was first introduced as an element of concern (CCME 1987). The remaining element guidelines were either identical to the WQC (1972) report's 20-year thresholds or, in the case of molybdenum, removed from the list entirely (CCME 1987). At this point one must note that all studies pertaining to irrigation water qualities focused almost entirely focused on the effect of trace elements on crop growth, yield and yield quality (NAS-NAE 1973, CCME 1987). While some studies did investigate trace element accumulation in the edible plant parts (Ter Haar 1970), the reports could not categorically state whether or not the food was safe for human consumption, because food safety guidelines for trace elements would only emerge in the late 1990's and early 2000's.

In 1992, the draft irrigation water quality guidelines for Australia and New Zealand was published and marked the introduction of mercury into irrigation water quality guidelines (Hart et al. 1992). Four years later, South Africa published its own set of irrigation water quality guidelines that incorporated trace element thresholds from all three of the aforementioned guidelines (DWAF 1996). As discussed in section 2.4, it was only as recently as 1997 that potentially hazardous trace element contamination in food stuffs was first discussed at the Joint FAO/WHO Expert Committee on Food Additives (JEFCA 1997). In 1998, the first general limit for Pb in foods was set at 2 $mg.kg^{-1}$ (JECFA 2002). The following year, a general limit of 1 $mg.kg^{-1}$ for Cd and Hg and "none" for As was proposed (JECFA 2002). Therefore, due to limited information on the impacts of potentially hazardous trace elements on the food safety of fresh produce at the time (1996), the South African Irrigation Water Quality Guidelines could not have quantitatively included food safety in the development of the guidelines.

The next advancement came in the year 2000, where the latest version of the Australian and New Zealand guidelines presented soil cumulative contaminant loading (CCL) limits for some metals and metalloids (ANZECC and ARMCANZ 2000). Since then, international guideline revisions have focused primarily on the development of software packages and the addition of biological contaminants, while little attention has been paid to the original trace element concentrations (Britz and Sigge 2012a-d, WRC 2014, du Plessis et al. 2017). The 2017 update to the South African irrigation water quality guidelines has included a risk evaluation system (based on soil, irrigation and crop parameters), included *E-coli* as a food safety parameter, and developed a software package to monitor irrigation water quality, which is freely available to farmers (du Plessis et al. 2017). The update has yet to address the food safety risk of potentially hazardous trace elements. Nor has this been addressed in any other irrigation water quality guideline updates, so far as the author is aware, despite numerous publications which have demonstrated that crops grown in trace element rich environments pose a food safety risk (Singh et al. 2012, Amin et al. 2013, Yañez et al. 2019).

One may speculate that the next advancement in irrigation water quality guideline development will be the inclusion of food safety with regard to potentially hazardous trace elements.

2.6.2 Fundamental trials for modern irrigation water quality guidelines

Irrigation water quality guidelines are derived from a multitude of fundamental trials performed between 1913 and 1987 which investigated the effect of elevated trace elements in soil or nutrient solutions on crop growth, yield and yield quality (NAS-NAE 1973, CCME 1987, US EPA 1976). The aim of the majority of these trials was to quantify the point at which soil contaminant loads would result in a marked yield reduction in a variety of crops.

Thereafter, a maximum trace element concentration in soil was decided upon and guidelines for element loads in irrigation water were calculated accordingly. For example, Table 2.5 presents the results derived from six fundamental trials referenced by the Water Quality Criteria (1972) report as justification for maximum allowable As loads in irrigation water (NAS-NAE 1973). The results therefrom have since been adopted by all subsequent irrigation water quality guidelines, including those of the FAO, USA, Canada, Australia and New Zealand, and South Africa (Westcot and Ayers 1984, CCME 1987, US EPA 1994, DWAF 1996, ANZECC and ARMCANZ 2000, CCME 2008, FAO 2017). It should be noted that all subsequent guidelines limit the irrigation period based on cumulative element loading in-field, beyond which, the field is no longer agronomically productive. This demonstrates that without appropriate remediation strategies, irrigation with trace element rich water is fundamentally unsustainable.

TABLE 2.5: Fundamental trial results for the determination of As loads in irrigation water (NAS-NAE 1973)

Dosing Method	Crop	Concentration	Findings	Source
Nutrient solutions	Cowpeas	0.5 $mg.L^{-1}$	Reduced root growth.	(Albert and Ardnt 1931)
	Cowpeas	1.0 $mg.L^{-1}$	Reduced root and shoot growth.	
Nutrient solutions	Pine	0.5 $mg.L^{-1}$	Toxicity symptoms.	(Rasmussen and Henry 1965)
	Apple	0.5 $mg.L^{-1}$	Toxicity symptoms.	
	Orange	0.5 $mg.L^{-1}$	Toxicity symptoms.	
Nutrient solutions	Tomatoes	0.5 $mg.L^{-1}$	80 % yield reduction	(Clements and Heggeness 1939)
Nutrient solutions	Citrus	10 $mg.L^{-1}$	Reduced root and shoot growth.	(Liebig et al. 1959)
	Citrus	5 $mg.L^{-1}$	Reduced root and shoot growth.	
Nutrient solutions	Beans	1.2 $mg.L^{-1}$	Growth suppression.	(Machlis 1941)
	Sudan Grass	12 $mg.L^{-1}$	Growth suppression.	
Added to Top Soil	Barley	60 $mg.kg^{-1}$	50% yield reduction in sandy loam.	(Crafts and Rosenfels 1939)
	Barley	95 $mg.kg^{-1}$	50% yield reduction in loam.	
	Barley	115 $mg.kg^{-1}$	50% yield reduction in clay loam.	
	Barley	145 $mg.kg^{-1}$	50% yield reduction in clay.	

Irrigation water quality guidelines are near identical among countries, with many seeming to adopt their guidelines from those previously determined elsewhere in the world (CCME 1987, DWAF 1996, ANZECC and ARMCANZ 2000, CCME 2008, FAO 2017). Table 2.6 clearly demonstrates the trend by comparing the South African irrigation water quality guidelines to those of the FAO, Australia and New Zealand, and Canada.

When compared on an individual basis, the South African irrigation water quality guidelines share 74% similarity with the FAO guidelines, 76% similarity with the Australian and New Zealand guidelines, and 82% similarity with the Canadian guidelines. Overall, 97% of the South African water quality guidelines are grounded in foreign publications.

This level of similarity may prove problematic, for example: the Canadian guidelines, of which South Africa shares 82% similarity, were specifically developed for use under Canadian conditions (CCME 1987), which have appreciably different soil morphologies

TABLE 2.6: Similarities between South African and international irrigation water quality guidelines

Element	Irrigation Program Duration (years)	South African Irrigation WQGs (1994)	Water Quality Criteria (1972) & FAO (1984)	Australian and NZ Irrigation WQGs (2000)	Canadian Irrigation WQGs (1987 – 2008)
		($mg.L^{-1}$)			
Al	100	5	●	●	●
	20	20	●	●	●
As	100	0.1	●	●	●
	20	2	●	●	●
B	100	0.5	1	●	●
	20	1 - 15	2	●	6
Be	100	0.1	●	●	●
	20	0.5	●	●	●
Cd	100	0.01	●	●	●
	20	0.05	●	●	0.01
Co	100	0.05	●	●	●
	20	5	●	0.1	●
Cr	100	0.1	●	●	●
	20	1	●	●	0.1
Cu	100	0.2	●	●	●
	20	5	●	●	●
F	100	2	1	1	1
	20	15	●	2	●
Fe	100	5	●	0.2	●
	20	20	●	10	●
Li	100	2.5	●	●	●
	20	2.5	●	●	●
Mn	100	0.2	●	●	●
	20	10	●	●	●
Mo	100	0.01	●	●	ND$^{\Delta}$
	20	0.05	●	●	ND$^{\Delta}$
Ni	100	0.2	●	●	●
	20	2	●	●	●
Pb	100	0.2	5	2	●
	20	2	10	5	●
Se	100	0.02	●	●	●
	20	0.05	0.02	●	0.02
Ur	100	0.01	ND$^{\Delta}$	●	●
	20	0.1	ND$^{\Delta}$	●	●
Va	100	0.1	●	●	●
	20	1	●	0.5	●
Zn	100	1	2	2	●
	20	5	10	●	●
Similarity to South African WQGs:			74 %	76 %	82 %

*Authors of the South African irrigation water quality guidelines have confirmed that the 0.02 mg. L^{-1} value for manganese was a typing error and will be corrected to 0.2 mg. L^{-1} in the next update. $^{\Delta}$ Not determined. ● Identical to South African IWQGs.

and climatic conditions. Since temperature and rainfall play a major role in biophysicochemical transformations of trace elements in soil (Brady and Weil 2006), one may need to revisit such guidelines from within a South African context.

Of the 19 trace elements and 38 limits presented in the South African irrigation water quality guidelines, only 1 could not be found in either the FAO, Australian and New Zealand, or Canadian guidelines: fluorine's 100-year threshold of 2 $mg.L^{-1}$. A possible explanation for this is that while the fluorine limit of 2 $mg.L^{-1}$ is presented in the Australian Guidelines as the 20-year threshold, it is more likely that the South Africans adjusted their 100-year threshold upwards to cater for the exceptionally high 15 $mg.L^{-1}$ 20-year threshold, which they adopted from the FAO and Canadian guidelines (Westcot and Ayers 1984, CCME 1987).

As demonstrated in Table 2.6, few irrigation water quality guidelines have been updated since the WQC report of 1972 and the introduction of the Canadian Guidelines in 1987. Therefore, a critical review of the data from which these guidelines were initially developed could be of great benefit to modern agriculture, since method detection limits and the level of precision within investigative procedures have made significant advancements since those early trials from 1913 to 1987.

Many irrigation water quality guidelines were derived from the results of soil loading trials. Guidelines were typically set at the soil trace element concentration which resulted in economically significant yield losses in a variety of crops. The findings of such trials were converted into irrigation water quality guidelines according to the following assumptions (Westcot and Ayers 1984, CCME 1987, DWAF 1996, ANZECC and ARMCANZ 2000):

1. Irrigation rate of 1 000 mm per year.
2. Trace elements retained within the top 0.15 m of soil.
3. Soil bulk density of 1 500 $kg.m^{-3}$.

Evans et al. (1979) studied trace element mobility in a soil to which sewage sludge had been applied for over 80 years, illustrated in Figure 2.5. Although trace elements did mobilize beyond 0.15 m, there was a definite accumulation around the top 0 – 0.25 m of soil, confirming the 0.15 m limit to be a fair general assumption (Evans et al. 1979).

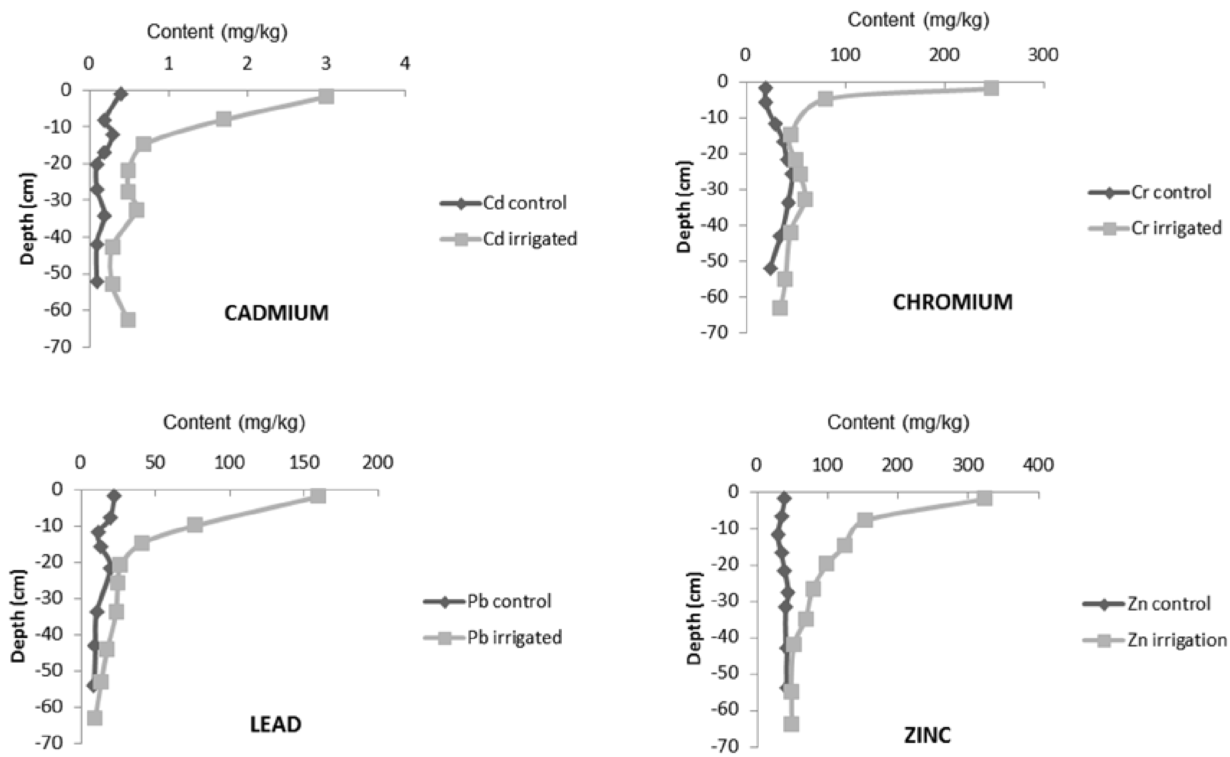

FIG 2.5: Heavy metal content of the soil profile after 80 years of irrigation with wastewater (after Evans et al. 1979)

If it is assumed that each trace element irrigation water quality guideline was based on a critical soil cumulative element loading limit, one would expect the final element accumulation to be near equal to both the 100 year and 20 year element thresholds. A logical explanation could be made for greater 100 year soil cumulative element loads, due to sequestration and transformation processes reducing element bioavailability (Brady and Weil 2006). However, this does not seem to be the case for all trace element thresholds.

Consider As and Pb: both have the same 20 year threshold of 2 mg.L^{-1}; however, the 100 year threshold for As is 0.1 mg.L^{-1} and 0.2 mg.L^{-1} for Pb. Therefore, while the 20-year soil cumulative element load for both As and Pb is 177.8 mg.kg^{-1}, the guidelines only permit 88.9 mg.kg^{-1} Pb and, even less, 44.4 mg.kg^{-1} As to accumulate over 100 years. Future revisions of the guidelines may need to investigate the details behind such discrepancies, as the author could not find clarification from the literature.

Taking the aforementioned assumptions into account, Table 2.7 illustrates the allowable accumulation of As and Pb in the top 15 cm of the soil profile at the end of each permissible irrigation period. For purpose of comparison, As and Pb rates as defined by the Permissible Utilisation and Disposal of Sewage Sludge Guidelines, Edition 1 (1997) have been included, along with South African soil screening values of As and Pb from the National Environmental Management: Waste Act (59/2008).

TABLE 2.7: Allowable maximum As and Pb accumulation in soils according to various South African regulatory guidelines

Regulatory body	Threshold scenario	As (mg.kg^{-1})	Pb (mg.kg^{-1})
South African Irrigation Water Quality Guidelines (1996)	100 years irrigation at "target water quality"	44.4 *	88.9 *
	20 years irrigation at "maximum permissible water quality"	177.8 *	177.8 *
Permissible Utilisation and Disposal of Sewage Sludge Edition 1 (1997)	Maximum permissible content in soil	2.0	6.6
	25 years of sludge application	1.33 •	3.77 •
	Classification as Type D sludge (for agricultural use)	15	50.5
South African soil screening values of metals, National Environmental Management: Waste Act (59/2008)	SSV1: All land uses protective of the water resource	5.8	20
	SSV2: Informal residential	23	110
	SSV2: Standard residential	48	230
	SSV2: Commercial/ industrial	150	19 000

* Accumulates in top 0.15 m of soil, 1000 mm irrigation inputs per year and soil bulk density of 1500 kg.m^{-3} (DWAF 1996).

• 8 tons of Type D sludge per hectare per year, accumulates in top 0.15 m of soil and soil bulk density of 1500 kg.m^{-3} (TT 85/97).

It is worth mentioning that the effects of other trace elements or major constituents (e.g.: cation effects, ligand effects, redox chemistry and complexation) do not seem to have been investigated or included in any of the guidelines mentioned in Table 2.3. For example, the oxidation state of As is not considered, even though the chemistry of As^{3+} and As^{5+} is vastly different. Other examples include the fact that Cd mobility is enhanced by chloride, Pb mobility should be greatly reduced in the presence of phosphate and U is mobilised by carbonate through complexation (Brady and Weil 2006). It may be worthwhile to include a level of chemistry knowledge to guide stakeholders in their decision making when confronted with contaminated irrigation water, sewage sludge or soil.

The sewage sludge guidelines provide by far the most conservative As and Pb soil loading rates of all the regulatory guidelines and seem to have been adopted from the German guidelines for sewage sludge (WRC 1997). The content of As and Pb in soils after irrigation, in accordance with the South African irrigation water quality guidelines, seem to fit around the "SSV2: Standard residential" and "SSV2: Commercial/ industrial" As and Pb screening values (Act 59/2008). According to definitions within the National Environmental Management: Waste Act (59/2008), SSV1 (Soil Screening Value 1) refers to soil quality values that are protective of human health as well as eco-toxicological risk for multi-exposure pathways, including contaminant migration to a water resource. SSV2 (Soil Screening Value 2) makes reference to soil quality values that are protective of risk to human health in the absence of a water resource. Similar to the soil screening values of the National Environmental Management: Waste Act (59/2008), a pragmatic approach to determining the cumulative soil loading rates of As and Pb via irrigation water, could be to differentiate loading rates according to the intended use of the field, such as for the production of foods for human consumption versus animal feeds.

2.6.3 Limitations of irrigation water quality guidelines

Irrigation water quality guidelines play a crucial role in protecting natural and agricultural resources. The guidelines serve as a common basis from which water quality objectives may be derived to empower all role-players to act in harmony and thereby ensure that fitness of water for specific uses is maintained, soil integrity and productivity is conserved, and the impacts on environmental and public health are mitigated. However, as alluded to previously, there are limitations within the irrigation water quality guidelines that need to be addressed:

1. The age of data (1913 - 1987) presented as trials from which the clear majority of guidelines are based should be revisited and updated to conform to modern analytical standards.
2. Guidelines developed for colder climates likely do not reflect the biophysicochemical conditions of soil and water in warmer climates. (This will be discussed in more detail in the upcoming sections).
3. The accumulation of potentially hazardous trace elements in soils, as permitted by the South African Irrigation Water Quality Guidelines, do not reflect that of the National Environmental Management: Waste Act (59/2008) or the Permissible Utilisation and Disposal of Sewage Sludge Guide (1997).
4. The quantity of trace element that are allowed to accumulate in the soil after 20 years of use at the upper threshold or 100 years of use at the lower threshold irrigation do not always result in approximately equal soil cumulative element loading.
5. Finally, due to the limited availability of food safety data at the time, the irrigation water quality guidelines could not have included food safety of potentially hazardous trace elements into consideration. Therefore, as food safety guidelines have developed, irrigation water quality guidelines for potentially hazardous trace elements should be updated to include the results of more recent studies which have linked trace element rich soils and irrigation water to crops that are unfit for human consumption.

The latter statement underpins the core of this dissertation, where the primary objective thereof is to assess the food safety risk of crops produced under conditions that are within the allowable limits of As and Pb in irrigation water, according to South African, and international, irrigation water quality guidelines.

Having discussed the origins and development of modern food and feed safety guidelines and irrigation water quality guidelines, the next section introduces the importance of having such guidelines within the South African context, where irrigation water is scarce, and the impacts of mining and industry have resulted in elevated levels of As and Pb in the local waterways.

2.7 The South African Water Crisis

Irrigated agriculture is the largest consumer of water in South Africa, utilising 61 % of the nation's available water (WWF-SA 2016). Being a semi-arid country, the average rate of South Africa's potential evaporation is three times higher than the average annual rainfall, therefore protecting this limited resource is of vital importance (WWF-SA 2016). With failing urban infrastructure and a rapidly expanding population, it is estimated that water demand will outstrip supply by 2030 (WWF-SA 2016).

South African farmers employ a variety of water sources to irrigate their crops, including surface waters (rivers and dams), groundwater and even treated sewage effluent (Thiam et al. 2015). To help address the limited supply, high density sludge treated mine water is also being evaluated as a new irrigation source for farmers (Annandale et al. 2009). Since numerous irrigation programs in South Africa are situated at the lower end of drainage basins, the water received is often polluted by upstream degradation activities (DWAF 1996). It is therefore probable that future irrigators will have to contend with both diminishing supply and deteriorating quality of irrigation water (Thiam et al. 2015).

If one considers the geology of the South Africa, the potential for heavy metal and metalloid contamination becomes apparent (Ashton et al. 2001). Seventy different ore minerals have been identified in the Witwatersrand goldfields conglomerates. The most abundant of which, after pyrite (FeS_2), are uraninite (UO_2), brannerite ($UO_3Ti_2O_4$), arsenopyrite (FeAsS), cobaltite (CoAsS), galena (PbS), pyrrhotite (FeS), gersdofite (NiAsS) and chromite ($FeCr_2O_4$). Arsenopyrite and galena are the primary sources of As and Pb respectively, in related mine waters (Naicker et al. 2003). Arsenopyrite, a water-soluble arsenic-containing sulphide, is present in the Greenstone Formations that are strongly associated with the gold and coal mines within the Olifants, Vaal and Limpopo

River Catchments (Murcott 2012). More than fifty occurrences of lead deposits have been reported in South Africa (Snodgrass 1986). The deposits occur almost exclusively in dolomite or quartz veins and have the potential to enter waterways through mining and excavations (Snodgrass 1986). There are currently two Pb mines and one Pb refinery in active operation in South Africa (Mbendi 2016).

There is scarce data on the concentration of As and Pb in water sources, particularly within the catchment zone of mining enterprises (Sami and Druzynski 2003, Ahoule et al. 2015). While there may be limited published data on water quality around South African mining towns, the impacts thereof on the local population have been documented. For example, children living in the Pb mining town of Aggeneys in the Northern Cape were recorded to have elevated Pb levels in their blood (> 18 µg/dl), which was directly correlated to greater learning difficulties and school failure rates (Von Schirnding et al. 2003). Glass (1990) detected Pb at 0.77 $mg.L^{-1}$ in the Edendalespruit River downstream of the abandoned Edendale Pb mine site, nearby to two local schools. Mzini and Winter (2015) recorded 1.0 $mg.L^{-1}$ Pb in potable water sampled from the Umtata Dam in the Eastern Cape and 46 $mg.L^{-1}$ Pb in the grey water used by Umtata residents to water their vegetable gardens. These values are considerably higher than the Pb irrigation “target water quality” of 0.2 and the Umtata greywater is well in excess of the 2.0 $mg.L^{-1}$ “maximum acceptable” Pb concentration for irrigation water (DWAF 1996).

Dzoma et al. (2010) found an average of 0.119 $mg.L^{-1}$ of As over a 1 km stretch of the Koekemorespruit in the North-West Province, which exceeds the “target water quality” threshold of 0.1 $mg.L^{-1}$. It is likely that the level of As contamination would increase considerably if investigations took place closer to the source of degradation activities. A study by Kempster et al. (2007) presented a synopsis of As data from 8 380 groundwater sites and 6 360 surface water sites across South Africa. Most groundwater sites had no detectable As, however 59 boreholes exceeded the “maximum acceptable” As concentration of 0.1 $mg.L^{-1}$ (Kempster et al. 2007) and are recorded in Figure 2.6.

For example:

- 0.341 mg.L^{-1} As in Victoria West, Central Karoo, in the Northern Cape.
- 0.520 mg.L^{-1} As in Sishen, near the Sishen Iron Mine in the Northern Cape.
- 1.269 mg.L^{-1} As near Uitenhage, Port Elizabeth, in the Eastern Cape.
- 1.664 mg.L^{-1} As near Kwa Nobuhle, Port Elizabeth, in the Eastern Cape.
- 10.00 mg.L^{-1} As near Durbanville, Cape Town, in the Western Cape.

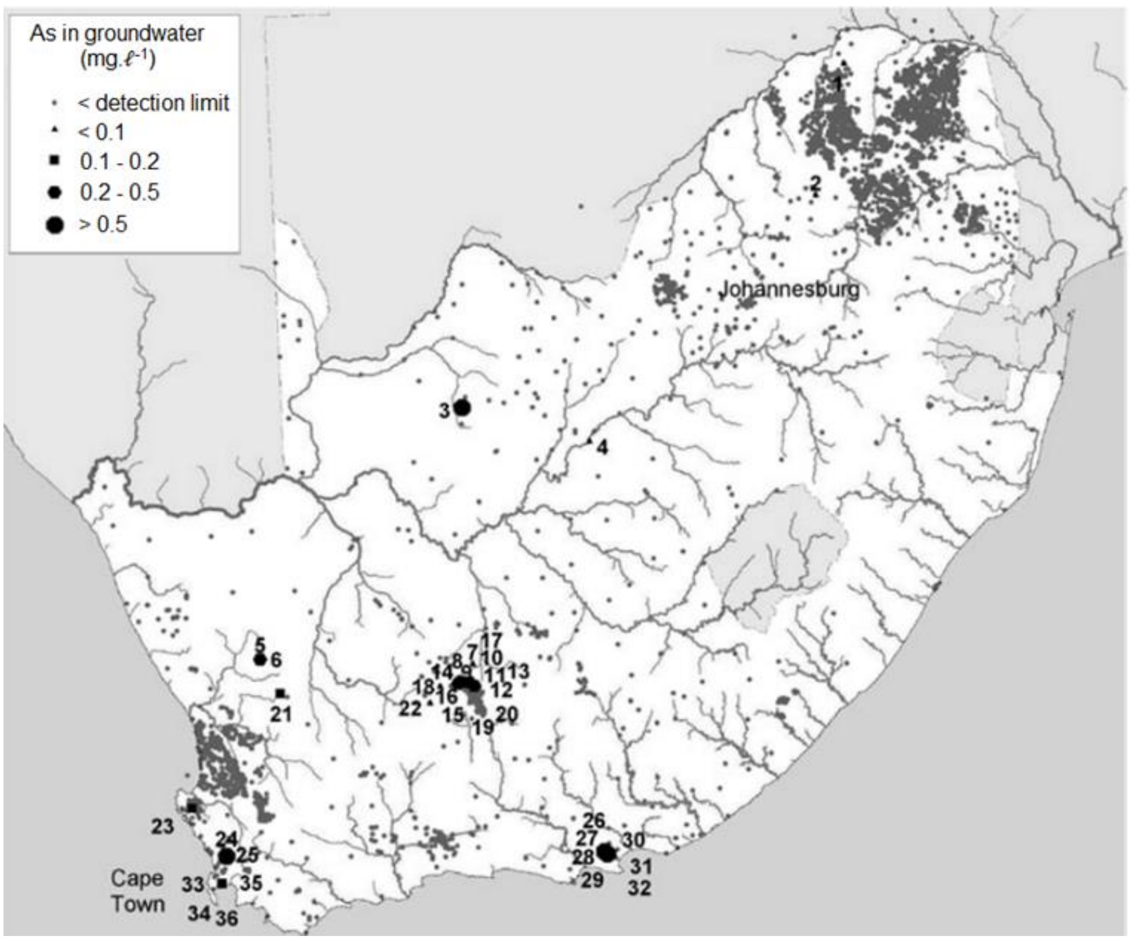

FIG 2.6: Groundwater arsenic data for South Africa (Kempster et al. 2007)

Most South African surface water sites also have no detectable As; however, sites with elevated As in surface water have been linked to industrial activities in Gauteng, see Figure 2.7 (Kempster et al. 2007).

For example:

- 0.160 mg.L^{-1} As at the Nigel-Delmas road bridge in Gauteng;
- 0.850 mg.L^{-1} As in the Roodeplaat Canal, Pretoria North, in Gauteng;
- 0.950 mg.L^{-1} As in the Roodeplaat Dam, Pretoria North, in Gauteng;
- 1.233 mg.L-1 As in the Welbedacht Dam, near Wepener, in the Free State.

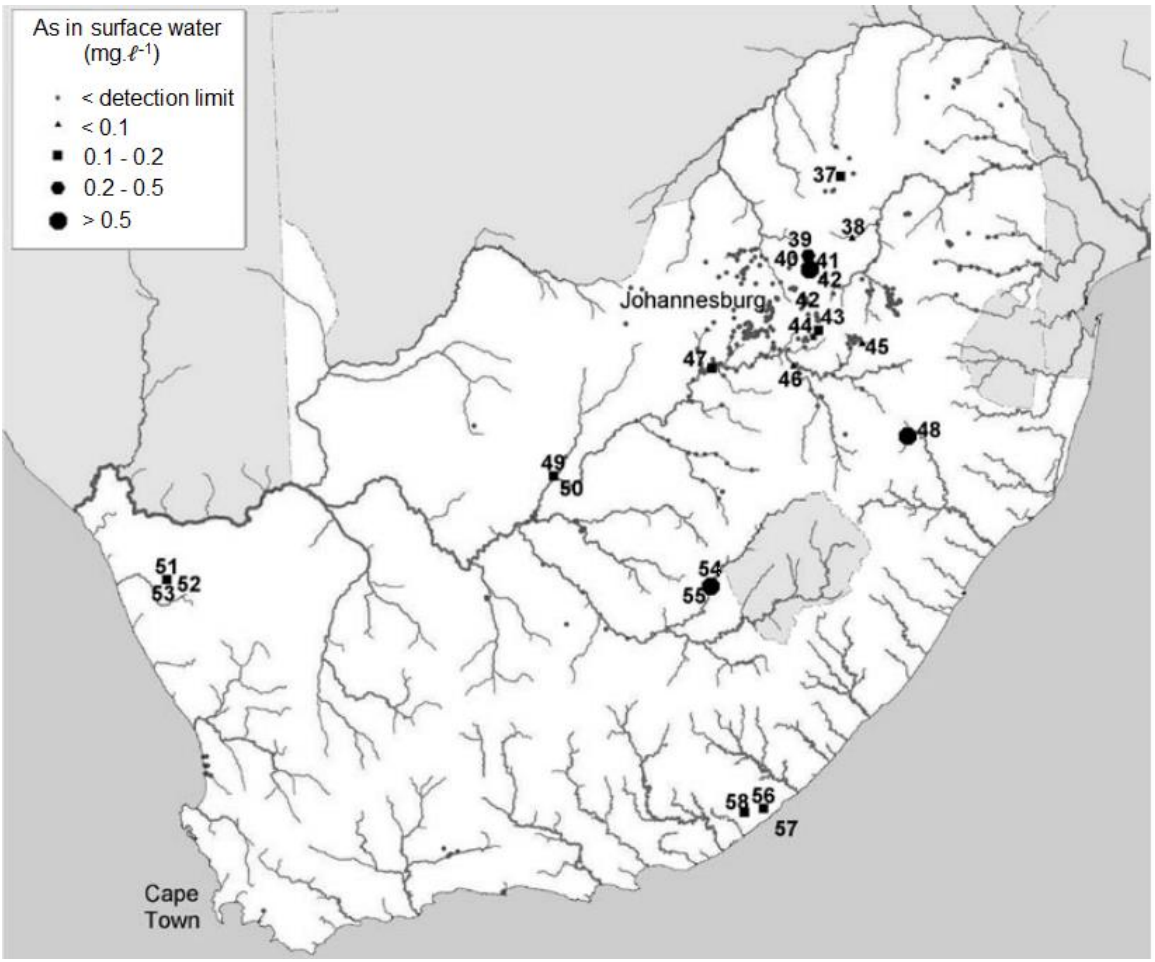

FIG 2.7: Surface water arsenic data for South Africa (Kempster et al. 2007)

In other parts of Africa, As in surface water has been recorded at 25.9 mg.L^{-1} in the mining region of Tarkwa, Ghana, and 28 mg.L^{-1} in borehole water in the mining region of Tsumeb, Namibia (Ato et al. 2010, Madec et al. 2016).

To put these values into context, the irrigation water quality guideline for As ranges from 0.1 to 2.0 $mg.L^{-1}$ and the guideline for Pb ranges from 0.2 $mg.L^{-1}$ to 2.0 $mg.L^{-1}$ (DWAF 1996). It is therefore necessary to test the food safety of crops irrigated with water containing the "target water quality" (0.1 $mg.L^{-1}$ As and 0.2 $mg.L^{-1}$ Pb) and "maximum acceptable" concentrations (2.0 $mg.L^{-1}$ for both As and Pb) for use as irrigation water, because local water sources have been shown to exhibit As and Pb at concentrations that are in excess of these thresholds.

The remaining sections of this review will cover how As and Pb speciation and mobility in soil and water may impact the mechanisms through which crops absorb, translocate and accumulate As and Pb. The outcome of such biophysicochemical transformations ultimately determine the extent to which crops may accumulate As and Pb, and therefore be deemed fit or unfit for human or animal consumption.

2.8 Arsenic and Lead in Soil and Water

In this study, both the short-term effect of irrigating crops with As and Pb loaded irrigation water, as well as the long-term effect of growing crops in soils that have accumulated As and Pb over a prolonged irrigation period, were investigated. It is essential to fully understand both foliar and root uptake pathways of As and Pb into the edible parts of crops, as both may result in the edible parts of crops exceeding human food safety guidelines (Warren et al. 2003, Alexander et al. 2006, Nayek et al. 2010, Baig and Kazi 2012, Schreck et al. 2014, Yañez et al. 2019).

2.8.1 Chemical speciation of arsenic and lead in irrigation water

Plants absorb elements in ionic form via roots as well as aerial plant parts, such as the stems, leaves and fruit (Fernández et al. 2013, Yang and Hinner 2016). Foliar uptake can occur in a variety of ways, be it direct agronomic inputs, such as foliar sprays, atmospheric deposition, or through overhead irrigation systems (Fernández et al. 2013). In this dissertation, the effects of trace element loaded irrigation water on food safety was investigated. In particular, the glasshouse foliar absorption trial (results presented in Chapter 4) focused on the contribution of element-loaded irrigation water applied to the aboveground biomass on the food safety of crops. The chemical speciation, particularly oxidation state, of these elements in typical irrigation water needs to be understood as speciation plays a role in the absorption process (Yang and Hinner 2016).

Irrigation water typically contains inorganic cations and anions which are readily available for plant uptake (NAS-NAE 1973). Arsenic in water occurs as oxyanions: arsenate (AsO_4^{3-}) in in the presence of oxygen and arsenite (AsO_3^{3-}) in reducing environments (FAO JECFA 2011, Smedley and Kinniburgh 2002, Wang et al, 2012). Lead in water typically occurs in cationic form (Pb^{2+}) under low to neutral pH (Wang et al. 2017). At pH 8, approximately half of Pb is present as Pb^{2+} ions and half are in the form of $PbOH^{+}$. At pH 9.5, Pb^{2+} ions are no longer present, instead one third are in the form of $PbOH^{+}$, one third is $Pb(OH)_2$ (aq) and the final third is comprised of $Pb_3(OH)_4^{2+}$ (Wang et al. 2017). This is the hydrolysis of Pb^{2+} in the absence of other ligands.

After element-loaded irrigation water enters the soil, a vast array of chemical, physical and biological transformations take place which influence the bioavailability of each element (Kunhikrishna et al. 2012). With the primary focus of this dissertation being the effect of irrigation water on crop food safety, the factors that affect the bioavailability of As and Pb in soil will be discussed, because these factors ultimately determine the extent to which root uptake of each element may take place. The glasshouse root uptake trial (results in Chapter 5) was implemented to determine the impact of root uptake on food safety in representative crops.

2.8.2 Factors affecting plant availability of arsenic in soil

On a mass basis, plant roots take up As from the soil in the following order: arsenite > arsenate > DMA > MMA (Pushon et al. 2017). Arsenite is the primary As species in anaerobic soils and is a close chemical analogue to silicic acid, while arsenate is the primary As species in aerobic soils and is a close chemical analogue to phosphate

(Wang et al. 2002, Davis et al. 2017). Similarities between arsenite and silicic acid, and between asrenate and phosphate, govern their entry into root cells (Davis et al. 2017).

Since plant roots cannot distinguish between phosphate and arsenate, both oxyanions compete at active transporter protein sites, such as $H_2PO_4^-/H^+$ symporters, along the cell membrane (Smith et al. 2003, Sadee et al. 2016). Therefore, a possible solution under short-term irrigation programmes is the addition of phosphate to the soil before irrigating with As loaded water (Azouzi et al. 2017), as the competitive ion effect may result in reduced As uptake by the plant (Bergqvist 2011). However, phosphate and arsenate also compete for adsorption sites on positively charged soil particles, such as iron, manganese and aluminium oxides and oxyhydroxides (Bolan et al. 2013). Therefore, studies have shown that the addition of phosphate in As contaminated soils may increase plant uptake of As, due to phosphate mediated desorption of arsenate (Cao and Ma 2004, Bolan et al. 2013). Although phosphate additions may be beneficial in the beginning of an irrigation program, the reverse effect may occur over the long term.

Arsenate and silicon compete for the same aquaporin channel in the symplastic uptake pathway (Dai et al. 2016). In rice, Low Silicon 1 (OsLsi1) and OsLsi2 are silicic acid transporters which also inadvertently adsorb molecules with similar chemical structure, namely: arsenite, MMA and DMA (Davis et al. 2017). Both Bogdan and Schenk (2008) and Dai et al. (2016) demonstrated reduced As in rice grains after the addition of silicic acid to the soil. However, both trials took place in in soils with low soluble levels of Si^{4+} in the form of H_4SiO_4, therefore further research is needed to confirm the effect of silicate amendments under conditions of silicate sufficiency.

Like phosphate, arsenate partakes in inner-sphere complexation with metal oxide surfaces via the formation of a stable binuclear bridge (Brady and Weil 2008). The addition of these oxides in the form of mineral salts or oxidised metal grits has been shown to reduce As concentrations in vegetable crops (Warren et al. 2003, Hartley and Lepp 2008). However, there are several challenges to this approach: 1) iron, manganese and aluminium oxides also reduce phosphate availability, requiring increased phosphorous applications, which increase the solubility of arsenate; 2) newly precipitated oxides are amorphous and have a very high sorption capacity, but over time they reorganise into crystalline structures in oxidising conditions and thereby exhibit lower sorption capacities; 3) other soil constituents, including silicates, humates and organic acids, complex with oxide surfaces, effectively reducing the number of available sorption sites and limiting the capacity for arsenate complexation (Warren et al. 2003, Hartley and Lepp 2008). One advantage of South African soils is that they typically contain high concentrations of iron, aluminium and manganese oxides, and thereby naturally exhibit an elevated phosphate and arsenate sorption capacity (FERTASA 2016). Lowering the pH of these soils may increase the sorption capacity of iron oxides through amorphotisation and thereby decrease the bioavailability of As (Bai et al. 2008).

The presence of arbuscular mycorrhizal (AM) fungi has been shown to limit As uptake in barley (Christophersen et al. 2012). The infection of roots with AM fungi was shown to increase phosphate uptake, which lead to the downregulation of $H_2PO_4^-/H^+$ symporters and effectively decreased active arsenate uptake via the roots (Christophersen et al. 2012). Bai et al. (2008) found similar results, but attributed the change to decreased soil pH after AM infection.

Finally, maintaining well aerated, oxidising soil environments is essential to limiting the solubility of As, as arsenite is considerably more mobile than arsenate (Brady and Weil 2006). Therefore, site selection when irrigating with waters that contain elevated levels of As is key. Sites with poor drainage, sub-surface compaction, perched water tables or ponding should be avoided to mitigate the level of As uptake by crops.

2.8.3 Factors affecting plant availability of lead in soil

Per the findings of Romero-Freire et al. (2018), the soil properties with the highest influence on Pb extractability are pH, organic matter and calcium carbonate content. Pb^{2+} has the rare ability to form innersphere complexes with silicates. The lead silicate bond has a stronger covalent character than most other cations and is the same reason why Pb^{2+} shows an affinity for functional groups on organic matter. When compared to other potentially hazardous metals in three cropping systems: rice, wheat and canola, Pb consistently exhibited the lowest transfer ability from soil to crop (Chen et al. 2016). Pb in soil is classified as a weak Lewis acid, which indicates a strong covalent character to the ionic bonds it forms in soils and plants (Sharma and Dubey 2005). Pb present in the soil is typically either bound to organic or colloidal material or in a precipitated form, all of which serve to reduce the availability of Pb for uptake by plant roots (Sharma and Dubey 2005).

Due to the complexity of Pb transformations in soil, it is impossible to accurately predict the rate and nature of such transformations on potential crop uptake. Therefore, site specific trials to evaluate cultivar response are recommended in soils containing elevated levels of Pb. Romero-Freire et al. (2015) demonstrated that calcareous soils dosed with Pb salts effectively reduced water-extractable Pb concentrations to below 0.2% of the total Pb content. Gypsiferous soils have also been proven capable of Pb removal via precipitation of Pb sulphates (Kameda et al. 2017). Therefore, calcareous and gypsiferous soils of the Northern Cape, South Africa, may effectively buffer Pb loaded irrigation water for a longer duration than soils in the rest of the country. Additionally, the application of gypsum or lime to irrigated fields may aid in the sequestration of Pb inputs (Romero-Freire et al. 2015, Kameda et al. 2017). The efficacy of such amendments is also dependent on chloride content, as Pb-Cl complexes are more soluble.

The addition of phosphate has also been shown to effectively immobilise Pb in soils through the precipitation of insoluble pyromorphite, $Pb_5(PO_4)_3Cl$ (Li et al. 2013). Lead is analogous to calcium and follows the same calcium uptake pathways in plants (Kerper and Hinkle 1997). Therefore, the addition of calcium has been shown to result in antagonistic ion competition at plant uptake sites and thereby reduces Pb accumulation in plants (Ding et al. 2016). The combination of these remediation strategies has been investigated by Li et al. (2013) under the hypothesis that calcium ions would reduce Pb adsorption through competition at adsorption sites of the soil. For example, ryegrass grown in Pb contaminated calcareous soil that received the phosphate amendment showed the lowest Pb uptake and highest yield (Li et al. 2013). Li et al. (2013) attributed their findings to the development of reactive, amorphous calcium-phosphate compounds with a strong affinity for Pb at circumneutral pH, along with the precipitation of pyromorphite. The antagonistic effect between Pb and Ca at the root cell membrane likely influenced the result as well. Although the Pb may not be plant available, the increased mobility may cause other environmental impacts. In fact, the cellular uptake of Pb has been shown to be activated by depletion of intracellular calcium stores (Kerper and Hinkle 1997). Therefore, adequate calcium fertilisation is vital to limiting the accumulation of Pb in crops.

The absorption of Pb in soil follows the Langmuir relation and increases with increasing pH between 3.0 to 8.5 (Sharma and Dubey 2005). At circumneutral to alkaline soil pH, the complexation of Pb ions by inorganic anions such as Cl^-, OH^-, and HCO_3^-, is generally considered potentially available for uptake across root cell membranes (Kim et al. 2015). Therefore, Pb may become more plant available soon after liming or in saline environments. In moderately acidic to slightly alkaline soils, Pb exists primarily as specifically absorbed at the hydroxyl surfaces of iron, aluminium and manganese oxides and hydroxides and clay minerals (Kim et al. 2015). Therefore, like As, the addition of iron, aluminium and manganese oxides may reduce the bioavailability of Pb. The specific adsorption is mainly due to the formation of inner sphere complexes of Pb hydroxides and is generally immobile and unavailable for plant uptake (Brady and Weil 2006). Baes and Mesmer (1976) found that the higher the solubility constant, K_{sp}, of metal ions, the lower the metal mobility at circumneutral pH (Cd^{2+} (10.1) < Ni^{2+} (9.9) < Zn^{2+} (9.0) < Cu^{2+} (7.7) < Pb^{2+} (7.7) < Cr^{3+} (4.0). Consequently, Pb ions tend to exhibit greater mobility than most other metals. At low pH, Pb is non-specifically adsorbed at cation exchange binding sites on oxides and clay minerals via electrostatic bonds (Yan et al. 2017). Therefore, Pb becomes more mobile with decreasing pH (Yan et al. 2017).

The addition of organic matter to soil has been shown to reduce the water-extractable fraction of Pb through specific absorption at organic functional groups that show a significant degree of polarisation (ionisation), such as carboxylate groups. (Romero-Freire et al. 2015). Dissolved organic matter, such as humic and fluvic acids, will result in the formation of Pb-organic complexes with a net negative charge (Kim et al. 2015). Due to the negative charge, at low pH, the solubility of these complexes decreases (Kim et al. 2015). In slightly acidic to alkaline soils, Pb-organic complexes are both stable and soluble (Lang and Kaupenjohann 2003). As a result, the addition of organic acids through organic matter decomposition may significantly increase the mobility of Pb (Lang and Kaupenjohann 2003). However, the complexed species were often found to be too large to pass through root cell membranes (Kim et al. 2015). While Pb-organic complexes may be highly mobile, they are not plant available (Kim et al. 2015). In summary, the addition of organic matter may effectively immobilise Pb in the short-term. However, over the long-term, Pb mobility is likely to increase as organic matter is decomposed into humates and fulvates, although the chelated Pb may not be available for uptake across root cell membranes.

2.9 Root and Foliar Uptake, Translocation and Partitioning Mechanisms

Trace elements may enter a plant via two pathways: root uptake and translocation mechanisms, as well as foliar absorption and phloem mobilization (Schreck et al. 2014). The uptake pathway (root or shoot) may have different impacts on As and Pb compartmentalization and speciation in plants, which consequently influences As and Pb bioavailability and toxicity (Schreck et al. 2014).

2.9.1 Root uptake mechanisms

For root uptake, As or Pb ions in the soil solution are first adsorbed onto the root surfaces, followed by their binding to polysaccharides of the rhizodermal cell surface or carboxyl groups of mucilage uronic acid (Shahid et al. 2017). After adsorption onto the root surface, As and Pb may penetrate the roots passively by diffusion through translocating water streams, or by active uptake and translocation mechanisms intended for their chemical analogues (Shahid et al. 2017).

Arsenate (AsO_4^-) is analogous to phosphate (PO_4^-) and thereby follows phosphate uptake and translocation mechanisms. Arsenate is adsorbed through the root surface via apoplastic and symplastic pathways. Once the Casparian strip is reached, arsenate, the dominate soil species under aerobic conditions, actively enters root cells through phosphate transporters (such as $H_2PO_4^-/H^+$ symporters and aquaglyceroporins), while the markedly more mobile arsenite, the dominant redox form under anaerobic conditions, enters root cells via silicic acid transporters (Carey et al. 2011). Organic forms of As, MMA and DMA, have been shown to enter root cells through aquaglyceroporins (Bergqvist 2011). Thereafter, arsenate and arsenite is transported via the xylem to the aboveground biomass of the crop, where it either influences phosphate metabolism or is actively accumulated in vacuoles (Page and Feller 2015). Later, arsenate and arsenite may be redistributed via the phloem to accumulate in the aboveground biomass (Page and Feller 2015).

According to Ding et al. (2016), Pb ions (Pb^{2+}) are analogous to calcium ions (Ca^{2+}) and therefore compete with calcium to enter plant roots via the apoplastic pathway or calcium ion channels (Ding et al. 2016). However, this is not entirely accurate, as even though both Pb^{2+} and Ca^{2+} are divalent cations, Pb is a B-type cation with extensive orbitals and thus far more complex chemical behaviour. The diversity of bonds that Pb^{2+} can achieve is likely the reason why it can “mimic” simpler alkali earth metals like Ca^{2+}. Kabata-Pendias (2011) determined that Pb is preferentially absorbed through roots via passive uptake. Due to the net negative charge of cell walls, once in the roots, Pb ions tend to sequester in root cells (Ding et al. 2016). Only a limited amount of Pb is translocated from roots to shoots via the xylem, due to the presence of natural barriers in the root endodermis such as the Casparian strip (Pourrut et al. 2011). Dollard and Lepp (1980) demonstrated that, like Ca, Pb is not remobilised through the phloem. Therefore, lower concentrations of Pb are expected in fruit, seeds and grains when taken up via roots, as demonstrated by Chen et al. (2016) who found that the concentration of Pb in the roots of rice, wheat and canola were an order of magnitude higher than in the grain.

2.9.2 Epidermal structures and cuticle morphology

The absorption of trace elements, in the form of solubilised mineral salts, through the aerial surface of plants typically follows a stepwise process (Fernández et al. 2013). When water is applied through overhead irrigation, a portion of trace elements contained therein are effectively adsorbed onto the cuticular surface. Thereafter, the adsorbed elements may be transported through the leaf cuticular surface into the cellular space where they may affect plant metabolism and physiology. This transportation process is referred to as “foliar uptake” (Kannan 2008). According to Wojcik (2004), the movement of low-molecular-weight solutes (such as As and Pb ions) from the leaf surface to the epidermal cell wall is a passive process driven by diffusion and electrochemical potential formed by a negative charge increase across the cuticular membrane. Therefore, one would expect to observe an increased foliar uptake of cations, such as Pb, when compared to anions, such as As (Shahid et al. 2017). There are a variety of foliar uptake pathways through which ions in solution may enter the cellular space and these will be discussed in detail in the sections that follow.

Cuticle morphology and epidermal structures, such as ectodesmatas, trichomes, lenticels and stomata, influence a plant’s foliar absorption potential, shown in Figure 2.8 (Fernández et al. 2013). The cuticle is a hydrophobic waxy surface that covers leaves, stems and fruit to limit water loss (Kannan 2008). While the cuticle is comprised primarily of a hydrophobic biopolymer matrix with waxes, approximately 20 % of the cuticle is made up of hydrophilic polysaccharides, such as cellulose and pectin (Popp et al. 2005). The cellulose microfibrils and other hydrophilic structures are secreted from epidermal cells to form branched, tortuous pathways throughout the waxy cuticle (Popp et al. 2005). These hydrophilic pathways allow for limited passive diffusion of small water soluble constituents, such as ions (Popp et al. 2005). The hydrophobic pathways are well documented, but will not be discussed in this review, as they do not apply to the uptake of As or Pb in irrigation water (Popp et al. 2005, Fernández et al. 2013). Micro imperfections, cracks and scales on the cuticle surface have also been shown to enhance foliar uptake of ions and larger chemical species (Shahid et al. 2017). Ectodesmata, which are negatively charged pores in the cuticle with a diameter of less than 1 nm, also allow for the permeation of cations from the leaf surface to the intercellular space (Wojcik 2004). Therefore, cations such as Pb may be absorbed through the ectodesmatal pathway, while arsenate anions may not (Shahid et al. 2017).

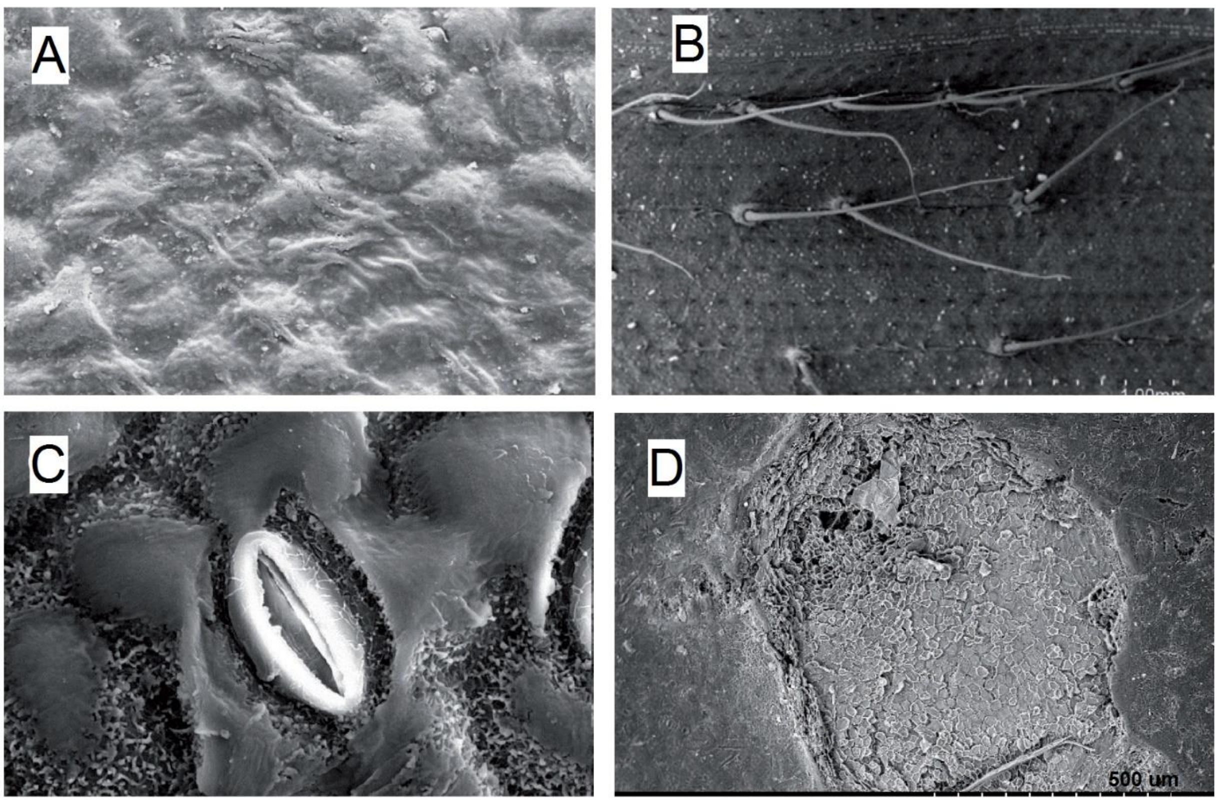

FIG 2.8: Scanning electron micrographs of (A) peach leaf cuticular surface, (B) trichomes on maize leaf, (C) stomatal pore on rose leaf, (D) lenticel on apple fruit skin (Fernández et al. 2013)

Trichomes are unicellular or multicellular pubescent appendages originating from epidermal cells, shown in Figure 2.8 (B). Trichomes vary greatly in structure and function: some have been shown to absorb water (Grammatikopoulos and Manetas 1994, Ju et al. 2012), while others have exhibited a hydrophobic effect (Burrows et al.

2013). As such, the impact of trichomes on the uptake of ions in solution is crop and cultivar dependent (Li et al. 2018).

Stomata are modified epidermal cells that control leaf transpirational water losses and gaseous exchange (van der Merwe 2016). Stomata generally occur on the abaxial leaf surface, but in amphistomatic plant species, including maize and soybean, stomata are also present on the adaxial leaf surface (Fernández et al. 2013). Stomata have been shown to increase the rate of foliar uptake of ions in solution, particularly under conditions favouring stomatal opening, such as increased sunlight (Fernández and Ebert 2005). Some authors have attributed this phenomenon to the direct penetration of water and solutes through open stomatal pores (Fernández and Eichert 2009, Fernández et al. 2013). Others suggest that the peristomatal cuticle has a higher general permeability and thus allows for diffusion to take place (Schlegel et al. 2005). The most recent publications have put forward the hypothesis that infiltration through stomatal pores occurs by diffusion through trans-stomatal water clusters (Eichert and Goldbach 2008).

Lenticels are macroscopic structures that form on the epidermis of stems, pedicels and certain fruits, after the periderm has formed (Fernández et al. 2013). These epidermal structures tend to form ligneous cracks and scales in the waxy cuticle, which may thereby allow water and solutes to penetrate the cellular space, although this pathway has yet to be rigorously tested (Fernández et al. 2013).

Once elements have been transported through the leaf cuticular surface into the cellular space, they must penetrate cell walls and plasma membranes of the leaf mesophyll. Cell walls in leaf tissue are continuous and thereby allow for the apoplastic movement of ions (Wojcik 2004). Arsenate moves across the cell membrane via the same mechanisms as phosphate, primarily through active phosphate transporters, while arsenite enters the cell via aquaporins (Yang and Hinner 2015, Li et al. 2016). Arsenic has been shown to be transported in both the xylem and the phloem (Ye et al. 2010). Therefore, As taken up through the aboveground biomass may be translocated to roots and *vice versa*. Since phloem mediated transport is crucial for the remobilisation of assimilates from leaves to fruit, seeds and grains, foliar applied As may exhibit higher concentrations in edible plant parts.

Lead has been shown to cross through leaf cuticles and enter the cellular space, although the exact mechanisms remain unclear (Shahid et al. 2017). Once through the cuticle, Pb ions tend to accumulate in the apoplast due to the net negative charge of cell walls, but may also follow the calcium ion pathway across cell membranes to a limited extent (Kerper and Hinkle 1997, Ding et al. 2016). Lead is not remobilised in the phloem and is therefore expected to remain at the point of contact when applied to the aboveground biomass (Dollard 1986).

2.9.3 Environmental factors affecting foliar uptake mechanisms

Numerous environmental factors influence foliar absorption rates, including: light intensity, temperature, humidity and wind speed. Generally, increases in light intensity are associated with higher rates of foliar uptake owing to stomatal opening. However, in regions of regular exposure to high light intensities, seasonal accumulation of epicuticular waxes may diminish a leaf's propensity for foliar uptake (Wojcik 2004). Under abnormally high temperatures, stomatal closure and increased evaporation of irrigation water from leaf surfaces results in the rapid crystallisation of dissolved minerals into surface deposits which are no longer available for foliar absorption (Ronen 2014). However, Wojcik (2004) speculated that high temperatures may increase viscosity of the waxy cuticle, and thus temporarily increase the rate of foliar uptake before crystal deposits are formed. As humidity increases, the rate of evaporation decreases, thereby prolonging the period during which water remains on the leaf surface. The longer water persists on the leaf surface, the longer dissolved species are able to remain in solution and be chemically available for foliar absorption processes (Ronen 2014, Wojcik 2004). Wind increases evaporation rates and so reduces foliar absorption. Heavy winds also can blow irrigation droplets off the leaf surface.

2.10 Conclusions

A review of the literature has confirmed that some local water sources contain As and/or Pb in excess of the South African Irrigation Water Quality Guideline limits, particularly in areas that have been impacted by mining and industry (Glass 1990, Kempster et al. 2007, Dzoma et al. 2010, Mzini and Winter 2015). Furthermore, previous studies have demonstrated that the edible parts of crops grown on soils, or irrigated with water, containing elevated levels of As and/or Pb, repeatedly exceeded international food safety guidelines (Warren et al. 2003, Alexander et al. 2006, Nayek et al. 2010, Baig and Kazi 2012, Schreck et al. 2014, Yañez et al. 2019). Finally, it has been suggested that it is unlikely that the South African Irrigation Water Quality Guidelines incorporated the impacts of food safety into the guidelines, since food safety guidelines for potentially hazardous trace elements only emerged after the publication of the South African Irrigation Water Guidelines in 1996 (DWAF 1996, JECFA 2002). As such, a critical review of the data from which these guidelines were initially developed could be of great benefit to modern agriculture.

Drawing from the literature, this dissertation adopted a multidisciplinary approach and, for the first time as far as the author is aware, investigated the direct impact of irrigation water quality guidelines on the food safety of fresh produce. Hence, the primary objective of this dissertation was to provide empirical evidence, by conducting foliar absorption and root uptake trials, to determine whether the South African Irrigation Water Quality Guidelines are compatible with the more recently established food safety guidelines. In doing so, it contributes to the gap in the literature with regards to the relationship between permissible irrigation water trace element concentrations and food safety thresholds.

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Research Strategy

A quantitative methodology employing a deductive approach was used in this dissertation to empirically test the food safety component of modern irrigation water quality guidelines. Two randomised complete block design (RCBD) glasshouse trials were performed to evaluate the effect of irrigation water quality on food safety. The first trial was conducted to establish the foliar absorption effect of crops encountering arsenic or lead contaminated irrigation water over one growing season, hereafter referred to as the “glasshouse foliar absorption trial”. The second was conducted to determine the effect of the long-term accumulation of such elements in the soil on food safety via root uptake mechanisms into crops over one growing season, hereafter referred to as the “glasshouse root uptake trial”. Four winter crops were selected to broadly represent the following food groups: grains, leafy vegetables, root vegetables and legumes. Crops were harvested at agronomic maturity. Both wet and dry mass of plant parts (leaf, stem, grain, pod, pea and root) were measured to combine food science and agronomic science norms and standards. Dry mass was the preferred method of presenting data as it avoided the concentration effect (de Anicésio et al. 2015). Finally, plant parts were analysed for total arsenic and lead content. Results are displayed in chapters four and five on a dry mass basis, while fresh mass was utilised to employ conversion factors for food safety guidelines.

3.2 Research Objectives and Hypotheses

The primary objective of this study was to evaluate whether arsenic (As) or lead (Pb) present in irrigation water at the concentrations that have been deemed acceptable by the South African Irrigation Water Quality Guidelines, or present in soil as a result of medium- to long-term irrigation inputs, would result in fresh produce that would comply with Food Safety Guidelines. To solve that objective the following hypotheses were set:

Null Hypothesis 1: Arsenic or lead present in irrigation water does not result in edible plant parts exceeding food safety thresholds for As or Pb.

Hypothesis 1: Arsenic or lead present in irrigation water results in edible plant parts exceeding food safety thresholds for As or Pb.

Null Hypothesis 2: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, does not result in edible plant parts exceeding food safety thresholds for As or Pb.

Hypothesis 2: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, results in edible plant parts exceeding food safety thresholds for As or Pb.

While not the primary focus of this study, a secondary set of hypotheses were developed to assess whether the resulting crops, if used as animal feed, would comply with Animal Feed Guidelines.

Null Hypothesis 3: Arsenic or lead present in irrigation water, does not result in the crop exceeding animal feed thresholds for As or Pb.

Hypothesis 3: Arsenic or lead present in irrigation water results in the crop exceeding animal feed thresholds for As or Pb.

Null Hypothesis 4: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, does not result in the crop exceeding animal feed thresholds for As or Pb.

Hypothesis 4: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, results in the crop exceeding animal feed thresholds for As or Pb.

Two sets of glasshouse pot trials were designed to address the temporal aspects of the hypotheses. The first was designed to test the effect of As or Pb loaded irrigation water which settles onto the aboveground biomass as a result of overhead irrigation (such as sprinkler or centre pivot). Synthetic As or Pb loaded irrigation water, with two treatment levels each, was sprayed onto crops to the point of run-off to imitate overhead irrigation while minimising element loading into the soil. The second was designed to test the effect of As or Pb present in soil as a result of medium- to long-term irrigation inputs. Soils were dosed with the equivalent As or Pb concentration that would have accumulated over 19 years of irrigation at the "maximum acceptable concentration" level or 99 years of irrigation at the "target water quality" concentration.

3.3 Design of the Glasshouse Trials

Glasshouse trials are commonly employed in agricultural science research (Ter Haar 1970, Warren et al. 2003, Baig and Kazi 2012, Yañez et al. 2019). Such trials effectively control confounding factors that would otherwise be difficult to manage in field trials, such as soil heterogeneity or extreme weather events. On a more practical level, a glasshouse pot trial design was chosen in order to contain the effects of the As and Pb treatments on the environment and prevent university staff and students from entering the designated trial area.

3.3.1 General design considerations

When designing the glasshouse trials to investigate the food safety component of modern irrigation water quality guidelines, the following criteria were considered:

1. Soil used in both pot trials had to satisfy the requisite "forgiving" soil criteria of fine textured, neutral to alkaline, as presented in the South African Irrigation Water Quality Guidelines (1996).
2. Pots had to be large enough to support the growth of all crops to agronomic maturity (harvest).
3. Chosen crops had to broadly represent the following food groups: grains, leafy vegetables, root vegetables and legumes.

4. The arsenic and lead salts used to simulate contamination had to be water-soluble.
5. Reasonable measures had to be taken to limit root uptake of arsenic or lead present in irrigation water for the glasshouse foliar absorption trial.
6. Great care had to be taken to prevent contamination of the environment and all people who could potentially come into contact with arsenic and lead utilised in the trials.

3.3.2 Safety precautions

The salts used in both trials to replicate arsenic and lead loading in irrigation water and soil were as follows: 90% sodium arsenite ($NaAsO_2$) and 99.92% lead(II) nitrate ($Pb(NO_3)_2$). The material safety datasheets (MSDS) for both compounds used in the trials were present in the laboratory and glasshouse. Personal protective equipment (PPE) employed were in accordance with the MSDS and included: full-face gas mask with inorganic and dust air filter, rubber gloves, lab coat and closed shoes. All glass wear and bottles containing salts in solution were clearly marked. Gloves and other non-reusables were kept separately, pots were lined with plastic bags, and contaminated soil was disposed of via the services of an outsourced hazardous waste company. Care was taken to place hazards and no-entry signs at all access points of the glasshouse and farm employees were made aware that no one could enter the premises without prior arrangement and the correct PPE.

3.3.3 Experimental site selection

To exclude the effects of soil heterogeneity, unwanted weather events, and to contain all possible arsenic and lead contamination, the trials were conducted in a temperature-controlled glasshouse at Phytotron D, Hatfield Experimental Farm, University of Pretoria, South Africa. The farm is located at 25^{o}45'S and 28^{o}16'E with an average elevation of 1327m above sea level and average annual rainfall of 670 mm (Annandale et al. 1999). The glasshouse was isolated from all other trials and personnel. A sandy clay loam soil of the Hutton form with a clay content of 24 % was selected for the purpose of the trials. Note that the soil had been previously utilized for crop production.

The moderate clay content was selected in order to provide a higher buffer capacity, while calcitic lime was added to the soil to increase pH to acceptable neutral to alkaline levels.

3.3.4 Crop selection

Since no research has been done to evaluate the effect of irrigation water quality guidelines on the food safety of crops produced under such guidelines, a wide variety of crops were selected to offer a broad overview of potential food safety concerns. Crops chosen for both trials had to fulfil each of the following criteria: winter growing season, locally produced, easily available seed and broadly representative of one the following food groups: grains, leafy vegetables, root vegetables and legumes. Swiss chard (leafy vegetable), beetroot (root vegetable) and garden pea (legume) fulfilled the above criteria; however, there was no locally produced winter grain. Barley is currently being considered for introduction to the Mpumalanga Highveld and was therefore chosen as the representative grain crop.

3.3.5 Selection of reference food safety thresholds

International food safety guidelines have only set standards for a maximum of six potentially hazardous trace elements: As, Cd, Cr, Hg, Pb and Sn (CODEX STAN 2015, FRLI 2011, GOC 2018, US FDA 2018). In South Africa, the Department of Agriculture, Forestry and Fisheries has adopted The Commission of the European Communities' food safety guidelines (DAFF 2011). These guidelines provide limits for As, Cd, Hg, Pb and Si; however, they only specify limits for Pb in agronomic fresh produce. An As threshold is only specified for polished rice, not vegetables, other grains and pulses. Therefore, for the purpose of this study, the Chinese Food Safety Guidelines were selected due to the fact that they are the most comprehensive in terms of listed trace elements and are the only guidelines to specify limits according to fruit, vegetable, grain and pulse sub-groups (Clever and Jie 2014). Furthermore, these guidelines are commonly utilised by non-Chinese researchers in food safety publications (Paltseva et al. 2018).

Note that the results regarding As and Pb accumulation in plant material were presented on a dry mass basis. Therefore, the As and Pb food safety thresholds were adapted from fresh mass to a dry mass according to the moisture content of the crops at harvest (refer to Appendix E for conversion factors).

3.4 Glasshouse Trials: Materials and Methods

Both trials were designed according to a two factorial, randomised complete block design and blocked according to crop type to avoid shading effects. Both trials took place from 22 May to 25 October 2017. For each trial, four crops were grown under five treatments (Table 3.1). The foliar absorption trial investigated the effects of crops irrigated with As or Pb loaded irrigation water on food safety, with the following treatments: control; As (0.1 $mg.L^{-1}$); As (2.0 $mg.L^{-1}$); Pb (0.2 $mg.L^{-1}$); Pb (2.0 $mg.L^{-1}$). The root uptake trial investigated the effect of crops grown in As or Pb loaded soils on food safety, with the following treatments: control; As [43.5 $mg.kg^{-1}$]; As [168.9 $mg.kg^{-1}$]; Pb (88.1 $mg.kg^{-1}$); Pb (168.9 $mg.kg^{-1}$). Due to the limited availability of larger pots, 8 L pots were used to contain the larger crops (barley and garden pea) and 3 L pots were used to grow the smaller crops (Swiss chard and beetroot). Pots were placed at 3 pots per row at a 15 cm inter- and intra-row spacing. A total of 120 pots were used for the purpose of this dissertation.

TABLE 3.1: Experimental design of glasshouse trials

Trial element	Glasshouse foliar absorption trial	Glasshouse root uptake trial
Treatments	5	5
Crops	4	4
Replicates	3	3
Total pots	60	60

3.4.1 Fertilizer and lime requirements

Fertilisers were applied per crop nutritional demand (see Appendix C) for the full fertiliser regimen. Lime was applied to increase soil pH to circumneutral conditions four weeks prior to planting.

3.4.2 Simulated long-term soil contamination

It has been recognized that the spiking of soils with soluble salts and the use of pots, instead of long-term field contaminated soils, may increase the availability of heavy metals in soil (Brunetti et al. 2011). Furthermore, the toxicity response of plants grown in laboratory spiked soils is frequently higher than in situ contaminated soils (Romero-Freire et al. 2015). In effect, the spiked approach simulates a single pollution event rather than the long-term pollutant build up that would realistically occur as a result of 20 or 100 years of irrigation. However, due to temporal, environmental and safety considerations, soils in pots were artificially dosed with trace element treatments three weeks prior to planting.

The South African Irrigation Water Quality Guidelines (1996) which were applied when this trial was designed in January 2017, do not specify a limit for the duration of irrigation with trace element loaded water. However, the Canadian and Australian guidelines do and were thus incorporated in the trial design (CCME 1987, ANZECC and ARMCANZ 2000). Trace element loads equivalent to the South African "target water quality" are referred to as the "100 year threshold" in the Canadian and Australian guidelines, while the South African "maximum acceptable" water quality is referred to as the "20 year threshold" in the Canadian and Australian guidelines and applies to crops grown in fine textured, neutral to alkaline soils (CCME 1987, DWAF 1996, ANZECC and ARMCANZ 2000). These guidelines state that irrigation regimes at such trace element loads must cease after 20 or 100 years of continuous irrigation (CCME 1987, ANZECC and ARMCANZ 2000). For example, a field irrigated with the "maximum acceptable" 2 $mg.L^{-1}$ As should no longer be agronomically productive, nor receive further irrigation inputs, after 20 years. Just as a field irrigated with the "target water quality" of 0.1 $mg.L^{-1}$ As should no longer be agronomically productive, nor receive further irrigation inputs, after 100 years. Therefore, the level of soil contamination utilised for the purpose of the glasshouse root uptake trial simulated the maximum allowable irrigation period, minus 1 year, to investigate plant growth in the final year of the irrigation program. These target concentrations are presented in Table 3.2. Fortuitously, by the time this trial was well underway, the South African Irrigation Water Quality Guidelines were revised to adopt the same 20-year and 100-year approach (du Plessis et al. 2017).

Soluble salts were dissolved in water at predetermined concentrations. After dissolving, an amount containing the requisite dosing concentration per pot was transferred to individual glass bottles and marked. Two weeks after liming, each pot received its allocated dose and was brought to field capacity. After being left to dry for 2 weeks, the soil in each pot was mixed to homogenise the dose. Thereafter, a sample was taken from each triplicate and analysed (Table 3.2).

TABLE 3.2: Target versus achieved soil element dosing for root uptake trial

Element	Target concentration ($mg.kg^{-1}$)	Achieved concentration ($mg.kg^{-1}$) ± SD	Achieved concentration minus control ($mg.kg^{-1}$)
As	43.5	45.63 ± 4.03	45.63
	168.9	165.00 ± 15.89	165.00
Pb	88.1	87.33 ± 16.04	85.76
	168.9	162.84 ± 19.45	161.27

The control soil contained no detectible arsenic and 1.57 $mg.kg^{-1}$ of lead. The goal in preparing the soil was to simulate 19 and 99 years of irrigation, rather than bring the total soil concentration up to the "target concentration". Therefore, in the case of Pb, where the soil contained residual amounts prior to dosing, the total achieved concentration was effectively higher than the target. After subtracting the control As and Pb concentrations from the achieved As and Pb concentrations, the effective dosing for all treatments was within 5 % of the desired amount.

3.4.3 Planting, germination and thinning

Fifteen barley seeds and five Swiss chard, garden pea and beetroot seeds were planted per pot. Pots were filled to field capacity and a clear plastic bag was secured over each pot to prevent evaporative losses. Bags were removed after emergence. Two weeks after emergence, seedlings were thinned to mimic in-field planting densities: seven seedlings per pot for barley, two seedlings per pot for garden pea and one seedling per pot for Swiss chard and beetroot.

3.4.4 Irrigation regime

Pots were irrigated according to crop demand. Pots were weighed and refilled to field capacity weekly. For the remaining days, five representative pots per crop were weighed and irrigated to field capacity. Thereafter, remaining pots received the average volume of water applied to the representative pots. Effectively, barley was irrigated approximately every third day. Garden peas were irrigated every second day, while Swiss chard and beetroot were irrigated daily.

3.4.5 Artificially contaminated irrigation water

Stock solutions (100 $mg.L^{-1}$) for As and Pb were stored in 5 L volumetric flasks. Flasks were gently shaken weekly and checked for precipitation. Volumetric flasks (1 L) were made-up with the exact concentration of each treatment and used to fill 1 L spray bottles which were stored in a light-proof box inside the glasshouse. Water within the spray bottles was occasionally collected and tested to ensure that the correct trace element concentrations were being applied (Appendix D).

During irrigation events, the abaxial leaf surfaces, stems, grains and fruits were sprayed to the point of runoff to simulate an irrigation event, while attempting to ensure that as little water as possible dripped off aboveground biomass and onto the soil surface. Thereafter, pots were filled to field capacity using a beaker filled with borehole water. Care was taken so that only the soil was wetted to ensure that the irrigation treatments were not washed off the aboveground biomass during watering. Note that the trial took place in a glasshouse, therefore only irrigation water came into contact with the aboveground biomass. The crops chosen for this trial were winter crops grown in a summer rainfall region, further justifying the fact that no rainfall influenced the results this trial.

After harvest, a mass balance of the Swiss chard pots was calculated to determine what proportion of As or Pb from irrigation water, at a concentration of 2.0 $mg.L^{-1}$ As or Pb, ended-up in the aboveground biomass and in the soil, shown in Table 3.3. Note that over the course of the growing season, an average of 4.24 L of As loaded irrigation water and 4.16 L of Pb loaded irrigation was applied as a spray to the aboveground biomass of each Swiss chard plant.

TABLE 3.3: Mass balance of As and Pb applied via irrigation (to the point of runoff) of the aboveground biomass of Swiss chard

Mass balance parameter	**As ($mg.kg^{-1}$)**	**Pb ($mg.kg^{-1}$)**
Mass in soil	0.446	0.402
Mass in aboveground biomass	0.401	0.429
Total mass	0.847	0.831

Despite best efforts to avoid runoff, approximately 53 % of the total As inputs and 48 % of the total Pb inputs accumulated in the soil. To quantify exactly where in the soil profile the As and Pb accumulated, each pot was dissected at various soil depths and analysed. For the first measurement, only the top 0.5 cm of soil surface was scraped off for analysis. Thereafter, depths of 0.5 – 2.0 cm, 2.0 – 5.0 cm and 5.0 – 10.0 cm were sampled. Note that the soils sections were sampled in their entirety and mixed in a bag before being sent for analysis. The results of which are shown in Table 3.4.

All of the arsenic that entered the soil via irrigation runoff was contained within the top 0.5 cm of the soil. Even though the highly mobile arsenite was applied as in the irrigation treatments, the results suggest that it was readily oxidised to arsenate and immobilised once it made contact with iron and manganese oxides on the soil surface.

Similarly, all of the lead was also contained within the top 0.5 cm of soil. Weaver and Bruner (1927) extensively mapped the root systems of Swiss chard and beetroot and reported that only a negligible proportion of the root system occupied the first 0.5 cm of soil, with the maximum root volume occupying soil at depths between 35 – 50 cm beneath the surface. Therefore, even though the soil crust accumulated approximately 4 $mg.kg^{-1}$ As or Pb over the entire growing season, it is unlikely that the root uptake of As or Pb via irrigation runoff would have had a noticeable effect on the results of the foliar uptake trial.

TABLE 3.4: Soil contamination of As and Pb at various depths through irrigation (to the point of runoff) of the aboveground biomass of Swiss chard

Element	Irrigation water concentration ($mg.L^{-1}$)	Soil depth (cm)	Achieved concentration ($mg.kg^{-1}$) ± SD
As	2.0	0 – 0.5	4.17 ± 0.33
		0.5 – 2	BDL
		2 – 5	BDL
		5 – 10	BDL
Pb	2.0	0 – 0.5	3.76 ± 0.64
		0.5 – 2	BDL
		2 – 5	BDL
		5 – 10	BDL

BDL: Below method detection limit of 0.70 $mg.kg^{-1}$ for As or 1.40 $mg.kg^{-1}$ for Pb

3.4.6 Harvest

Crops were harvested at maturity: 70 days after emergence for Swiss chard, garden pea and beetroot, and 110 days after emergence for barley. Swiss chard and beetroot were harvested fresh, garden pea and barley were harvested dry. The whole plant was harvested from approximately 1 – 2 cm above the soil. Roots were not collected, as the red soil was too difficult to wash off. All dust and soil particles were brushed off each plant with a soft brush, plants were then quickly rinsed with deionised water for to wash off surface residues. Thereafter, each whole plant was divided according to plant part: stem, leaf, root (beetroot), pea, pod and grain. Each plant part was weighed fresh to determine the fresh mass. Plant parts were then dried in brown paper bags at 60 degrees Celsius for 48 hours. Beetroot roots were dried for one week. Dried plant parts were then weighed to determine dry mass. Note that beetroot roots were peeled before drying according to CODEX Standard Food Safety Methods, while barley grains were not de-husked (CODEX STAN 2015).

3.4.7 Moisture content and fresh mass to dry mass conversion factors

The combined average moisture content of each plant part from both the foliar absorption and root uptake trials is presented in Table 3.5.

TABLE 3.5: Mean moisture content per crop part at harvest

Crop part	Mean fresh mass (g)	Mean dry mass (g)	Mean moisture content (%)
Barley grain	1.82	1.54	6.51
Barley leaf	7.36	6.84	9.59
Barley stem	19.04	12.83	24.99
Beetroot leaf	12.43	2.61	80.98
Beetroot root	12.52	1.22	89.87
Garden pea leaf	4.08	1.60	64.81
Garden pea pea	12.58	2.95	75.42
Garden pea pod	4.98	1.21	76.82
Garden pea stem	8.21	2.12	74.20
Swiss chard leaf	14.43	2.26	83.73

From the average moisture content of each plant part, conversion factors were calculated to transform the Chinese Food Safety Guideline (presented in fresh mass) to dry mass values, which will be discussed in relation to the results of this study on a dry mass basis in Chapters 4 and 5. Chinese Food Safety Guidelines had the advantage of providing different As and Pb thresholds for a wide range of processed and fresh foods. In terms of classifying the edible parts investigated in this study, barley grains fell under "grains (excluding paddy rice)" with a fresh mass total As threshold of 0.5 $mg.kg^{-1}$. The remaining edible parts were classified under "fresh vegetables", which also had a fresh mass total As threshold of 0.5 $mg.kg^{-1}$. In terms of Pb, barley grains were classified under "grains and their products" with a fresh mass Pb limit of 0.2 $mg.kg^{-1}$. Swiss chard and beetroot leaves were classified as "leaf vegetables" and therefore had a fresh mass limit of 0.3 $mg.kg^{-1}$ Pb. Garden pea pods and peas fell under "leguminous vegetables" with a fresh mass limit of 0.2 $mg.kg^{-1}$ Pb. Finally, beetroot roots did not fall under any vegetable sub-category and was therefore classified under the general "fresh vegetables" category, with a fresh mass limit of 0.1 $mg.kg^{-1}$ Pb. The conversion factors to transform the Chinese Food Safety Guidelines from fresh mass to dry mass are presented in Table 3.6.

TABLE 3.6: Food safety threshold conversion from fresh mass to dry mass

Trace element	Edible part	Fresh mass limit (mg.kg^{-1})	Factor	Dry mass limit (mg.kg^{-1})
As	Barley grain	0.5	1.07	0.54
	Beetroot leaf	0.5	5.26	2.63
	Beetroot root	0.5	9.87	4.94
	Garden pea pea	0.5	4.07	2.04
	Garden pea pod	0.5	4.31	2.16
	Swiss chard leaf	0.5	6.15	3.08
Pb	Barley grain	0.2	1.07	0.21
	Beetroot leaf	0.3	5.26	1.58
	Beetroot root	0.1	9.87	0.99
	Garden pea pea	0.2	4.07	0.81
	Garden pea pod	0.2	4.31	0.86
	Swiss chard leaf	0.3	6.15	1.85

Regarding animal feed, the South African Animal Feed Guidelines stipulate a standard moisture content of 12 % for all animal feed As and Pb thresholds. Therefore, to convert the feed guidelines to a dry mass basis, a standard conversion factor of 1.14 was applied (Table 3.7). In terms of crop part classification, all leaf and stem material was classified under “hays, straw, lucerne, roughages and bagasse” with a maximum As content of 4.0 mg.kg^{-1} relative to a farm feed with a moisture content of 120g.kg^{-1}. While grains, roots, peas and pods were classified under general “feed ingredients”, with an As threshold of 2.0 mg.kg^{-1} at 12 % moisture content.

The feed classifications were slightly different for Pb, where only the subcategory of “green roughages” was applicable to the crops investigated in this trial. “Green roughages” are defined by the dairy industry as containing moisture from 60 – 90 % and classified into various types, such as: pasture, cultivated fodder crops, tree leaves, roots and crops" (DKP 2019). Green roughages were allocated a much higher feed safety threshold (40.0 $mg.kg^{-1}$ Pb at 12 % moisture), likely in order to compensate for the high moisture content when consumed by animals. Garden pea, beetroot and Swiss chard fell under the green roughage sub-category, while due to its low moisture content, barley was defined as a dry roughage and therefore fell under the general "feed ingredients" category with a Pb threshold of 10.0 $mg.kg^{-1}$ with 12 % moisture.

TABLE 3.7: Animal feed safety guideline conversion to dry mass

Trace element	Crop part	Limit with 12% moisture (mg.kg^{-1})	Factor	Dry mass limit (mg.kg^{-1})
As	Barley grain	2.0	1.14	2.27
	Barley leaf	4.0	1.14	4.55
	Barley stem	4.0	1.14	4.55
	Beetroot leaf	4.0	1.14	4.55
	Beetroot root	2.0	1.14	2.27
	Garden pea leaf	4.0	1.14	4.55
	Garden pea pea	2.0	1.14	2.27
	Garden pea pod	2.0	1.14	2.27
	Garden pea stem	4.0	1.14	4.55
	Swiss chard leaf	4.0	1.14	4.55
Pb	Barley grain	10	1.14	11.36
	Barley leaf	10	1.14	11.36
	Barley stem	10	1.14	11.36
	Beetroot leaf	40	1.14	45.45
	Beetroot root	40	1.14	45.45
	Garden pea leaf	40	1.14	45.45
	Garden pea pea	40	1.14	45.45
	Garden pea pod	40	1.14	45.45
	Garden pea stem	40	1.14	45.45
	Swiss chard leaf	40	1.14	45.45

3.5 Laboratory Analysis of Plant Materials

Dried plant parts were ground using a Bosch MKM6003 Grinder and transferred into small plastic Ziplock bags.

The US EPA Method 3052 was chosen over US EPA Method 3051A for the total decomposition of plant tissues in order to avoid the production of an amorphic siliceous precipitate. The precipitate was deemed to have the potential to adsorb trace elements and thereby underestimate the total extractable concentration.

Precisely 0.3 g of dried plant material was digested in 9 mL of concentrated nitric acid and 3 mL of hydrofluoric acid and microwaved for 15 minutes in an Ultrawave microwave acid digestion system. After cooling, the contents were decanted into a 50 mL plastic disposable test tube and diluted to 35 mL with deionised water. Samples were than analysed by inductively coupled plasma - optical emission spectrometry (ICP-OES) and results were compared against standard series. Originally, the samples were to be analysed using inductively coupled plasma mass spectrometry (ICP-MS) which has a significantly lower method detection limit; however, the university ICP-MS was undergoing routine maintenance at the time of analysis. The ICP-OES method achieved a method detection limit (MDL) of 0.70 $mg.kg^{-1}$ for As or 1.40 $mg.kg^{-1}$ for Pb. All plant samples where As or Pb was detected below the MDL were sent to an external laboratory for ICP-MS analysis. Thirty random plant samples whose results were above the MDL were also sent to the external laboratory for method validation purposes.

The ICP-MS analysis was done by an ISO 17025 accredited (SANAS) referral laboratory. One gram of dry powdered material was added to a mixture of 13 mL nitric acid and 6 mL hydrochloric acid and microwave acid digested. Samples were then analysed by ICP-MS with a Limit of Quantification (LoQ) of 0.001 $mg.L^{-1}$. Note that the LoQ is a concentration higher than the MDL, where the results are aimed at attaining maximum precision and accuracy. Both accuracy and precision deteriorate as concentrations move lower than the LoQ towards the MDL.

3.6 Laboratory Analysis of Soil and Water Samples

3.6.1 Routine chemical soil analysis

All soil analyses were performed in triplicate. The routine soil chemical analysis was undertaken two weeks after the addition of lime, but before fertilization and planting.

The pH (water) of the soil was measured using a pH meter and electrode system. 25 mL of deionized water was added to 10 g of air-dried soil (1:2.5 soil/water ratio), shaken on a reciprocal shaker for 20 minutes, left to stand for one hour, then pH readings were taken. Electrical conductivity (EC) was then measured from the same sample. The same procedure was followed for pH (0.01 M $CaCl_2$); however, 25 mL of deionised water was substituted for 25 mL of 0.01 M $CaCl_2$ solution.

The standard Walkley-Black method was employed to determine percent organic carbon of the soil samples (Nelson and Sommers, 1996; Non-Affiliated Soil Analysis Work Committee, 1990a).

The ammonium acetate extraction method was employed to determine the amount of soluble and exchangeable bases (Ca, Mg, Na and K). Twenty millilitres of 1 M NH_4OA_C was added to 2 g of air-dried soil in a 50 mL centrifuge tube. The samples were then shaken on a reciprocal shaker for 2 hours. Thereafter, samples were centrifuged for 10 minutes at 6000 rpm, filtered through Whatman 44 filter paper and analysed by ICP-OES (Van Reeuwijk 2002).

The Bray No. 1 extraction method was utilised to determine available phosphorus. Seven millilitres of Bray Extracting Solution was added to 1 g of air-dried soil placed into a 50 mL centrifuge tube, vigorously shaken for 1 minute, then centrifuged for 5 minutes at 6000 rpm. Samples and blanks were then analysed with ICP-OES (Bray and Kurtz 1945, Menage and Pridmore 1973).

Nitrogen was extracted according to US EPA Method 1686 (2001). Water is added to soil, passed through a Cd-Cu reduction column to reduce all nitrate to nitrite. Nitrate concentration is then determined by diazotizing with sulphanilimide and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride to form a highly coloured azo dye which is measured colourimetrically.

The calcium phosphate extraction method was used to determine elemental sulphur content of the soil. 15 mL of 0.01 M calcium phosphate was added to 3 g of air-dried soil in a 50 mL centrifuge tube, shaken for 1 hour in a reciprocal shaker, filtered with Whatmann 44 filter paper and analysed by ICP-OES (AGRILASA 2004).

Prior to potting, three representative soil samples were taken and pH was analysed to determine the lime requirement. Two weeks after the addition of lime, pH was measured again, as well as other chemical properties that could impact the sequestration and mobility of As and Pb (Table 3.8).

TABLE 3.8: Chemical properties of soil used in pot trials

Property	Mean	Unit
pH_{water}	7.19 *	
pH_{CaCl2}	6.64 *	
EC	0.071	$mS.m^{-1}$
Organic carbon	0.58	%
CEC	6.80	$cmol_c.kg^{-1}$
N	22.2	$mg.kg^{-1}$
P	38.9	$mg.kg^{-1}$
K	196.7	$mg.kg^{-1}$
Na	70.1	$mg.kg^{-1}$
Mg	218.1	$mg.kg^{-1}$
Ca	829.6	$mg.kg^{-1}$
SO_4	36.7	$mg.kg^{-1}$

* Before liming: pH water = 5.28 and pH $CaCl_2$ = 4.77.

Soil used for the pot trials was initially too acidic for agronomic use. Four weeks after liming, the soil pH was remeasured to confirm effective liming to bring the pH of the soil up to circumneutral conditions. The original low pH soil would have significantly increased the mobility of added lead, while reducing the mobility of arsenic. However, at the amended pH, fit for agronomic use, As and Pb soil mobility would be closer to that which would be expected in a well-managed field.

The electrical conductivity (EC) of the soil was relatively low, consequently crops grown were not expected to exhibit osmotic stress or phytotoxicity symptoms related to saline soil conditions.

The organic carbon content of the trial soil was less than 1 %, which is typical of South African Highveld soils (FERTASA 2016). Low organic carbon equates to low organic matter, which suggests that the low mobility of As and Pb could not have been attributed to the effect of organic compounds in the trial soils, but other factors. Due to the clay content of the selected soil, the cation exchange capacity (CEC) was moderate to low. This is typical of soils with kaolinitic clay and low organic matter (Ketterings et al. 2007).

Regarding the nutritional status of the soil, potassium was in excess, phosphorus was adequate, and nitrogen was low. However, a crop maintenance fertilizer regime including N, P and K was applied to simulate a typical fertilizer program (Appendix C). According to Dai et al. (2016) phosphorus applications are likely to decrease the mobility of lead, through the precipitation of insoluble pyromorphite. Additionally, it was anticipated that the root uptake of arsenic would likely decrease with the addition of phosphorus, due to the competitive anion effect (Wang et al. 2002). The impact of exchangeable cations is presented in Table 3.9.

TABLE 3.9: Estimated exchangeable cation percentage of cations in soil used for pot trials after liming

Cation	Occupied CEC (%)
Ca	60.7
Na	4.48
K	7.41
Mg	26.3

The ideal percentages for cations on the CEC for an optimal reserve of nutrients are as follows: 0.5 – 3 % Na, 2 – 7.5 % K, 10 – 20 % Mg and 60 – 70 % Ca (FERTASA 2016). The soil used for the pot trials generally fulfilled the ideal CEC requirements, with slightly excessive Na and Mg.

However, the estimated exchangeable Na percentage of 4.48 %, combined with the EC of 70.77 $\mu S.m^{-1}$, demonstrates that the soil used in these trials was neither saline nor sodic and was therefore fit to grow all classes of crops (Brady and Weil 2008). Ideally, the CEC should comprise of less than 3 % Na; however, due to the fact that the soil contained in excess of 60 % Ca, it was expected that crops grown would be able to tolerate the slightly elevated Na levels (FERTASA 2016).

3.6.2 Routine physical soil analysis

Soil colour was determined qualitatively utilising the Munsell colour chart.

Particle size analysis was determined via hydrometer. Twenty millilitres of hydrogen peroxide was added to 50 g of air-dried soil. Beakers were then placed on a hot plate and the samples were stirred while deionised water was added to make the volume up to 150 mL. Samples were then dried, washed with deionized water and calgon, placed in a mixing cup and mixed for 5 minutes using an electric mixer. The mixture was then washed and passed through a 0.053 mm sieve into a 1 L cylinder. The cylinder was filled and mixed thoroughly using a plunger. After 6 hours and 35 minutes, a hydrometer was carefully inserted into the solution without mixing it and a reading was taken. The suspension was then mixed again and after 40 seconds a second reading was taken. Percent sand, silt and clay was calculated thereafter (Bouyoucos 1962).

A Pressure Plate apparatus was utilised for determination of field capacity (FC): the water content held in soil after excess water has been drained, and permanent wilting point (PWP): the soil water content at which most plants will wilt and fail to recover turgor upon rewetting (Abbott 1985). The difference between FC and PWP gives a good indication of plant available water.

Prior to potting, three representative soil samples were taken and analysed for basic physical properties. The results are shown in Table 3.10.

TABLE 3.10: Physical properties of soil used in pot trials

Soil colour		Particle size distribution (%)			Texture	Water content (m^3/m^3)	
Dry	Moist	Clay	Silt	Sand		FC	PWP
2.5 YR 4/8	5YR 3/4	23.9	14.3	61.8	Sandy clay loam	0.324	0.155

A sandy clay loam was chosen for the research trials as it has a moderate clay content while maintaining good drainage. Furthermore, most crops can be grown in a sandy clay loam and soils with moderate to low clay are better suited to irrigation than heavy clay soils. While a higher clay content and 2:1 clay minerology may have been a better fit in terms of the South African irrigation water quality guidelines' definition of a forgiving soil, care was taken not to select an exceptionally high clay soil to avoid waterlogging within pots. Waterlogged, reducing conditions are atypical growth environments of the crops used in these trials and such conditions strongly influence the mobility and bioavailability of arsenic and lead. Therefore, to limit confounding factors in this research, the use of high clay soils was avoided.

3.6.3 Acid ammonium oxalate and dithionite-citrate-bicarbonate extraction

The acid ammonium oxalate (in the dark) extraction was performed to determine the amount of amorphous and very poorly crystalline Fe, Al and Mn forms in the soil. The reaction was carried out in the absence of light to avoid photodecomposition (Courchesne and Turmel, 2007) of the oxalate solution as well as the dissolution of crystalline Fe oxides (Kersten and Forstner, 1990).

Twenty millilitres of acid ammonium oxalate solution (a mixture of 0.2 M ammonium oxalate solution and 0.2 M oxalic acid solution at a ratio of 1.3:1, adjusted to pH 3) was added to 0.5 g of air-dried soil. Samples were agitated in a reciprocal shaker for 4 hours, then centrifuged for 10 minutes at 6000 rpm. The extracts were then membrane filtered using 0.45 um pore size filter paper and analysed using ICP-AES (Courchesne and Turmel, 2007).

The dithionite-citrate-bicarbonate extraction was performed to determine the total Fe, Al and Mn forms in the soil, including crystalline iron oxides as well as much of the amorphous materials (McKeague and Day 1996). 40 mL of 0.3 M sodium citrate and 5mL of $NaHCO_3$ was added to 4 g of air-dried soil in a 125 mL centrifuge tube, placed in a water bath and warmed to 77 degrees Celsius, then 1 g of dithionite ($Na_2S_2O_4$) powder was added and stirred rapidly for 1 minute. The sample remained in the water bath for a further 15 minutes while being stirred intermittently. Thereafter, samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was then decanted into a 200 cm^3 volumetric flask. 60 mL of deionised water was added to the residue in the centrifuge and the aforementioned process was repeated to generate additional supernatant which was added to the 200 cm^3 volumetric flask, which was then made up to volume with deionised water. The solution was then analysed by ICP-OES (AGRILASA 2002). The results are shown in Table 3.11.

TABLE 3.11: Dithionite and oxalate extractable iron, aluminium and manganese

Cation	**Dithionite extractable ($g.kg^{-1}$) ± SD**	**Oxalate extractable ($g.kg^{-1}$) ± SD**	**Percent amorphous (%)**
Fe	23.2 ± 1.54	6.54 ± 0.96	28.2
Al	4.98 ± 1.06	1.45 ± 0.07	29.1
Mn	0.45 ± 0.08	0.18 ± 0.04	40.0

Approximately 2.3 % of the soil used in the trials comprised of iron, 0.5 % aluminium and 0.05 % manganese. These values are typical of mineral soils (Clark and Peck 1986). The acid ammonium oxalate and dithionite-citrate-bicarbonate extraction methods for metal oxyhydroxides developed by McKeague and Day (1996) give an indication of the extent of crystallisation of soil minerals containing Fe, Al and Mn. The oxalate extraction dissolved much of the amorphous, poorly crystalline material, but very little crystalline oxides, while the dithionite extraction was able to dissolve crystalline oxides and amorphous materials, thereby providing a total extractable figure for Fe, Al and Mn residing with oxides. More crystalline metal oxides possess fewer adsorption sites, while amorphous metal oxyhydroxides have far more reactive sites and contribute to a soil's anion exchange capacity (Brady and Weil 2008). In this soil, approximately 30 % of all Fe and Al oxyhydroxides were determined to be amorphous as well as 40 % of the Mn oxyhydroxides. These amorphous mineral surfaces would exhibit a high affinity for As and Pb and thereby reduce plant uptake of those elements, and in doing so, aid in the fulfilment of the forgiving soil conditions required for this study's trials.

3.6.4 Acid and salt extraction of As and Pb

A 100 g representative soil sample was taken from each pot used in the trial. From the representative sample, one sub-sample was subjected to microwave assisted acid digestion and another sub-sample was subjected to a salt extraction. The microwave assisted acid digestion was performed to determine the total As or Pb in each soil sample. The salt extraction was performed to estimate potentially phytoavailable concentrations of As or Pb in each soil sample.

The US EPA Method 3052 was chosen for the total acid extraction method of soil samples, as described in Section 3.5. For the estimation of potentially phytoavailable concentrations, 1 g of soil was shaken on a reciprocal shaker with 10 mL of the 0.01 M $CaCl_2$ extractant for 40 minutes, centrifuged at 3000 rpm for 10 minutes, filtered through Whatman 44 filter paper, acidified with 9 mL of concentrated nitric acid to minimise the presence of amorphous siliceous deposits, diluted to the 35 mL mark with deionised water and analysed by ICP-OES. The method achieved a method detection limit (MDL) of 0.70 $mg.kg^{-1}$ for As and 1.40 $mg.kg^{-1}$ for Pb.

3.7 Laboratory Analysis of Water Samples

Sampled in triplicate, water samples were filtered through Whatmann 44 filter paper, 20 mL of filtrate was then transferred to a 50 mL centrifuge tube, stabilised with 9 mL of concentrated nitric acid, diluted to the 35 mL mark with deionised water and analysed for As and Pb by ICP-OES.

Borehole water was sampled from the tap of the glasshouse in July and September 2017. The results indicated that As and Pb were consistently found to be below the method detection limit of 0.04 $mg.kg^{-1}$ for As or 0.07 $mg.kg^{-1}$ for Pb. For quality control purposes, irrigation water used for the glasshouse foliar uptake trial was sampled monthly for the duration of the trial. The results are shown in Appendix D.

3.8 Data Processing

To investigate the crops' ability to extract As and Pb from the soil into the aboveground biomass, the bioaccumulation factor (BAF) was calculated (Eq. 3.1). The BAF refers to the ratio between the element concentration in the aboveground organs ($mg.kg^{-1}$) and that of the soil (Liñero et al. 2017). BAF values greater than 1 indicate a strong extraction of the element from the soil into the aboveground biomass.

$$BAF = \frac{\text{element concentration in aboveground organs}}{\text{element concentration in soil}} \qquad \text{... Eq. 3.1}$$

To determine the transferability of trace elements from the roots to the aboveground biomass, the transfer coefficient (TC) was determined (Chen et al. 2016, Liñero et al. 2017). The TC is defined as the ratio between element concentration in the aboveground organs ($mg.kg^{-1}$) and that of the roots. This equation could only be applied to beetroot, because the roots of the other three crops were not analysed. This coefficient is defined in equation 3.2.

$$TF = \frac{\text{element concentration in aboveground organs}}{\text{element concentration in roots}} \quad \text{... Eq. 3.2}$$

To determine the extent of translocation of As and Pb applied as an irrigation treatment to the aboveground biomass to other plant parts, the mobility index (MI) of Kumar et al. (2009) was adapted. The MI refers to the ratio of element concentration in the receiving level to that of the source level (Eq. 3.3). In the case of the glasshouse foliar uptake trial, the source level was the leaves and stems and receiving levels were the roots, grains or peas (excluding pods).

$$MI = \frac{\text{element concentration in the receiving level}}{\text{element concentration in the source level}} \quad \text{... Eq. 3.3}$$

Data for each trial was analysed separately using NCSS 12 (data analysis software system). Two-way analysis of variance (ANOVA) conducted to evaluate the effect of irrigation treatment and crop part on potentially hazardous trace element accumulation in plant tissues ($\alpha = 0.05$). There were 5 irrigation treatments, 10 crop parts analysed per treatment and 3 replicates, resulting in 149 total degrees of freedom. Fisher's Least Significant Difference (LSD) Test at the 5% significance level ($\alpha = 0.05$) was used to determine statistically significant differences between the means.

3.8.1 Quality control

Quality control measures were taken to assess contamination and reliability of data. Blank and drift standards were run after five determinations to calibrate the ICP-OES. The coefficients of variation of replicate analysis were determined for precision of analysis; the variations were found to be less than 10 %. Precision and accuracy of analysis was further assured by sending 30 random dried and powdered plant samples to an external ISO 17025 accredited (SANAS) referral laboratory for analysis by ICP-MS for comparison against the ICP-OES results. The original ICP-OES results were found within ± 2 % of the ICP-MS results.

CHAPTER 4: GLASSHOUSE FOLIAR ABSORPTION TRIAL

4.1 Introduction

The purpose of the glasshouse foliar absorption pot trial was to evaluate whether arsenic (As) or lead (Pb) present in irrigation water at the “target water quality”, or “maximum acceptable” concentrations, would result in crop plant parts exceeding modern food safety thresholds for those elements over one growing season. The “target water quality” is the concentration of As or Pb that may be utilized for agricultural irrigation on a field for up to 100 years (0.1 $mg.L^{-1}$ As, 0.2 $mg.L^{-1}$ Pb). The “maximum acceptable water quality” is the concentration of As or Pb that may be utilized for agricultural irrigation on a field for up to 20 years (2.0 $mg.L^{-1}$ As, 2.0 $mg.L^{-1}$ Pb). The crops selected for this trial were chosen to demonstrate the response of grains, legumes, leafy vegetables and root vegetables to overhead irrigation with As or Pb loaded water. Synthetic As or Pb contaminated water was sprayed on crops to the point of run-off every 1 to 3 days to imitate overhead irrigation. This trial was specifically designed to investigate foliar uptake of these elements based on the methodology outlined by Bondada and Ma (2004). Soil was wet to field capacity with borehole water, while care was taken not to wet the aboveground biomass in order to limit runoff and therefore limit the influence of root uptake pathways.

In this chapter, the yield response of crops to foliar spray treatments is discussed, followed by notes on crop quality. Thereafter, the accumulation of As or Pb per plant part is discussed with reference to food safety and animal feed thresholds. Note that food safety thresholds were adapted from a wet mass basis to a dry mass basis for purpose of comparison. Results on a wet mass basis can be found in Appendix B1. Finally, the salient findings of the trial are presented in the conclusion.

4.2 Material and Methods

The glasshouse foliar absorption trial comprised of 60 pots. Four crops (barley, beetroot, Swiss chard and garden pea) were evaluated against 5 treatments (control, As irrigation at 0.1 $mg.L^{-1}$, As irrigation at 2.0 $mg.L^{-1}$, Pb irrigation at 0.2 $mg.L^{-1}$ and Pb irrigation at 2.0 $mg.L^{-1}$) with 3 replicates each. Data from the crops was collected for a total of 10 different plant parts (barley grain, barley leaves, barley stems, beetroot root, beetroot leaves, Swiss chard leaves, peas, pea pods, pea leaves and pea stems), which yielded 150 samples for analysis. The effects of irrigation treatments were evaluated using analysis of variance (ANOVA) and differences among sources were compared with Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$).

4.3 Results and Discussion

4.3.1 Yield response

Results of yield response for the four different crops under foliar irrigation with either As or Pb loaded irrigation water are recorded in Appendix A1.

No significant difference in yield response was found between the control and treatment yields for all crops (barley, garden pea, Swiss chard and beetroot). The foliar treatments had no effect on dry mass accumulation in plant parts (stem, leaf, grain, pea, pod and root), in all crops. These results were expected, as it was anticipated that the element concentrations applied in the foliar treatments were too low to influence crop growth and yield. The results thus confirm that foliar applications at the concentrations applied in this study do not influence yield.

4.3.2 Influence of irrigation treatments on crop quality

Crop quality was visually assessed by qualitatively assessing phenology, colour (Munsell) and the presence or absence of treatment residues. No noticeable differences between control and treated plants were observed for each of the four crops under investigation. As and Pb applied via irrigation water did not result in a visible deposit or residue. No difference in size, shape and quantity of barley grains and pea pods was observed. Similarly, the size, shape and colour of Swiss chard, beetroot leaves and beetroot roots did not differ from the control plants.

4.3.3 Arsenic accumulation in plant parts

Arsenic accumulation in the aboveground biomass was analysed according to plant part (Table 4.1). The As irrigation treatments were as follows: 0.0 mg.L^{-1} (control), 0.1 mg.L^{-1} ("target water quality") and 2.0 mg.L^{-1} ("maximum acceptable water quality"). Note that the Chinese, not South African, food safety guidelines were used to determine the human food safety thresholds for As and Pb. While both guidelines share the same range of allowable As in human foods, the Chinese guidelines specify trace element limits for fruits, vegetables and grains, while the South African guidelines do not. Food safety guidelines are presented on a fresh mass basis. For the purpose of this study, the food safety guidelines were converted from fresh mass to dry mass (DM) according to the average moisture content of each plant part at harvest.

TABLE 4.1: Mean arsenic (± standard deviation) in crops irrigated with As loaded water, with reference to food safety thresholds

Crop	Plant part	Treatment (mg As. L^{-1})	Plant tissue (mg As. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg As. kg^{-1} DM$^{\Delta}$)
Barley	Grain	0.0	0.01 ± 0.00	a	0.54
		0.1	0.12 ± 0.04	a	
		2.0	0.28 ± 0.09	a	
Beetroot	Leaf	0.0	0.07 ± 0.02	a	2.63
		0.1	1.64 ± 0.07	ab	
		2.0	10.77 ± 2.36	c	
	Root	0.0	0.01 ± 0.00	a	4.94
		0.1	0.01 ± 0.00	a	
		2.0	0.03 ± 0.01	a	
Garden pea	Pea	0.0	0.01 ± 0.00	a	2.04
		0.1	0.01 ± 0.00	a	
		2.0	0.05 ± 0.02	a	
	Pod	0.0	0.04 ± 0.01	a	2.16
		0.1	0.09 ± 0.03	a	
		2.0	0.68 ± 0.13	a	
Swiss chard	Leaf	0.0	0.06 ± 0.01	a	3.08
		0.1	1.56 ± 1.02	ab	
		2.0	33.38 ± 8.96	d	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM$^{\Delta}$ dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

The general trend showed increased As in crop plant tissues with increased As irrigation treatments, however most edible parts did not accumulate enough As to pose a health risk.

Whole barley grains (inclusive of bran) did not accumulate As above the food safety threshold at any treatment level and were therefore deemed fit for human consumption. This trend is in-line with previous studies, which showed that arsenate and arsenite are poorly translocated from leaves to grains (Carey et al. 2011). Arsenic found in the tissue of garden pea pods and peas did not exceed the food safety threshold at both treatment levels. As would be expected, beetroot roots did not accumulate any more As than the control at either irrigation treatment level. While certain organic arsenic species may be remobilised from vacuoles, they are typically redistributed to the aboveground biomass and not the roots (Page and Feller 2015).

Swiss chard and beetroot leaves irrigated with 0.1 $mg.L^{-1}$ As did not exceed the food safety threshold of 3.08 $mg.kg^{-1}$. However, similar to the findings of Mahmood and Malik (2014), the Swiss chard and beetroot leaves which received the 2 $mg.L^{-1}$ As irrigation treatment readily absorbed foliar applied As and accumulated the largest amount of As of all the crops and plant parts. Swiss chard accumulated the most As, with an average of 33.38 $mg.kg^{-1}$ As on a dry mass basis. This concentration was an order of magnitude greater than the regulated food safety threshold. Literature suggests that this was likely due to the large surface area of Swiss chard and beetroot leaves (Nayek et al. 2010, Mahmood and Malik 2014). All results which exceeded the food safety guideline thresholds were found to be statistically significant according to Fisher's LSD Test.

While not the primary focus of this study, the implications of using these crops for animal feed is shown in Table 4.2. The animal feed guidelines used here are from Annexure 4 of the South African Department of Agriculture, Forestry and Fisheries (DAFF) Act No. 36 of 1947. Wherein a 12% water content is assumed for all feed. For the purpose of this study, the feed guidelines were converted to a dry mass (DM) basis and compared to the dry mass results of each plant part. All crops were evaluated according to the “hays, straws, lucerne, roughages and bagasse” sub-category threshold of feed ingredients.

TABLE 4.2: Mean arsenic (± standard deviation) in crops irrigated with As loaded water, with reference to animal feed safety thresholds

Crop	Plant part	Treatment (mg As. L^{-1})	Plant tissue (mg As. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg As. kg^{-1} DM*)
Barley	Grain	0.0	0.01 ± 0.00	a	4.55
		0.1	0.12 ± 0.04	a	
		2.0	0.28 ± 0.09	a	
	Leaf	0.0	0.11 ± 0.02	a	4.55
		0.1	10.32 ± 1.83	c	
		2.0	21.46 ± 4.85	d	
	Stem	0.0	0.04 ± 0.00	a	4.55
		0.1	0.61 ± 0.26	a	
		2.0	0.65 ± 0.37	a	
Beetroot	Leaf	0.0	0.07 ± 0.02	a	4.55
		0.1	1.64 ± 0.07	a	
		2.0	10.77 ± 2.36	c	
	Root	0.0	0.01 ± 0.00	a	4.55
		0.1	0.01 ± 0.00	a	
		2.0	0.03 ± 0.01	a	
Garden pea	Leaf	0.0	0.10 ± 0.02	a	4.55
		0.1	4.04 ± 2.08	bc	
		2.0	19.50 ± 6.38	c	
	Pea	0.0	0.01 ± 0.00	a	4.55
		0.1	0.01 ± 0.00	a	
		2.0	0.05 ± 0.02	a	
	Pod	0.0	0.04 ± 0.01	a	4.55
		0.1	0.09 ± 0.03	a	
		2.0	0.68 ± 0.13	a	
	Stem	0.0	0.05 ± 0.01	a	4.55
		0.1	0.55 ± 0.26	a	
		2.0	2.20 ± 1.37	ab	
Swiss chard	Leaf	0.0	0.06 ± 0.01	a	4.55
		0.1	1.56 ± 1.02	ab	
		2.0	33.38 ± 8.96	e	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

In general, leaves, and to a lesser extent, stems, accumulated most of the As applied to the aboveground biomass. Barley leaves at both As irrigation treatments accumulated As above the regulated 4.55 mg.kg^{-1} DM for hays, straws, lucerne, roughages and bagasse, while the stems did not (DAFF Act 36 of 1947). Similarly, pea, Swiss chard and beetroot leaves which received the 2 mg.L^{-1} As treatment far exceeded the animal feed threshold, while leaves which received the 0.1 $mg.L^{-1}$ As treatment did not. Barley grains, garden pea pods and peas, and beetroot roots were all well below the As thresholds for use as animal feed at all treatment levels. All results which exceeded the animal feed thresholds were found to be statistically significant according to Fisher's LSD Test.

The highest rate of As absorption occurred on the leaf surfaces of all four crops. This is likely due to the larger surface area and complex cuticle morphology, as well as the increased concentration of epidermal structures through which As may be absorbed (Kannan 2008, Nayek et al. 2010, Fernández et al. 2013, Mahmood and Malik 2014). The lower rate of As uptake via stems may also suggest that arsenite was oxidised to arsenate when applied to the stem surface, which may then have strongly associated with preexisting iron and aluminium oxide soil and dust particles and later been washed off the leaf surface during subsequent irrigations or during the sample washing process in the laboratory after harvest.

Owing to the small number of replicates per treatment (three for each plant part of each crop), mean values may not have accurately reflected true values where large variability among the replicates occurred. To evaluate the validity of using the means, the medians were also investigated and are recorded in Table 4.3. Comparison of the median and mean values demonstrated that in all instances, the selection of the statistical parameter did not change the result of whether the treatment exceeded the dry mass food safety threshold. Owing to the similarities in results between the median and mean values, both serve to validate the other, thereby justifying the use of means for this data set.

TABLE 4.3: Median arsenic in crops irrigated with As loaded water, with reference to food and animal feed safety thresholds

Crop	Plant part	Treatment (mg As. L^{-1})	Plant tissue (mg As. kg^{-1} DM)	Food Threshold (mg As. kg^{-1} DM^{Δ})	Feed Threshold (mg As. kg^{-1} DM*)
Barley	Grain	0.0	0.01	0.54	4.55
		0.1	0.15		
		2.0	0.34		
	Leaf	0.0	0.10		4.55
		0.1	9.62		
		2.0	24.26		
	Stem	0.0	0.04		4.55
		0.1	0.69		
		2.0	0.81		
Beetroot	Leaf	0.0	0.08	2.63	4.55
		0.1	1.66		
		2.0	10.22		
	Root	0.0	0.01	4.94	4.55
		0.1	0.01		
		2.0	0.03		
Garden pea	Leaf	0.0	0.08		4.55
		0.1	2.74		
		2.0	16.81		
	Pea	0.0	0.01	2.04	4.55
		0.1	0.01		
		2.0	0.04		
	Pod	0.0	0.03	2.16	4.55
		0.1	0.08		
		2.0	0.66		
	Stem	0.0	0.05		4.55
		0.1	0.43		
		2.0	1.25		
Swiss chard	Leaf	0.0	0.05	3.08	4.55
		0.1	1.16		
		2.0	29.01		

DM^{Δ} dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

The real-world applications of these results are discussed and presented in the form of food and feed safety consequence matrices in Chapter 6.

4.3.4 Lead accumulation in plant parts

Lead accumulation in the aboveground biomass was analysed according to plant part (Table 4.4). The effects of irrigation treatments were evaluated using analysis of variance (ANOVA) and differences among sources were compared with Fisher's Least

Significant Difference (LSD) test (α = 0.05). The Pb irrigation treatments were as follows: 0.0 $mg.L^{-1}$ (control), 0.2 $mg.L^{-1}$ ("target water quality") and 2.0 $mg.L^{-1}$ ("maximum acceptable water quality"). For the purpose of this study, the food safety guidelines were converted from fresh mass to dry mass (DM) according to the average moisture content of each plant part at harvest.

TABLE 4.4: Mean lead (± standard deviation) in crops irrigated with Pb loaded water, with reference to food safety thresholds

Crop	Plant part	Treatment (mg Pb. L^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg Pb. kg^{-1} DM$^{\Delta}$)
Barley	Grain	0.0	0.01 ± 0.00	a	0.21
		0.2	0.15 ± 0.01	a	
		2.0	0.17 ± 0.02	a	
Beetroot	Leaf	0.0	0.06 ± 0.00	a	1.58
		0.2	13.72 ± 2.43	b	
		2.0	36.46 ± 6.52	c	
	Root	0.0	0.02 ± 0.00	a	0.99
		0.2	0.02 ± 0.00	a	
		2.0	0.03 ± 0.01	a	
Garden pea	Pea	0.0	0.01 ± 0.00	a	0.81
		0.2	0.02 ± 0.00	a	
		2.0	0.02 ± 0.00	a	
	Pod	0.0	0.01 ± 0.00	a	0.86
		0.2	0.11 ± 0.02	a	
		2.0	0.36 ± 0.26	a	
Swiss chard	Leaf	0.0	0.05 ± 0.00	a	1.85
		0.2	32.42 ± 5.94	c	
		2.0	62.41 ± 14.41	d	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM$^{\Delta}$ dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

The general trend showed increased Pb in crop plant tissues with increased Pb irrigation treatments; however, most edible parts did not accumulate enough Pb to pose a health risk.

Swiss chard and beetroot leaves accumulated Pb well beyond the food safety guidelines at both Pb irrigation treatments. These results are comparable to Singh et al. (2010), where cauliflower florets accumulated 17.9 $mg.kg^{-1}$ Pb (dry mass) when irrigated with water containing 0.09 $mg.L^{-1}$ Pb. All other edible crop parts were within the food safety guideline limits and therefore fit for human consumption. A likely explanation for the finding that roots, grains and peas accumulated negligible Pb is because Pb is not remobilised in the phloem (Dollard 1986) and would therefore be expected to remain at the point of contact when applied to the aboveground biomass. All results which exceeded the food safety thresholds were found to be statistically significant according to Fisher's LSD Test.

As mentioned in the previous section, although the implications of using these crops for animal feed was not the primary focus of this study, the results were analysed in terms of the animal feed guidelines and are shown in Table 4.5 on the following page. The guidelines for Pb refer to "green roughage", which, while not defined in the DAFF document, is defined by the dairy industry as "containing moisture from 60 – 90 % and classified into various types, such as: pasture, cultivated fodder crops, tree leaves, roots and crops" (DKP 2019). Green roughages are allocated a much higher feed safety threshold, likely in order to compensate for the high moisture content when consumed by animals. Garden pea, beetroot and Swiss chard fell under the green roughage sub-category, while due to its low moisture content, barley was defined as a dry roughage and therefore fell under the general "feed ingredients" category. Hence the difference in recorded feed threshold values. Note all crop moisture contents can be found in Table 3.5.

Barley leaves and stems exceeded the feed threshold when irrigated with water containing 2.0 $mg.L^{-1}$ Pb, while those irrigated with 0.2 $mg.L^{-1}$ Pb did not. Even though beetroot and garden pea leaves accumulated high concentrations of Pb at both treatment levels, only Swiss chard leaves exceeded the feed safety threshold of green roughage when irrigated with water containing 2.0 $mg.L^{-1}$ Pb. In the case of Pb, more leaves and stems were considered fit for animal consumption, not because they accumulated less Pb when compared to the As irrigation trials, but because the Pb feed guidelines are significantly higher (10 $mg.kg^{-1}$ or 40 $mg.kg^{-1}$ at 12% moisture) than the As feed guidelines (2 $mg.kg^{-1}$ or 4 $mg.kg^{-1}$ at 12% moisture).

TABLE 4.5: Mean lead (± standard deviation) in crops irrigated with Pb loaded water, with reference to animal feed safety thresholds

Crop	Plant part	Treatment (mg Pb. L^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg Pb. kg^{-1} DM*)
Barley	Grain	0.0	0.01 ± 0.00	a	11.36
		0.2	0.15 ± 0.01	a	
		2.0	0.17 ± 0.02	a	
	Leaf	0.0	0.15 ± 0.03	a	11.36
		0.2	7.79 ± 1.47	b	
		2.0	46.26 ± 17.56	d	
	Stem	0.0	0.04 ± 0.00	a	11.36
		0.2	6.46 ± 0.11	b	
		2.0	20.90 ± 3.04	c	
Beetroot	Leaf	0.0	0.06 ± 0.00	a	45.45
		0.2	13.72 ± 2.43	bc	
		2.0	36.46 ± 6.52	d	
	Root	0.0	0.02 ± 0.00	a	45.45
		0.2	0.02 ± 0.00	a	
		2.0	0.03 ± 0.01	a	
Garden pea	Leaf	0.0	0.11 ± 0.01	a	45.45
		0.2	26.19 ± 3.84	cd	
		2.0	34.64 ± 2.68	d	
	Pea	0.0	0.01 ± 0.00	a	45.45
		0.2	0.02 ± 0.00	a	
		2.0	0.02 ± 0.00	a	
	Pod	0.0	0.01 ± 0.00	a	45.45
		0.2	0.11 ± 0.02	a	
		2.0	0.36 ± 0.26	a	
	Stem	0.0	0.07 ± 0.01	a	45.45
		0.2	0.44 ± 0.16	a	
		2.0	1.94 ± 0.54	a	
Swiss chard	Leaf	0.0	0.05 ± 0.00	a	45.45
		0.2	32.42 ± 5.94	d	
		2.0	62.41 ± 14.41	e	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

As with the arsenic analysis, median values were also investigated to evaluate the validity of using the mean scores and are recorded in Table 4.6. Comparison of the median and mean values demonstrated that the selection of the statistical parameter did not change the result of whether the treatment exceeded the dry mass food safety threshold, thereby justifying the use of means for this data set.

TABLE 4.6: Median lead in crops irrigated with Pb loaded water, with reference to food and animal feed safety thresholds

Crop	Plant part	Treatment (mg Pb. L^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Food Threshold (mg Pb. kg^{-1} DM^{Δ})	Feed Threshold (mg Pb. kg^{-1} DM*)
Barley	Grain	0.0	0.01	0.21	11.36
		0.2	0.14		
		2.0	0.18		
	Leaf	0.0	0.15		11.36
		0.2	8.77		
		2.0	44.93		
	Stem	0.0	0.04		11.36
		0.2	6.44		
		2.0	21.96		
Beetroot	Leaf	0.0	0.06	1.58	45.45
		0.2	14.90		
		2.0	35.65		
	Root	0.0	0.02	0.99	45.45
		0.2	0.02		
		2.0	0.02		
Garden pea	Leaf	0.0	0.11		45.45
		0.2	26.90		
		2.0	33.55		
	Pea	0.0	0.01	0.81	45.45
		0.2	0.02		
		2.0	0.02		
	Pod	0.0	0.01	0.86	45.45
		0.2	0.08		
		2.0	0.18		
	Stem	0.0	0.08		45.45
		0.2	0.53		
		2.0	1.56		
Swiss chard	Leaf	0.0	0.05	1.85	45.45
		0.2	33.63		
		2.0	52.50		

DM^{Δ} dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.
DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

The real-world applications of these results are discussed and presented in the form of food and feed safety consequence matrices in Chapter 6.

4.3.5 ANOVA table

Table 4.7 presents the results of a two-way analysis of variance (ANOVA) conducted to evaluate the effect of irrigation treatment and crop part on potentially hazardous trace element accumulation in plant tissues (α = 0.05). There were 5 irrigation treatments, 10 crop parts analysed per treatment and 3 replicates, resulting in 149 total degrees of freedom. The results indicate that there was a statistically significant difference ($p < 0.001$) for the interaction effect between treatment and crop part. The two main effects were also statistically significant ($p < 0.001$). Therefore, the relationship between choice of crop and concentration of As or Pb in irrigation water was found to play a crucial role in determining whether the edible parts of crops would accumulate As or Pb in excess of food (or feed) safety thresholds.

Table 4.7: Two-way Analysis of Variance (ANOVA) of foliar absorption trial

Source	***SS***	***df***	***MS***	***F***	***P-value***	***F critical***
Treatment	7 607.27	4	1 901.82	79.80	< 0.001	2.46
Crop part	12 141.99	9	1 349.11	56.61	< 0.001	1.97
Interaction	10 418.10	36	289.39	12.14	< 0.001	1.54
Error	2 383.25	100	23.83			
Total	32 550.60	149				

4.4 Comparison to Previous Studies

The results of the Pb irrigation treatments showed a similar trend to that of the As irrigation treatments, with leaves accumulating by far the greatest quantity of trace element applied via irrigation water. However, the major difference between the two treatments was that leaves irrigated with the Pb treatments tended to accumulate Pb at a rate of almost double that of the As treatments. One possible explanation is that the source of Pb, lead nitrate, used when formulating the irrigation treatments was more easily adsorbed via the leaf cuticular surface than the As source, sodium arsenite. However, the complexation of Pb and nitrate is relatively low (category 5) according to Sposito (2008). This trend can be explained by the literature, where the movement of low-molecular-weight solutes (such as arsenic and lead ions) from the leaf surface to the epidermal cell wall has been described as a passive process, driven by diffusion and electrochemical potential formed by a negative charge increase across the cuticular membrane (Wojcik 2004, Shahid et al. 2017). Therefore, one would expect to observe an increased foliar uptake of cations, such as lead, when compared to that of anions, such as arsenic.

The results of the foliar absorption trial were similar to that of Baig and Kazi (2012) who investigated As uptake in peas and Swiss chard irrigated with 0.1 $mg.L^{-1}$ As. While Baig and Kazi (2012) detected As in garden pea, the level of Pb in garden peas of this trial was below the detection limit, but the results for Swiss chard were comparable: 0.98 $mg.kg^{-1}$ As, on a fresh weight basis, versus 1.56 $mg.kg^{-1}$, on a dry mass basis. The findings of Das et al. (2004) exhibited similar contamination concentrations to this study, but on different crops: when irrigated with water containing 0.52 $mg.L^{-1}$ As, peeled potatoes accumulated 0.73 $mg.kg^{-1}$ As and rice grains accumulated 0.14 mg.kg-1 As (on a dry mass basis). Another interesting study worth mentioning is that of Bondada and Ma (2004) which investigated foliar As applications of a hyperaccumulator fern (*Pteris vittata* L.) and recorded a maximum concentration of 116 $mg.kg^{-1}$ As, on a dry mass basis, four weeks after exposure to a once-off application of As at 400 $mg.L^{-1}$. Bondada and Ma's (2004) results are far greater than those of this study which suggests that none of the crops investigated were hyperaccumulators.

With regards to Pb, Amin et al. (2013) and Mahmood and Malik (2014), investigated the effect of irrigating crops with water containing 0.18 and 0.43 $mg.L^{-1}$ Pb, respectively. The results from those trials ranged from 0.49 $mg.kg^{-1}$ Pb in peeled potato, to 14.3 $mg.kg^{-1}$ Pb in brinjal, on a dry mass basis. Those results are comparable to the findings of this study, which ranged from 0.01 $mg.kg^{-1}$ in peas and beetroot root, to 62.4 $mg.kg^{-1}$ of Pb in Swiss chard leaves irrigated with water containing 2.0 $mg.L^{-1}$ Pb. Note that the concentration of Pb in irrigation water investigated in this dissertation was four times greater than that of the Mahmood and Malik (2014) study, which suggests that the absorption of Pb in leaves may be directly proportional to the concentration of Pb in irrigation water.

4.5 Mobility Index

The mobility index (MI) is the ratio between the concentration of element in the receiving level and the concentration of element in the source level (Kumar et al. 2009). MI values greater than one suggest a strong mobility of the element from the source level (leaves) to the receiving level (grains, peas or roots). MI values for the foliar absorption trial are recorded in Table 4.8.

TABLE 4.8: Mobility index (MI) of As and Pb from leaf to grain, seed or root

Crop	Plant part	Mobility Index			
		As [0.1]	**As [2.0]**	**Pb [0.2]**	**Pb [2.0]**
Barley	Grain	0.0116	0.0130	0.0193	0.0037
Garden pea	Pea	0.0025	0.0026	0.0008	0.0006
Beetroot	Root	0.0061	0.0028	0.0015	0.0008

In all cases, the amount of As or Pb that accumulated within the receiving level plant part (barley grains, garden pea seed excluding pods, beetroot roots) was recorded below the method detection limit. Therefore, the MI values reported in Table 4.8 are the maximum possible index if the As or Pb concentration within the grain, seed or root was measured at the method detection limit. In reality, the MI values are likely to be far lower.

All MI's are well below 1 which therefore indicates practically zero mobility of As or Pb from the leaves to the grain, seed or root. Both As and Pb may absorb onto leaf surfaces through epidermal structures such as ectodesmatas, trichomes, lenticels and stomata (Fernández et al. 2013). Thereafter, the adsorbed elements may be transported through the leaf cuticular surface into the cellular space where they may affect plant metabolism and physiology (Kannan 2008). Arsenate has been shown to move across the cell membrane via active phosphate transporters, while arsenite may enter the cell via aquaporins (Yang and Hinner 2015, Dai et al. 2016). Once foliar uptake has taken place, As has been shown to be transported in both the xylem and the phloem of certain plants (Ye et al. 2010). However, the results of this study suggest that the extent of phloem remobilisation of As in the leaves of barley, garden pea and beetroot is extremely low. Therefore, the leaves of these crops effectively acted as a sink for foliar applied As.

Lead has been shown to cross through leaf cuticles and enter the cellular space, although the exact mechanisms remain unclear (Shahid et al. 2017). Once through the cuticle, lead ions tend to accumulate in the apoplast due to the net negative charge of cell walls but may also follow the calcium ion pathway across cell membranes to a limited extent (Kerper and Hinkle 1997, Ding et al. 2016). According to Dollard (1986), Pb is not remobilised in the phloem and is therefore expected to remain at the point of contact when applied to the aboveground biomass. The findings of Dollard (1986) are supported by the results of this trial, as the MI of Pb in barley, garden pea and beetroot was almost zero. Therefore, like As, the leaves of these crops acted as a strong sink for Pb when applied via overhead irrigation. This has strong implications for irrigation management practices and crop choice which will be discussed in Chapter 6.

4.6 Limitations of the Trial

This trial investigated the effects of irrigation on winter crops in a summer rainfall region and therefore did not account for the potentially positive effects of rainfall when growing summer crops under As and Pb loaded irrigation water.

In this study, the impact of two potentially hazardous trace elements in irrigation water on four crops was investigated. Increasing the number of potentially hazardous trace elements to include Cd, Hg and U, as well as increasing the number of crops, would allow for a more extensive evaluation of how irrigation quality guidelines may impact food and feed safety. However, extending the research to include additional trials was outside the scope of this dissertation.

Finally, adding more treatment concentrations per trace element, could have allowed for the quantification of crop-specific trace element concentrations at which the food safety risk could pose, for example, a 10 % risk of edible parts being unfit for human consumption.

4.7 Conclusions

The general trend showed increased As or Pb in crop plant tissues with increased As or Pb irrigation treatments; however, most edible parts did not accumulate enough As or Pb to pose a health risk if consumed by humans or animals. Excluding the controls, 17 % of crops irrigated with As treated water and 33 % of crop parts irrigated with Pb treated water were unfit for human consumption. Similarly, 20 % of crops irrigated with As treated water and 15 % of crop parts irrigated with Pb treated water were unfit for use as animal feed.

The crop parts that exceeded food or feed guidelines are summarised as follows: Beetroot and Swiss chard leaves irrigated with 2 $mg.L^{-1}$ As, as well as beetroot and Swiss chard leaves irrigated with 0.2 $mg.L^{-1}$ and 2 $mg.L^{-1}$ Pb, exceeded the food safety thresholds. Regarding As in animal feed, all crop leaves irrigated with 2 $mg.L^{-1}$ As and barley leaves irrigated with 0.1 $mg.L^{-1}$ As exceeded the thresholds. In terms of Pb, barley leaves, barley stems and Swiss chard leaves irrigated with 2 $mg.L^{-1}$ Pb were deemed unfit for use as animal feed.

The result of this study show that the foliar absorption pathway should not be ignored, as it currently is in irrigation water quality guidelines.

Having analysed the short-term impact of foliar uptake of As and Pb in contaminated irrigation water on food safety, the next step in this study was to compare root uptake of those trace elements as a consequence of long-term irrigation programs. The results of the root uptake trial are reported in the next chapter.

CHAPTER 5: GLASSHOUSE ROOT UPTAKE TRIAL

5.1 Introduction

The effect of medium- to long-term irrigation programs on food safety was simulated in this glasshouse pot trial by dosing soil with the equivalent quantity of arsenic or lead that would have accumulated over 19 years at the “maximum allowable water quality”, or over 99 years at the “target water quality”, at an irrigation rate of 1 000 mm per year. The “target water quality” is the concentration of As or Pb that may be utilized for agricultural irrigation on a field for up to 100 years (0.1 $mg.L^{-1}$ As, 0.2 $mg.L^{-1}$ Pb). The “maximum acceptable water quality” is the concentration of As or Pb that may be utilized for agricultural irrigation on a field for up to 20 years (2.0 $mg.L^{-1}$ As, 2.0 $mg.L^{-1}$ Pb). Due to temporal, environmental and safety constraints, soils in pots were synthetically dosed with a once-off application of As or Pb equivalent to 99 years of irrigation at the “target water quality” or 19 years of irrigation at the “maximum acceptable water quality” of each element. The choice of 99- and 19-year simulations was made to investigate plant growth in the “final year” of allowable irrigation. This trial was specifically designed to investigate root uptake of these elements based on the methodology outlined by McBride et al. (2013).

The crops treated in this trial were chosen to demonstrate the response of grains, legumes, leafy vegetables and root vegetables under As or Pb loaded soils as a result of medium- to long-term irrigation. It was assumed that 1000 mm of irrigation water per year would accumulate in the top 0.15 m of soil with a bulk density of 1500 $kg.m^{-3}$. Throughout the trial, soil was irrigated to field capacity with borehole water, while care was taken not to allow any leaching from the bottom of the pots to maintain the As and Pb levels of the soil.

In this chapter, the yield response of crops to soil dose treatments is discussed, followed by notes on crop quality. Thereafter, the accumulation of As or Pb per plant part is discussed with reference to human food safety and animal feed thresholds. Note that food safety thresholds were adapted from a wet mass basis to a dry mass basis. Results on a wet mass basis can be found in Appendix B2. Finally, the salient findings of the trial are presented in the conclusion.

5.2 Materials and Methods

The glasshouse root uptake trial comprised of 60 pots. Four crops (barley, beetroot, Swiss chard and garden pea) were evaluated against 5 treatments (control, soil dosed with 43.5 $mg.kg^{-1}$ As, soil dosed with 168.9 $mg.kg^{-1}$ As, soil dosed with 88.1 $mg.kg^{-1}$ Pb and soil dosed with 168.9 $mg.kg^{-1}$ Pb) with 3 replicates each. Data from the crops was collected for a total of 10 different plant parts (barley grain, barley leaves, barley stems, beetroot root, beetroot leaves, Swiss chard leaves, peas, pea pods, pea leaves and pea stems), which yielded 150 samples for analysis. The effects of soil treatments were evaluated using analysis of variance (ANOVA) and differences among sources were compared with Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$).

5.3 Effect of Treatment Applications on Soil Properties

Soil samples from pots were analysed approximately two weeks after harvest. A summary of the effect of the soil treatments on pH and EC is recorded in Table 5.1. As anticipated, the addition of As and Pb salts during the soil treatment dosing process increased the electrical conductivity (EC) of the soil. The dosing of sodium arsenite increased the soil EC from 0.071 $mS.m^{-1}$ to 0.219 $mS.m^{-1}$ at 43.5 $mg.kg^{-1}$ As and 0.229 $mS.m^{-1}$ at 168.9 $mg.kg^{-1}$ As. Similarly, the addition of lead nitrate salts increased the soil EC to 0.205 $mS.m^{-1}$ at 88.1 $mg.kg^{-1}$ Pb and 0.208 $mS.m^{-1}$ at 168.9 $mg.kg^{-1}$ Pb. The resulting increase in EC was below the 0.250 $mS.m^{-1}$ threshold at which salt sensitive crops may begin to show signs of osmotic stress (FERTASA 2016).

TABLE 5.1: Effect of As and Pb treatments on soil pH and EC

Element	Target concentration ($mg.kg^{-1}$)	pH_{water} ± SD	pH_{CaCl} ± SD	EC ± SD ($mS.m^{-1}$)
Control	0.0	6.71 ± 0.30	6.17 ± 0.21	0.071 ± 0.002
As	43.5	7.14 ± 0.17	6.56 ± 0.13	0.219 ± 0.047
	168.9	6.98 ± 0.36	6.66 ± 0.20	0.229 ± 0.099
Pb	88.1	7.03 ± 0.23	6.63 ± 0.14	0.205 ± 0.050
	168.9	6.96 ± 0.13	6.57 ± 0.16	0.207 ± 0.056

Approximately two weeks after harvest, soil from each pot in the root uptake glasshouse trial was sampled and analysed to determine the total (acid extractable) and bioavailable (salt extractable) concentrations of applied As or Pb (Table 5.2). Three pots dosed with 168.9 $mg.kg^{-1}$ As and three pots dosed with 168.9 $mg.kg^{-1}$ Pb were left unplanted and occasionally irrigated to simulate the effects of a fallow period on As and Pb sequestration.

TABLE 5.2: Bioavailability of As and Pb 22 weeks after soil application

Element	Target concentration ($mg.kg^{-1}$)	Crop	Acid extraction ($mg.kg^{-1}$)	Salt extraction ($mg.kg^{-1}$)
As	43.5	Barley	36.18	BDL
		Garden pea	34.66	1.71
		Swiss chard	36.26	BDL
		Beetroot	35.42	1.49
As	168.9	Barley	101.48	23.73
		Garden pea	127.96	28.45
		Swiss chard	156.95	33.97
		Beetroot	115.19	36.61
		Unplanted *	173.40	98.17
Pb	88.1	Barley	85.89	BDL
		Garden pea	77.97	BDL
		Swiss chard	93.54	BDL
		Beetroot	91.92	BDL
Pb	168.9	Barley	158.87	BDL
		Garden pea	182.53	BDL
		Swiss chard	152.78	BDL
		Beetroot	163.19	BDL
		Unplanted *	160.17	9.57

BDL: Below method detection limit of 0.70 $mg.kg^{-1}$ for As or 1.40 $mg.kg^{-1}$ for Pb.

On a mass balance basis, less than 1 % of the total applied As or Pb was incorporated into the plant biomass, while the rest underwent physicochemical transformations to be incorporated into the soil mineral structure. In pots dosed with 43.5 $mg.kg^{-1}$ As, only

3.1% of the arsenic was plant available after 22 weeks. While in pots dosed with 168.9 $mg.kg^{-1}$ As, 24.5 % of the total As was plant available. Therefore, the soil's ability to buffer As was dose dependent, as it could effectively immobilize approximately 95 $mg.kg^{-1}$ of As after 22 weeks. These results would suggest that arsenite, from the sodium arsenite source used to dose the soil, may have been oxidised to arsenate over time. Therefore, like phosphate, arsenate may have strongly associated with iron and aluminium oxyhydroxides, resulting in far less remaining exchangeable and salt extractable As.

The soil used in this trial exhibited a high propensity for Pb attenuation. When dosed with both 88.1 $mg.kg^{-1}$ and 168.9 $mg.kg^{-1}$ Pb, all salt extractable Pb was below the method detection limit. Pb immobilization was likely a result of multiple physicochemical factors: Pb may have been buffered by the soil's cation exchange; phosphate fertilizer applications may have resulted in insoluble lead phosphate precipitates; Pb may have undergone sorption onto the relatively high concentration of amorphous Fe, Al and Mn oxyhydroxides (Refer to Table 3.10 in Chapter 3).

Interestingly, soils in which crops were grown were shown to significantly increase the rate of As and Pb immobilization when compared to unplanted "fallow" soils. When dosed with the higher trace element rates, 56.6 % of the As and 6 % of Pb in the unplanted pots were salt extractable after 22 weeks, compared to 24.5 % As and 0 % Pb in planted pots. This significant decrease of bioavailability in planted pots may be attributed to the effect of plant root exudates and/or the more regular wetting and drying cycle of the soils in planted pots, which was shown to increase the incidence of reactive amorphous Fe, Al and Mn oxyhydroxides.

5.4 Results and Discussion

5.4.1 Yield response

Yield results for the four crops under the different treatments are recorded in Appendix A2. No significant difference in yield response was found between the control and As or

Pb treated pots for all crops (barley, garden pea, Swiss chard and beetroot). The soil dosing treatments of As had no effect on dry mass accumulation in plant parts (stem, leaf, grain, pea, pod and root), of all crops. However, while the Pb treatments did not affect yield, an increase in leaf dry mass was noted when barley and garden pea was grown in the higher Pb dosed soils (168.9 $mg.kg^{-1}$). This was probably a consequence of the increased nitrogen input from lead nitrate salt used to simulate lead accumulation.

5.4.2 Influence of soil dose treatments on crop quality

Crop quality was visually assessed by qualitatively assessing phenology, colour (Munsell) and the presence or absence of treatment residues. No noticeable differences between control and treated plants were observed for each of the four crops under investigation. No difference in size, shape and quantity of barley grains and pea pods was observed. Similarly, the size, shape and colour of Swiss chard, beetroot leaves and beetroot roots did not differ from the control plants.

5.4.3 Arsenic accumulation in plant parts

Arsenic accumulation in the aboveground biomass was analysed according to plant part (Table 5.3). The As soil treatments were as follows: 0.0 $mg.kg^{-1}$ As (control), 43.5 $mg.kg^{-1}$ As (to simulate 99 years of irrigation at “target water quality” for As) and 168.9 $mg.kg^{-1}$ As (to simulate 19 years of irrigation at “maximum acceptable water quality” for As). Note that the Chinese, not South African, food safety guidelines were used to determine the human food safety thresholds for As and Pb. While both guidelines share the same range of allowable As in human foods, the Chinese guidelines specify trace element limits for fruits, vegetables and grains, while the South African guidelines do not. Food safety guidelines are presented on a fresh mass basis. For the purpose of this study, the food safety guidelines were converted from fresh mass to dry mass (DM) according to the average moisture content of each plant part at harvest.

Similar to the foliar uptake trial, the general trend showed increased As in crop plant tissues with increased soil As treatments, however more edible parts were shown to accumulate enough As to pose a health risk.

TABLE 5.3: Mean arsenic (± standard deviation) in crops grown in As dosed soils, with reference to food safety thresholds

Crop	Plant part	Treatment (mg As. kg^{-1})	Plant tissue (mg As. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg As. kg^{-1} DM$^{\Delta}$)
Barley	Grain	0.0	0.01 ± 0.00	a	0.54
		43.5	0.80 ± 0.23	a	
		168.9	0.93 ± 0.42	a	
Beetroot	Leaf	0.0	0.07 ± 0.02	a	2.63
		43.5	3.03 ± 1.96	b	
		168.9	16.67 ± 6.20	cd	
	Root	0.0	0.01 ± 0.00	a	4.94
		43.5	4.48 ± 1.11	b	
		168.9	10.16 ± 0.94	c	
Garden pea	Pea	0.0	0.01 ± 0.00	a	2.04
		43.5	0.11 ± 0.01	a	
		168.9	0.19 ± 0.05	a	
	Pod	0.0	0.04 ± 0.01	a	2.16
		43.5	1.10 ± 0.37	ab	
		168.9	1.47 ± 0.33	ab	
Swiss chard	Leaf	0.0	0.06 ± 0.01	a	3.08
		43.5	3.85 ± 0.73	b	
		168.9	12.31 ± 2.22	c	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM$^{\Delta}$ dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

Barley grains, beetroot leaves and Swiss chard leaves grown in soils dosed with 43.5 mg.kg^{-1} As all exceeded the food safety threshold for As. However, in the case of barley grains, the results were not statistically differentiable from the control according to Fisher's LSD.

The edible parts of all crops grown in soils dosed with 168.9 mg.kg^{-1} As, except for garden pea, exceeded the food safety threshold for As. Beetroot leaves accumulated the most As, followed by Swiss chard leaves, then beetroot roots. All of which were over double the food safety threshold. Note that food safety testing standards require that all root vegetables be peeled first, therefore unpeeled beetroot may have shown considerably higher As contamination and posed an even greater food safety risk.

The results of this study indicated that only garden pea pods and peas grown in As loaded soils did not accumulate As beyond the food safety threshold. This highlights the importance of careful crop selection when growing food crops in soils that have accumulated As as a result of medium- to long-term irrigation with As loaded waters, even at As concentrations that are acceptable according to irrigation water quality guidelines.

While not the primary focus of this study, the implications of using these crops for animal feed is shown in Table 5.4. on the next page. As was discussed in the previous chapter, the animal feed guidelines used here are from Annexure 4 of the South African Department of Agriculture, Forestry and Fisheries (DAFF) Act No. 36 of 1947. Wherein a 12% water content is assumed for all feed. For the purpose of this study, the feed guidelines were converted to a dry mass (DM) basis and compared to the dry mass results of each plant part. All crops were evaluated according to the “hays, straws, lucerne, roughages and bagasse” sub-category threshold of feed ingredients.

Similar to the results of the foliar uptake trial in Chapter 4, the majority of the As that was taken up by each crop accumulated in the leaves. However, proportionally more As in the root uptake trial accumulated in the stems, compared to the foliar uptake trial. This was expected since all As was initially taken up by the roots then translocated to the leaves via the stems.

In terms of animal feed, garden pea leaves, pea stems and barley leaves exceeded the animal feed threshold at both As soil treatments. Beetroot roots, beetroot leaves and Swiss chard leaves were unfit for use as feed when grown in soils containing 168.9 $mg.kg^{-1}$ As. Barley stems did not accumulate As in excess of the regulated animal feed guidelines, nor did garden pea pods and peas.

TABLE 5.4: Mean arsenic (± standard deviation) in crops grown in As dosed soils, with reference to animal feed safety thresholds

Crop	Plant part	Treatment (mg As. kg^{-1})	Plant tissue (mg As. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg As. kg^{-1} DM*)
Barley	Grain	0.0	0.01 ± 0.00	a	4.55
		43.5	0.80 ± 0.23	a	
		168.9	0.93 ± 0.42	a	
	Leaf	0.0	0.11 ± 0.02	a	4.55
		43.5	12.64 ± 1.75	c	
		168.9	35.30 ± 6.35	e	
	Stem	0.0	0.04 ± 0.00	a	4.55
		43.5	1.49 ± 0.69	ab	
		168.9	2.99 ± 1.02	ab	
Beetroot	Leaf	0.0	0.07 ± 0.02	a	4.55
		43.5	3.03 ± 1.96	ab	
		168.9	16.67 ± 6.20	cd	
	Root	0.0	0.01 ± 0.00	a	4.55
		43.5	4.48 ± 1.11	b	
		168.9	10.16 ± 0.94	c	
Garden pea	Leaf	0.0	0.10 ± 0.02	a	4.55
		43.5	10.32 ± 1.24	c	
		168.9	17.40 ± 2.03	cd	
	Pea	0.0	0.01 ± 0.00	a	4.55
		43.5	0.11 ± 0.01	a	
		168.9	0.19 ± 0.05	a	
	Pod	0.0	0.04 ± 0.01	a	4.55
		43.5	1.10 ± 0.37	ab	
		168.9	1.47 ± 0.33	ab	
	Stem	0.0	0.05 ± 0.01	a	4.55
		43.5	4.64 ± 0.37	b	
		168.9	7.53 ± 1.05	bc	
Swiss chard	Leaf	0.0	0.06 ± 0.01	a	4.55
		43.5	3.85 ± 0.73	ab	
		168.9	12.31 ± 2.22	c	

$^{\infty}$ 95% probability level (Fisher's LSD test).
DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

The results of this trial indicated that As taken up by roots exhibited a tendency to accumulate in leaf and stem tissues in all four crops, which would be of concern if these crops were intended for use as animal feed.

Owing to the small number of replicates per treatment (three for each plant part of each crop), mean values may not have accurately reflected true values where large variability among the replicates occurred. To evaluate the validity of using the means, the medians were also investigated and are recorded in Table 5.5. Comparison of the median and mean values demonstrated that in all instances, bar one, the selection of the statistical parameter did not change the result of whether the treatment exceeded the dry mass food safety threshold. Garden pea stems grown in soils containing 45.3 $mg.kg^{-1}$ As were above the feed threshold as a mean, but below the threshold as a median. However, as the threshold value was within 3 % of the mean and median, the mean was used in the analysis.

TABLE 5.5: Median arsenic in crops grown in As dosed soils, with reference to food and animal feed safety thresholds

Crop	Plant part	Treatment (mg As. kg^{-1})	Plant tissue (mg As. kg^{-1} DM)	Food Threshold (mg As. kg^{-1} DM^{Δ})	Feed Threshold (mg As. kg^{-1} DM*)
Barley	Grain	0.0	0.01	0.54	4.55
		43.5	0.96		
		168.9	0.81		
	Leaf	0.0	0.10		4.55
		43.5	13.00		
		168.9	33.88		
	Stem	0.0	0.04		4.55
		43.5	1.18		
		168.9	2.56		
Beetroot	Leaf	0.0	0.08	2.63	4.55
		43.5	2.92		
		168.9	17.82		
	Root	0.0	0.01	4.94	4.55
		43.5	4.65		
		168.9	10.50		
Garden pea	Leaf	0.0	0.09		4.55
		43.5	10.85		
		168.9	16.05		
	Pea	0.0	0.01	2.04	4.55
		43.5	0.11		
		168.9	0.21		
	Pod	0.0	0.03	2.16	4.55
		43.5	0.98		
		168.9	1.33		
	Stem	0.0	0.05		4.55
		43.5	4.43		
		168.9	8.10		
Swiss chard	Leaf	0.0	0.05	3.08	4.55
		43.5	3.55		
		168.9	13.56		

DM^{Δ} dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

The real-world applications of these results are discussed and presented in the form of food and feed safety consequence matrices in Chapter 6.

5.4.4 Lead accumulation in plant parts

Lead accumulation in the aboveground biomass was analysed according to plant part (Table 5.6). The As soil treatments were as follows: 0.0 $mg.kg^{-1}$ Pb (control), 88.1 $mg.kg^{-1}$ Pb (to simulate 99 years of irrigation at "target water quality" for Pb) and 168.9 $mg.kg^{-1}$ Pb (to simulate 19 years of irrigation at "maximum acceptable water quality" for Pb). Note that the Chinese, not South African, food safety guidelines were used to determine the human food safety thresholds for As and Pb. While both guidelines share the same range of allowable Pb in human foods, the Chinese guidelines specify trace element limits for fruits, vegetables and grains, while the South African guidelines do not. Food safety guidelines are presented on a fresh mass basis. For the purpose of this study, the food safety guidelines were converted from fresh mass to dry mass (DM) according to the average moisture content of each plant part at harvest.

TABLE 5.6: Mean lead (± standard deviation) in crops grown in Pb dosed soils, with reference to food safety thresholds

Crop	Plant part	Treatment (mg Pb. kg^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg Pb. kg^{-1} DM$^{\Delta}$)
Barley	Grain	0.0	0.01 ± 0.00	a	0.21
		88.1	0.14 ± 0.00	a	
		168.9	0.37 ± 0.05	a	
Beetroot	Leaf	0.0	0.06 ± 0.00	a	1.58
		88.1	2.52 ± 0.86	ab	
		168.9	57.27 ± 6.09	d	
	Root	0.0	0.02 ± 0.00	a	0.99
		88.1	4.05 ± 2.01	b	
		168.9	15.80 ± 3.79	c	
Garden pea	Pea	0.0	0.01 ± 0.00	a	0.81
		88.1	0.03 ± 0.01	a	
		168.9	0.13 ± 0.05	a	
	Pod	0.0	0.01 ± 0.00	a	0.86
		88.1	0.24 ± 0.09	a	
		168.9	4.30 ± 0.40	b	
Swiss chard	Leaf	0.0	0.05 ± 0.00	a	1.85
		88.1	2.32 ± 0.83	ab	
		168.9	12.22 ± 4.72	c	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM$^{\Delta}$ dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.

Similar to the findings of the As root uptake trial, the majority of Pb taken up by each crop accumulated in the root, leaf and stem tissue, with proportionally less accumulating in the edible fruits (peas and pods) and grains (barley). However, many crop parts were found to accumulate Pb to the extent that they were not fit for human consumption.

Peeled beetroot roots, beetroot leaves and Swiss chard leaves grown in both Pb soil treatments exceeded the food safety thresholds. Therefore, these crops should not be grown for use as human food in soils that have been impacted by Pb. Barley grains and garden pea pods were deemed unfit for human consumption only when grown in soils containing 168.9 $mg.kg^{-1}$ Pb. This highlights the importance of careful crop selection when growing food crops in soils that have accumulated Pb as a result of medium- to long-term irrigation with Pb loaded waters, even at Pb concentrations that are acceptable according to irrigation water quality guidelines.

While the garden peas did not accumulate Pb beyond the food safety limit, it is interesting to note that the pods tended to accumulate far more As than the peas. While the mechanism is not fully understood, these results were similar to that of Singh et al. (2012), where pea pods were shown to accumulate the greatest proportion of Pb when grown in soils containing 52.7 $mg.kg^{-1}$ Pb. This could be due to the high fibre content of the pods, which may attract Pb ions to the negatively charge surfaces. Apart from barley grains, results which exceeded the food safety thresholds were found to be statistically significant according to Fisher's LSD Test.

While not the primary focus of this study, the implications of using these crops for animal feed was is shown in Table 5.7. The animal feed guidelines used here are from Annexure 4 of the South African Department of Agriculture, Forestry and Fisheries (DAFF) Act No. 36 of 1947. Wherein a 12% water content is assumed for all feed. For the purpose of this study, the feed guidelines were converted to a dry mass (DM) basis and compared to the dry mass results of each plant part. The guidelines for Pb refer to "green roughage", which, while not defined in the DAFF document, is defined by the dairy industry as containing moisture from 60 – 90 % and classified into various types, such as: pasture, cultivated fodder crops, tree leaves, roots and crops" (DKP 2019). Green roughages are allocated a much higher feed safety threshold, likely in order to compensate for the high moisture content when consumed by animals. Garden pea, beetroot and Swiss chard fell under the green roughage sub-category, while, due to its low moisture content, barley was defined as a dry roughage and therefore fell under the general "feed ingredients" category. Hence the different feed threshold values. Note all crop moisture contents can be found in Table 3.5.

TABLE 5.7: Mean lead (± standard deviation) in crops grown in Pb dosed soil, with reference to animal feed safety thresholds

Crop	Plant part	Treatment (mg Pb. kg^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Fisher's LSD$^{\infty}$	Threshold (mg Pb. kg^{-1} DM*)
Barley	Grain	0.0	0.01 ± 0.00	a	11.36
		88.1	0.14 ± 0.00	a	
		168.9	0.37 ± 0.05	a	
	Leaf	0.0	0.15 ± 0.03	a	11.36
		88.1	2.41 ± 0.27	ab	
		168.9	5.48 ± 0.45	b	
	Stem	0.0	0.04 ± 0.00	a	11.36
		88.1	2.63 ± 0.67	ab	
		168.9	3.96 ± 2.21	ab	
Beetroot	Leaf	0.0	0.06 ± 0.00	a	45.45
		88.1	2.52 ± 0.86	ab	
		168.9	57.27 ± 6.09	e	
	Root	0.0	0.02 ± 0.00	a	45.45
		88.1	4.05 ± 2.01	ab	
		168.9	15.80 ± 3.79	c	
Garden pea	Leaf	0.0	0.11 ± 0.01	a	45.45
		88.1	4.06 ± 0.79	ab	
		168.9	32.97 ± 10.53	d	
	Pea	0.0	0.01 ± 0.00	a	45.45
		88.1	0.03 ± 0.01	a	
		168.9	0.13 ± 0.05	a	
	Pod	0.0	0.01 ± 0.00	a	45.45
		88.1	0.24 ± 0.09	a	
		168.9	4.30 ± 0.40	b	
	Stem	0.0	0.07 ± 0.01	a	45.45
		88.1	3.44 ± 0.28	ab	
		168.9	14.10 ± 3.50	c	
Swiss chard	Leaf	0.0	0.05 ± 0.00	a	45.45
		88.1	2.32 ± 0.83	ab	
		168.9	12.22 ± 4.72	c	

$^{\infty}$ 95% probability level (Fisher's LSD test).

DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

The general trend showed increased Pb in crop plant tissues with increased soil Pb treatments.

Barley demonstrated low amounts of Pb in the aboveground biomass when grown in lead loaded soil, even when compared to the foliar uptake trial. While most Pb accumulated in the leaves and stem, the Pb concentrations were far below the animal feed threshold and therefore would be fit for animal consumption. This indicates that the translocation of Pb from roots to shoots may be limited in barley, and possibly other grains, making barley a good choice to grow for animal feed in Pb impacted soils. This trend was repeated in almost all crops, where the aboveground biomass accumulated Pb at concentrations below that of the animal feed threshold. The only exception was beetroot leaves grown in soil containing 168.9 $mg.kg^{-1}$ Pb.

As was the case of Pb in the foliar uptake trial, far more crops were considered fit for animal consumption when grown in Pb treated soils compared to those grown in As treated soils. This was not because the crops accumulated significantly less Pb when compared to the As irrigation trials, but because the Pb feed guidelines are far more lenient (10 $mg.kg^{-1}$ and 40 $mg.kg^{-1}$ at 12% moisture) than the As feed guideline (2 $mg.kg^{-1}$ or 4 $mg.kg^{-1}$ at 12% moisture).

As with the arsenic analysis, median values were also investigated to evaluate the validity of using the mean scores and are recorded in Table 5.8. Comparison of the median and mean values demonstrated that the selection of the statistical parameter did not change the result of whether the treatment exceeded the dry mass food safety threshold, so in accordance with the previous analyses, the means of this data set were used.

TABLE 5.8: Median lead in crops grown in Pb dosed soils, with reference to food and animal feed safety thresholds

Crop	Plant part	Treatment (mg Pb. kg^{-1})	Plant tissue (mg Pb. kg^{-1} DM)	Food Threshold (mg Pb. kg^{-1} DM$^{\Delta}$)	Feed Threshold (mg Pb. kg^{-1} DM*)
Barley	Grain	0.0	0.01	0.21	11.36
		88.1	0.14		
		168.9	0.39		
	Leaf	0.0	0.15		11.36
		88.1	2.53		
		168.9	5.17		
	Stem	0.0	0.04		11.36
		88.1	3.00		
		168.9	4.03		
Beetroot	Leaf	0.0	0.06	1.58	45.45
		88.1	1.92		
		168.9	59.76		
	Root	0.0	0.02	0.99	45.45
		88.1	5.29		
		168.9	14.42		
Garden pea	Leaf	0.0	0.11		45.45
		88.1	3.96		
		168.9	38.16		
	Pea	0.0	0.01	0.81	45.45
		88.1	0.03		
		168.9	0.10		
	Pod	0.0	0.01	0.86	45.45
		88.1	0.22		
		168.9	4.56		
	Stem	0.0	0.08		45.45
		88.1	3.61		
		168.9	12.92		
Swiss chard	Leaf	0.0	0.05	1.85	45.45
		88.1	2.20		
		168.9	12.53		

DM$^{\Delta}$ dry mass food safety threshold calculated from the National Food Safety Standards, People's Republic of China (2012), which is presented on a fresh mass basis.
DM* dry mass animal feed guidelines calculated from DAFF Act No. 36 of 1947 Annexure 4, which assumes 12% moisture content.

5.4.5 ANOVA table

A two-way analysis of variance (ANOVA) was conducted to evaluate the effect of soil treatment and crop part on potentially hazardous trace element accumulation in plant tissues ($\alpha = 0.05$) (Table 5.9). There were 5 soil treatments, 10 crop parts analysed per treatment and 3 replicates, resulting in 149 total degrees of freedom. The results indicate that there was a statistically significant difference ($p < 0.001$) for the interaction effect between treatment and crop part. The two main effects were also statistically significant at the level $p < 0.001$. Therefore, both choice of crop and the concentration of As or Pb in the soil as a result of medium- to long-term irrigation inputs were found play a critical role in determining whether the edible parts of crops would accumulate As or Pb in excess of food (or feed) safety thresholds.

TABLE 5.9: Two-way Analysis of Variance (ANOVA) of root uptake trial

Source	***SS***	***df***	***MS***	***F***	***P-value***	***F critical***
Treatment	4 425.96	4	1 106.49	118.60	< 0.001	2.46
Crop part	4 116.90	9	457.43	49.03	< 0.001	1.97
Interaction	8 207.08	36	227.97	24.44	< 0.001	1.54
Error	932.96	100	9.33			
Total	17 682.89	149				

5.5 Comparison to Previous Studies

The As concentration ranges of this trial were comparable to those found in the literature (Warren et al. 2003, Das et al. 2004, Baig and Kazi et al. 2012, Paltseva et al. 2018, Yañez et al. 2019), where vegetables grown in As contaminated soils exhibited As concentrations ranging from 0.02 $mg.kg^{-1}$ to 21.3 $mg.kg^{-1}$, on a dry mass basis. In this trial, As concentrations ranged from 0.01 $mg.kg^{-1}$ to 35.30 $mg.kg^{-1}$, on a dry mass basis. Warren et al. (2003) reported that lettuce leaves grown in soil containing 65 $mg.kg^{-1}$ As accumulated 17.81 $mg.kg^{-1}$ As. By comparison, the leaves of Swiss chard from this study, grown in 43.5 and 168.9 $mg.kg^{-1}$ As loaded soil, accumulated 3.85 and 12.31 $mg.kg^{-1}$ As, respectively. Similarly, Yañez et al. (2019) reported that broadbean beans grown in soils containing 49 $mg.kg^{-1}$ As accumulated relatively low amounts of As (0.42 $mg.kg^{-1}$ DM) compared to cabbage grown in the same soil (10.10 $mg.kg^{-1}$ DM). In this trial, garden pea pods grown in in soils containing 43.5 $mg.kg^{-1}$ As accumulated

0.11 mg.kg^{-1} As, while beetroot leaves grown in the same soil accumulated 3.03 mg.kg^{-1} As.

With regards to vegetables grown in Pb loaded soils, the results of this trial are supported by those in the published literature (Ter Haar 1970, Alexander et al. 2006, Mahmood and Malki 2014, Paltseva et al. 2018). In this trial, Pb in the edible parts ranged from 0.01 mg.kg^{-1} to 30.62 mg.kg^{-1}. In the literature referenced in Table 2.4, Pb in edible parts of crops grown in Pb loaded soils ranged from 0.3 to 19.68 mg.kg^{-1} Pb on a dry mass basis. Carrots and cabbage leaves grown in soil containing 17.1 mg.kg^{-1} Pb accumulated 2.1 and 5.8 mg.kg^{-1} Pb (DM) respectively (Ter Haar 1970). This is comparable to the results of this trial where beetroot roots and leaves grown in soil containing 88.1 mg.kg^{-1} Pb accumulated 11.56 and 2.52 mg.kg^{-1} Pb on a dry mass basis. Singh et al. (2012) reported that cabbage and peas grown in soil containing 52.7 mg.kg^{-1} Pb accumulated 34 mg.kg^{-1} and 19 mg.kg^{-1} Pb, respectively. By comparison, Swiss chard and peas in this study, grown in 88.1 mg.kg^{-1} Pb loaded soil, accumulated 0.03 and 2.32 mg.kg^{-1} Pb, respectively.

The general trend of the results of this trial were slightly below those of similar trials found in the literature. This is likely due to the selection of a “forgiving” soil (fine textured, neutral to alkaline pH) that was used in the trials for this dissertation. A “forgiving” soil is a prescribed condition when irrigating with trace element loaded waters above the “maximum acceptable” concentrations, according to the South African irrigation water quality guidelines (1996).

Since pots dosed with the highest treatment of 168.9 mg.kg^{-1} As or Pb, simulating 19 years of irrigation at 2 mg.L^{-1}, received the same quantity of As or Pb, it was anticipated that crops would accumulate a similar quantify of either As or Pb. However, crops grown in Pb loaded pots tended to accumulate slightly more Pb, on a mass basis, than crops grown in As loaded pots accumulated As. With the exception of barley grains.

Dollard and Lepp (1980) demonstrated that, like calcium, lead is not remobilised through the phloem. Therefore, lower concentrations of lead are expected in grains when taken up via roots. This was also shown by Chen et al. (2016) who found that the concentrations of Pb in the roots of rice, wheat and canola were an order of magnitude higher than in the grain.

With regard to crops accumulating more Pb than As at the 168.9 $mg.kg^{-1}$ soil treatment, there are a number of possible explanations for this. The addition of lead nitrate may have induced localized acidification on the micro-scale, which may have increased the bioavailability of Pb within those zones. Another possible explanation is that rhizosphere acidification would have likely increased Pb mobility at the root surface, thereby enhancing root uptake of Pb, while concurrently decreasing the mobility of As. Additionally, the addition of phosphorus fertilizer, as a band placement, while decreasing Pb mobility through precipitation reactions, would also reduce the uptake of As through plant roots by the competitive anion effect. However, the most plausible explanation is the fact that the rate of crop Pb uptake tended to increase with increased soil Pb, which may be indicative of an active uptake and translocation mechanism in Pb impacted soils. This will be discussed in the following section.

5.6 Bioaccumulation Factors, Transfer Coefficients and Translocation Factors

The bioaccumulation factor (BAF), also known as the bioconcentration factor (BCF) or soil-to-plant metal transfer factor (MTF), is the ratio between the element concentration in the aerial organs and that measured in the soil (Amin et al. 2013, Chen et al. 2016, Dai et al. 2016, Liñero et al. 2017, Rehman et al. 2017). The BAF results of this trial are presented in Table 5.10. The transfer coefficient (TC) has also been included for beetroot roots in Table 5.10 as it gives an indication of the transfer of trace elements from soil to roots (Chen et al. 2016).

Both the BAF and TC give an indication of heavy metal transfer in the soil-crop system. BAF and TC values greater than 1 indicate a strong extraction of the element from the soil to the roots (TC) or aerial organs (BAF) (Chen et al. 2016, Liñero et al. 2017). Note that BAF values with a "less than" symbol were derived from the method detection limit, because the concentration of As or Pb in those plant parts was deemed less than the method detection limit.

All BAF and TC values were well below 1 and therefore indicate limited As and Pb transfer from soil to crop.

TABLE 5.10: Bioaccumulation factors (BAF) and transfer coefficients (TC) of As and Pb from soil to plant parts

Crop	**Plant part**	**Bioaccumulation factor / Transfer coefficient**			
		As [43.5]	**As [168.9]**	**Pb [88.1]**	**Pb [168.9]**
Barley	Grain	0.02	0.01	≈ 0	≈ 0
	Leaf	0.29	0.21	0.03	0.03
	Stem	0.03	0.02	0.03	0.02
Beetroot	Leaf	0.07	0.10	0.03	0.34
	Root	0.10	0.06	0.05	0.09
Garden pea	Leaf	0.24	0.10	0.05	0.20
	Pea	≈ 0	≈ 0	≈ 0	≈ 0
	Pod	0.03	0.01	≈ 0	0.03
	Stem	0.11	0.04	0.04	0.08
Swiss chard	Leaf	0.09	0.07	0.03	0.07

Focusing on Pb, the BAF, particularly of leaves, tended to increase with increased soil Pb. This may indicate an active uptake and translocation of Pb from the soil and into leaves. These results are similar to those of Miller and Koeppe (1971), where maize was shown to actively translocate and accumulate progressively greater quantities of Pb in the leafy tissue, with increased soil Pb. These results are also supported by the trend demonstrated when previous literature was amalgamated (refer to Figures 2.2 and 2.3 of Chapter 2) to show that crop Pb uptake into the aboveground biomass continued to increase with increased soil Pb with no signs of plateau, even at soil Pb loads of 2000 $mg.kg^{-1}$. While these values do not indicate a strong extraction of the element from the soil to aerial organs, the trend may suggest an active Pb accumulation mechanism as soil Pb loads increase.

With regard to As, the bioaccumulation factors tended to decrease with increased soil As, which indicated that the crops investigated may exhibit a finite absorption capacity for As. These results are comparable to that of Dai et al. (2016) where BCF values for As in various wheat cultivars ranged from 0.005 to 0.05. These results are also supported by the trend demonstrated when previous literature was amalgamated (refer to Figures 2.2 and 2.3 of Chapter 2) to reveal that crop As uptake began to plateau at As soil loads of approximately 150 $mg.kg^{-1}$. The low BAF and TC values of As suggest that the soil conditions were well aerated and thus conducive to limiting the mobility of As by oxidising arsenite to arsenate (Davis et al. 2017). Since arsenate and phosphate compete for the same active transporter protein sites, adequate phosphate fertilization may also have contributed to reduced As transfer from soil to roots (Azouzi et al. 2017). Once inside the root, As is translocated to the rest of the plant via xylem loading (Page and Feller 2015). The results of this trial suggest that leaf tissue acted as the strongest sink for As in all four crops.

The translocation factor (TF) is defined as the ratio between the element concentration accumulated in aerial organs compared to the concentration in roots (Liñero et al. 2017). It is an indicator of the mobility of the element from the roots to the aboveground biomass, i.e.: translocation via xylem loading (Shahid et al. 2017). TF values greater than 1 suggest a strong translocation of the element from the roots to the aboveground biomass (Liñero et al. 2017). The TF from beetroot roots to leaves are presented in Table 5.11.

TABLE 5.11: Translocation factors (TF) of As and Pb from roots to the aboveground biomass of beetroot

Crop	**Translocation factor**			
	As [43.5]	**As [168.9]**	**Pb [88.1]**	**Pb [168.9]**
Beetroot	0.68	1.64	0.62	3.62

TF results from Liñero et al. (2017) ranged between 0.1 - 1 for Pb and 0.1 - 1.1 for As in Swiss chard in soils with low As and Pb, which is similar to that of this trial at the lower As and Pb soil treatments. However, at a soil concentration of 168.9 $mg.kg^{-1}$ As or Pb, the TF of beetroot suggested strong translocation of As and Pb from the roots to the aboveground biomass. Therefore, as the concentration of As or Pb in beetroot roots increases, the results suggest that the plant may actively translocate more As or Pb out of the roots and into the leaves, via xylem loading, possibly to limit As and Pb accumulation in the root.

5.7 Limitations of the Trial

Due to the temporal and practical challenges associated with simulating the biophysicochemical soil transformations that would realistically have taken place over 19 or 99 years of trace element accumulation through gradual irrigation inputs, soil dosing of As and Pb for this trial effectively simulated a once-off pollution event. Therefore, the results of this trial represent a “worst case scenario” of As and Pb uptake, as the bioavailability of these elements in the soil would be higher than expected over a 19- or 99-year period of accumulation.

Although food safety guidelines are based on peeled beetroot, a more comprehensive understanding of trace element root uptake and biomass accumulation could have been gained if the washed beetroot root peels had been tested for As and Pb as well.

This trial investigated the impact of two potentially hazardous trace elements in soils on four crops. Increasing the number of potentially hazardous trace elements to include Cd, Hg and U, as well as increasing the number of crops, while beyond the scope of this dissertation, would have allowed for a more extensive evaluation of how irrigation quality guidelines may impact food and feed safety.

Finally, adding more treatment levels per trace element could have allowed for the quantification of crop-specific trace element concentrations at which the food safety risk could pose, for example, a 10 % risk of edible parts being unfit for human consumption.

5.8 Conclusions

The general trend in the root uptake trial showed increased As or Pb in crop plant tissues, with increased As or Pb soil treatments. Unlike the foliar uptake trial, the majority of edible parts accumulated As or Pb above the food safety thresholds. Excluding the controls, 58 % of crop parts grown in As treated soils and 67 % of crops grown in Pb treated soils were unfit for human consumption. For use as animal feed, 45 % of crop parts grown in As treated soil and 5 % of crops grown in Pb treated soils exceeded the animal feed guidelines.

Barley grains, beetroot leaves and Swiss chard leaves grown in both As treatment levels exceeded food safety guidelines. Additionally, beetroot roots grown in soils dosed with 168.9 $mg.kg^{-1}$ As were deemed unfit for human consumption. Beetroot leaves, beetroot roots, and Swiss chard leaves grown in both Pb treatment levels, as well as barley grains and garden pea pods grown in soils containing 168.9 $mg.kg^{-1}$ Pb were also unfit for human consumption.

Overall, leafy greens have been shown to pose a high food safety risk, while garden peas were shown to pose the lowest food safety risk.

These results have demonstrated that even if lands with “forgiving” soils have been irrigated at a rate of 1 000 mm per year with waters containing As or Pb at acceptable concentrations, according to irrigation water quality guidelines, the resulting produce must be monitored as it may not be fit for human consumption. Therefore, when growing crops for food in As or Pb impacted soils, as a result of long-term irrigation inputs or otherwise, crop selection is vital to minimize potential health risks to those consuming the produce.

Regarding As in animal feed, barley leaves, pea leaves and pea stems grown in both As treatment levels and beetroot leaves, beetroot roots and Swiss chard leaves grown in soils dosed with 168.9 $mg.kg^{-1}$ As were unfit for use. However, in the case of Pb impacted soils, only beetroot leaves grown in soils containing 168.9 $mg.kg^{-1}$ Pb exceeded the animal feed thresholds.

Therefore, the results of this study suggest that farmers should avoid growing crops for animal feed in soils impacted by medium- to long-term As irrigation inputs, particularly when irrigated at the maximum acceptable threshold of 2.0 $mg.L^{-1}$ As. In such cases, an oil or fibre crop would be preferable. However, Pb impacted soils would be better utilised for the production of animal feed (particularly green roughage), instead of human food, due to the more lenient animal feed guidelines.

Having reported the findings of both the foliar absorption and root uptake trials, the real-world implications of the trials are discussed in the next chapter, focussing on the development of matrices to determine the consequence of irrigating with As or Pb loaded water on human food and animal feed safety.

CHAPTER 6: FOOD AND FEED SAFETY CONSEQUENCE MATRICES

6.1 Introduction

Du Plessis et al. (2017) introduced a risk-based model to evaluate, among other analyses, the fitness-for-use of trace elements in irrigation water according to the rate of critical soil cumulative element loading. This chapter adopts a similar approach to create a user-friendly tool which graphically presents the findings of this study's trials from a food and feed safety perspective. The matrix tool presented here is technically not a risk matrix, because the level of probability of the event occurring is not shown (Duijm 2015, Roughton and Crutchfield 2015). Therefore, the tool has been termed a "consequence" matrix, since only the consequence of each treatment on food or feed safety is shown.

In developing the matrices, the food or feed safety threshold was chosen as the limit at which crops should no longer be considered fit for use. Crop parts have been plotted on colour coded matrices to provide a quick, visual summary of the findings from a food or feed safety perspective.

This short chapter commences with a brief review of factors that may influence the accumulation of potentially hazardous trace elements in the edible parts of crops. Having been discussed in detail in previous chapters, the variables are summarised to provide context when evaluating the resulting matrices. The food and feed safety findings from both the foliar absorption and root uptake trials are then compiled into consequence matrices to demonstrate the real-world applications of this research.

6.2 Food Safety Related Parameters in Agricultural Systems

There are a variety of factors which play a role in the quantity of As or Pb which may accumulate in the edible parts of crops. These are summarised here to provide the context in which the developed matrices should be evaluated.

- **Soil texture**: fine textured soils (high clay and silt fraction) have a greater capacity to buffer chemical inputs, such as As and Pb, than coarse textured soils (high sand fraction). Soils used in these trials were fine textured and therefore likely to have limited the uptake of As and Pb via roots.
- **Soil pH**: circumneutral pH (6 to 7.5) soils are considered ideal for the bioavailability of essential plant nutrients while limiting to that of potentially hazardous trace elements. While not particularly pH dependent, As mobility has been shown to increase under alkaline soil conditions (Pigna et al. 2015). The bioavailability of Pb is pH driven and increases under acidic conditions (Somasundaram et al. 2006). Therefore, the circumneutral pH of the soil used in this trial may have limited the availability of As and Pb for plant uptake.
- **Soil redox status**: arsenic is significantly more available under reducing soil conditions (waterlogging). Similarly, Pb has been shown to be less mobile at higher redox potentials (Reddy and Patrick 1977). Soils used in these trials were kept aerated to maintain the aerobic field conditions which are ideal for the growth of the chosen crops. As a result, As and Pb bioavailability was reduced.
- **Duration of (or time passed since) trace element loading**: In general, the longer an element has been in soil, the more likely biophysicochemical transformations have occurred to reduce the mobility of that element (Romero-Freire et al. 2015). Therefore, soils that have received low doses of As or Pb over a long period of time (e.g.: 100 years of irrigation inputs) are likely to have less biologically available As or Pb than soils that have received the equivalent dose during a recent once-off event.
- **Rainy season**: crops chosen for this study were winter crops grown in a summer rainfall region. As a result, the potentially beneficial effects of rainfall washing As and Pb off the leaf surface did not occur. Therefore, the foliar uptake trial may have resulted in more crops deemed fit for human consumption if grown outdoors during a rainy season.
- **Temperature**: higher temperatures increase the rate of biophysicochemical transformations in the soil. Due to the complex nature of such transformations, the result may either increase or decrease the bioavailability of As or Pb. With regards to

the foliar uptake trial, higher temperatures reduce the time that droplets remain on the leaf surface, which may inhibit the foliar uptake of As or Pb.

- **Irrigation system**: The foliar uptake trial aimed to replicate the effects of overhead irrigation, such as centre pivot or sprinklers. If As or Pb is found to be elevated in the irrigation water, agricultural consultants could effectively avoid the problem of foliar uptake by recommending an irrigation system which would avoid wetting of the aboveground biomass, such as drip or subsurface irrigation.
- **Crop choice**: as demonstrated by this research, crop selection is critical in determining the accumulation of As and Pb in edible parts. The results of the four crops selected (barley, beetroot, garden pea and Swiss chard) are presented in the consequence matrices which follow. When the risk of producing crops for food or feed is too high, farmers should be advised to grow crops for fibre or fuel.

6.3 Development of the Consequence Matrix

The food and feed safety consequence matrices presented in this chapter have been developed to evaluate the fitness-for-use of crops according to the food or feed safety guidelines, as a consequence of As or Pb irrigation inputs, as investigated in this study. These matrices are specific to the trial conditions and results of this study and should therefore not be used as blanket recommendations. In line with du Plessis et al. (2017), a four-level, colour-coded, fitness-for-use class method was applied in the development of the tool to represent fitness-for-use in terms of the food/feed safety guidelines (Table 6.1).

TABLE 6.1: Fitness-for-use criteria based on crop part accumulation of As or Pb

	Fitness-for-use	**Level of crop part accumulation**
Trace element accumulation	Ideal	> 50 % below the food/feed safety threshold
	Acceptable	25 to 50 % below the food/feed safety threshold
	Tolerable	< 25 % below the food/feed safety threshold
	Unacceptable	Equal to or above the food/feed safety threshold

Such a model can also be used to provide recommendations along with the generated fitness-for-use output, based on potentially hazardous trace element concentrations in irrigation water as well as the duration of the irrigation program.

For example; crops that accumulate trace elements at the “ideal” level are most suitable for cultivation and should be recommended to the farmer. Crops which are determined to be accumulating trace elements at the “acceptable” level can also be planted, but edible parts should be sent for food safety analysis every third growing season to monitor potentially hazardous trace element accumulation. Owing to crops in the “tolerable” level accumulating contaminants close to threshold limits, it is recommended that, if grown, they be analysed for potentially hazardous trace element accumulation on a season-by-season basis. Any crops equal to, or exceeding, the food/feed safety thresholds should be considered “unacceptable” for human/animal consumption and not planted. With regard to laboratory analysis, only the potentially hazardous trace elements deemed by the model to pose a food/feed safety risk need be monitored.

6.4 Food and Feed Safety Consequence Matrices

A food or feed safety consequence matrix was developed for each of the four trials that formed the basis of this study: foliar absorption of As or Pb, followed by root uptake of As or Pb. Crops plotted on the matrices were coded as follows: B. (barley), BR. (beetroot), GP. (garden pea) and SC. (Swiss chard).

6.4.1 Consequence of foliar irrigation with arsenic enriched water on food/feed safety

The matrix presented in Table 6.2 ranks and prioritises the extent to which edible crop parts accumulated As, as a result of As rich irrigation water making contact with the aboveground biomass. Most edible crop parts fall within the low consequence categories (illustrated in blue and green). Therefore, it can be deduced that barley, beetroot (roots) and garden pea are well suited for production under As rich irrigation water. However, leafy greens, such as Swiss chard and beetroot leaves, should be avoided. Both of those crops irrigated at the “maximum acceptable” As concentration of 2 $mg.L^{-1}$ fall within the “unacceptable” fitness-for-use category.

TABLE 6.2: Food safety consequence matrix for foliar irrigation with As rich water

As Foliar irrigation treatment ($mg.L^{-1}$)	> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
2.0	BR. root GP. pea GP. pod	B. grain		BR. leaf SC. leaf
0.1	B. grain BR. root GP. pea GP. pod	B. leaf SC. leaf		
	Food Safety Threshold			

With regard to animal feed (Table 6.3), most leafy materials were “unacceptable” for use, particularly when irrigated at the highest acceptable concentration. However, grains, roots, and peas in the pod all fell under the “ideal” fitness-for-use category.

TABLE 6.3: Feed safety consequence matrix for foliar irrigation with As

As Foliar irrigation treatment ($mg.L^{-1}$)	> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
2.0	B. grain B. stem BR. root GP. pea GP. pod GP. stem			B. leaf BR. leaf GP. leaf SC. leaf
0.1	B. grain B. stem BR. leaf BR. root GP. pea GP. pod GP. stem SC. leaf		GP. leaf	B. leaf
	Feed Safety Threshold			

6.4.2 Consequence of foliar irrigation with lead enriched water on food/feed safety

The matrix presented in Table 6.4 ranks and prioritises the extent to which edible crop parts accumulated Pb as a result of Pb treated irrigation water making contact with the aboveground biomass. Peas in the pod and beetroot roots were categorised as "ideal" for use as food. Barley grain irrigated at 0.2 mg.L^{-1} were "acceptable"; however, those irrigated with water containing 2.0 mg.L^{-1} Pb should be monitored on a season-by-season basis for fitness-of-use. Farmers should be advised not to cultivate leafy greens, like Swiss chard and beetroot leaves, if Pb in irrigation water exceeds 0.2 mg.L^{-1}.

TABLE 6.4: Food safety consequence matrix for foliar irrigation with Pb

Pb Foliar irrigation treatment (mg.L^{-1})					
	2.0	BR. root GP. pea GP. pod		B. grain	BR. leaf SC. leaf
	0.2	BR. root GP. pea GP. pod	B. grain		BR. leaf SC. leaf
		> 50 % below limit	**25 to 50 % below limit**	**< 25 % below limit**	**≥ limit**
		Food Safety Threshold			

Similar to the As trial, animals may be fed barley grains and peas in pods when these crops are irrigated with Pb rich water (Table 6.5). A Pb concentration of 0.2 mg.L^{-1} in irrigation water is unlikely to produce crops unfit for use as animal feed; however, at 2.0 mg Pb.L^{-1}, foliage should be either be avoided or analysed for Pb every season, as such crop parts may pose an animal feed safety risk.

TABLE 6.5: Feed safety consequence matrix for foliar irrigation with Pb

Pb Foliar irrigation treatment (mg.L^{-1})	> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
2.0	B. grain BR. root GP. pea + pod GP. stem		BR. leaf GP. leaf	B. leaf B. stem SC. leaf
0.2	B. grain BR. leaf BR. root GP. pea + pod GP. stem	B. leaf B. stem GP. leaf SC. leaf		
	Feed Safety Threshold			

Current irrigation water quality guidelines do not consider foliar absorption; however, these results demonstrate that the phenomenon should not be ignored. An ideal way to model actual crop uptake via foliar absorption (necessary for consideration in future updates of the irrigation water quality guidelines), would be to quantify the relationship between trace element concentration and food safety risk, as well as the impact of other factors, such as rainfall, leaf cuticular morphology and evaporative demand on foliar uptake.

6.4.3 Consequence of root uptake of arsenic following irrigation with arsenic enriched water on food/feed safety

The food and feed safety consequences associated with root uptake of As in soils impacted by medium- to long-term irrigation inputs of As rich water are presented in Tables 6.6 and 6.7.

With regards to human consumption, only garden peas were categorised as “ideal” and only garden pea pods were categorised as “acceptable” (Table 6.6). Therefore, farmers growing crops in fields with high levels of As should only consider garden peas if the field is to be used to grow food for human consumption. Again, leafy greens were “unacceptable”, along with barley grains. Beetroot roots should either be monitored on a season-by-season basis or not planted at all.

TABLE 6.6: Food safety consequence matrix for soils with elevated As

Soil As ($mg.kg^{-1}$)		> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
	168.9	GP. pea	GP. pod		B. grain BR. leaf BR. root SC. leaf
	43.5	GP. pea	GP. pod	BR. root	B. grain BR. leaf SC. leaf
		Food Safety Threshold			

Only barley grains and peas in pod were considered "ideal" for use as animal feed (Table 6.7). Leafy crop tissues, roots and most stems, particularly in soils containing 168.9 mg $As.kg^{-1}$, could not be used as animal feed. Ultimately, decisions regarding which crops to plant are at the discretion of the farmer and agronomic advisor, based on the level of risk the farmer is willing to accept. However, in soils will elevated levels of As, the farmer may be best advised to grow fuel or fibre crops.

TABLE 6.7: Feed safety consequence matrix for soils with elevated As

As Soil treatment ($mg.kg^{-1}$)		> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
	168.9	B. grain GP. pea GP. pod	B. stem		B. leaf BR. leaf BR. root GP. leaf GP. stem SC. leaf
	43.5	B. grain B. stem GP. pea GP. pod	BR. leaf	BR. root SC. leaf	B. leaf GP. leaf GP. stem
		Feed Safety Threshold			

6.4.4 Consequence of root uptake of lead following irrigation with lead rich water on food/feed safety

Table 6.8 ranks the level of Pb accumulation in edible crop parts when grown in Pb impacted soils, here made to represent medium- to long-term Pb accumulation as a result of irrigation inputs. Most edible crop parts fall within the “unacceptable” fitness-for-use category. Beetroot leaves, beetroot roots and Swiss chard should not be grown for food in soils containing 88.1 mg.kg^{-1} Pb or higher. On soils dosed with 168.9 mg Pb.kg^{-1}, barley grains and garden pea pods were also unsuitable for consumption. Only garden peas without the pod were considered “ideal” and posed no risk to human health if eaten. With Pb impacted soils, the field might be better utilised to produce crops for animal feeds, fibre or fuel.

TABLE 6.8: Food safety consequence matrix for soils with elevated Pb

Pb Soil treatment (mg.kg^{-1})		> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
	168.9	GP. pea			B. grain BR. leaf BR. root GP. pod SC. leaf
	88.1	GP. pea GP. pod	B. grain		BR. leaf BR. root SC. leaf
		Food Safety Threshold			

Due to the relatively lenient feed safety guidelines for Pb, the vast majority of crops grown in Pb impacted soils were “ideal” for use as animal feed (Table 6.9). In soils containing 88.1 mg.kg^{-1} Pb, all crops were considered ideal. In soils containing 168.9 mg.kg^{-1} Pb, only garden pea leaves were “acceptable” and only beetroot leaves were “unacceptable”. Therefore, in fields with soils impacted by Pb, the farmer would be well advised to grow crops for animal feed instead of human food.

TABLE 6.9: Feed safety consequence matrix for soils with elevated Pb

Pb Soil treatment ($mg.kg^{-1}$)		> 50 % below limit	25 to 50 % below limit	< 25 % below limit	≥ limit
	168.9	B. grain B. leaf B. stem BR. root GP. pea GP. pod GP. stem SC. leaf	GP. leaf		BR. leaf
	88.1	B. grain B. leaf B. stem BR. leaf BR. root GP. leaf GP. pea GP. pod GP. stem SC. leaf			
		Feed Safety Threshold			

6.5 Real-World Applications

In terms of agricultural extension, the short-term impacts resulting from foliar uptake of irrigation water high in potentially hazardous trace elements could be mitigated by installing a drip or sub-soil irrigation system instead of an overhead irrigation system. However, fitness-for-use based crop choices will become far more important over the medium- to long-term as soils accumulate the elements to potentially hazardous levels.

The ultimate goal of the tool developed in this chapter would be to empower farmers with trace element impacted lands to make informed decisions regarding the choice of crop for human food, animal feed, fuel or fibre.

In the field, analysis of site-specific factors (as discussed in Section 6.2) together with irrigation rates, would enable a more accurate assessment of the consequence of irrigation treatments on fitness-for-use of crops. Application of the Decision Support

System (DSS) developed by du Plessis et al. (2017), would assist in this regard. Ultimately, incorporation of the food and feed safety consequence matrix into the DSS software could align irrigation water quality guidelines with those of food and feed safety.

Ideally, a database of food and feed consequence matrices should be developed for all major crops and potentially hazardous trace elements present in soil and irrigation water. It is advisable that the crops be tested on standardised “forgiving” soils as a “best-case-scenario”, from which the farmer and agronomic advisor may weigh up the risks against the farm’s site-specific factors. Farm soils that do not fall into the “forgiving” category should automatically assume a greater likelihood of crops being unfit for human or animal consumption, because those soils will have a lower As and Pb buffer capacity. Such a database would allow each farmer with potentially hazardous trace element impacted lands or water to make better informed crop choices with regards to food and feed safety. Alternatively, one could begin with Tier 1 recommendations being the most conservative, with worst case scenario assumptions. Followed by Tier 2 allowing for more relaxed guidelines under favourable site-specific conditions.

While most farmers may not be impacted by potentially hazardous trace elements today, as food safety guidelines become more widely utilised and enforced (as is already seen in the organic and other export markets), and as mining and industry continue to impact agricultural land and waterways, the demand for such a risk management database is expected to increase over time.

CHAPTER 7: CONCLUSIONS

7.1 Introduction

This dissertation has achieved its purpose of evaluating the impact of irrigating crops according to the South African Irrigation Water Quality Guidelines for As and Pb on food and feed safety as determined by current food safety guidelines.

Two glasshouse trials were conducted to achieve the research objectives. The foliar uptake trial determined the short-term impact of As or Pb loaded irrigation water making contact with the aboveground biomass and its effects on food/feed safety. The root uptake trial evaluated food/feed safety impacts of the medium- to long-term effects of growing crops in soils that have been irrigated with As or Pb loaded soils until one year before the land would be deemed unusable for agricultural purposes. A selection of winter season crops was grown to investigate the treatment effects on a variety of crop growth forms, namely: grains (barley), leafy greens (Swiss chard and beetroot), legumes (garden pea) and roots (beetroot).

In both trials, some crops were found to accumulate As or Pb in excess of the food/feed safety guidelines, while others were deemed fit for human or animal consumption. The range of As or Pb which accumulated in the crop parts presented in this dissertation fell within the same range of As or Pb from similar trials described in the literature review. Therefore, the results presented here are likely to be reliable and repeatable.

A new food and feed safety consequence matrix approach was developed to enable the simple extrapolation of valuable information which could assist a farmer or agricultural consultant in making informed crop choices and irrigation system decisions, based of the risk profile of their soils and irrigation water in terms of food and feed safety guidelines. This tool was specifically developed to present the As and Pb results of this dissertation but could be expanded to include other potentially hazardous trace elements with future research.

7.2 Evaluation of Hypotheses

The general trend in the foliar absorption trial showed increased As or Pb in crop plant tissues with increased As or Pb irrigation treatments; however, most edible parts did not accumulate enough As or Pb to pose a health risk if consumed by humans or animals. Excluding the controls, 17 % of crop parts irrigated with As treated water and 33 % of crop parts irrigated with Pb treated water were unfit for human consumption. Similarly, 20 % of crop parts irrigated with As treated water and 15 % of crop parts irrigated with Pb treated water were unfit for use as animal feed.

Null Hypothesis 1: Arsenic or lead present in irrigation water does not result in edible plant parts exceeding food safety thresholds for As or Pb.

Hypothesis 1: Arsenic or lead present in irrigation water results in edible plant parts exceeding food safety thresholds for As or Pb.

Therefore, with reference to null hypothesis 1, that As or Pb present in irrigation water does not result in edible plant parts exceeding the food safety thresholds, it is accepted for all crops irrigated with 0.1 $mg.L^{-1}$ As; all crops (except for leafy greens) irrigated with 2.0 $mg.L^{-1}$ As; as well as all crops (except for leafy greens) irrigated with 0.2 $mg.L^{-1}$ or 2.0 $mg.L^{-1}$ Pb.

Hypothesis 1 is accepted for beetroot and Swiss chard leaves irrigated with 2 $mg.L^{-1}$ As; as well as beetroot and Swiss chard leaves irrigated with 0.2 $mg.L^{-1}$ Pb and 2 $mg.L^{-1}$ Pb. Under those irrigation regimens, the resulting produce exceeds the food safety thresholds.

Null Hypothesis 3: Arsenic or lead present in irrigation water, does not result in the crop exceeding animal feed thresholds for As or Pb.

Hypothesis 3: Arsenic or lead present in irrigation water results in the crop exceeding animal feed thresholds for As or Pb.

Regarding null hypothesis 3, that As or Pb present in irrigation water does not result in edible plant parts exceeding the animal feed thresholds, the null hypothesis is accepted for all crop parts (except barley leaves) irrigated with 0.1 $mg.L^{-1}$ As. The null hypothesis is rejected for all crops irrigated with 2 $mg.L^{-1}$ As. The null hypothesis is accepted for all crops irrigated with 0.2 $mg.L^{-1}$ Pb, as well as all crops (except for barley leaves, barley stems and Swiss chard leaves) irrigated with 2 $mg.L^{-1}$ Pb.

Hypothesis 3 is accepted for all crops irrigated with 2 $mg.L^{-1}$ As, and barley leaves irrigated with 0.1 $mg.L^{-1}$ As; as well as barley leaves, barley stems and Swiss chard leaves irrigated with 2 $mg.L^{-1}$ Pb.

The general trend in the root uptake trial showed increased As or Pb in crop plant tissues, with increased As or Pb soil treatments. However, unlike the foliar uptake trial, the majority of edible parts accumulated As or Pb above the food safety thresholds. Excluding the controls, 58 % of crop parts grown in As treated soils and 67 % of crop parts grown in Pb treated soils were unfit for human consumption. For use as animal feed, 45 % of crop parts grown in As treated soil and 5 % of crops grown in Pb treated soils exceeded the animal feed guidelines.

Null Hypothesis 2: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, does not result in edible plant parts exceeding food safety thresholds for As or Pb.

Hypothesis 2: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, results in edible plant parts exceeding food safety thresholds for As or Pb.

Null hypothesis 2, that As or Pb present in soil as a result of medium- to long-term irrigation inputs does not result in edible plant parts exceeding food safety thresholds, is accepted for garden peas and beetroot roots grown in soils dosed with 43.5 $mg.kg^{-1}$ As; garden peas grown in soils dosed with 168.9 $mg.kg^{-1}$ As; garden peas and barley grown in soil dosed with 88.1 $mg.kg^{-1}$ Pb; as well as garden pea pods grown in soil with 168.9 $mg.kg^{-1}$ Pb.

Hypothesis 2 is accepted for barley grains, beetroot leaves and Swiss chard leaves grown at both soil As treatment levels; as well as beetroot roots grown in soils dosed with 168.9 $mg.kg^{-1}$ As. Hypothesis 2 is also accepted for beetroot leaves, beetroot roots, and Swiss chard leaves grown at both soil Pb treatment levels; as well as barley grains and garden pea pods grown in soils containing 168.9 $mg.kg^{-1}$ Pb. Under all these conditions, the resulting in fresh produce exceeds the food safety thresholds for As and Pb.

Null Hypothesis 4: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, does not result in the crop exceeding animal feed thresholds for As or Pb.

Hypothesis 4: Arsenic or lead present in soil as a result of medium- to long-term irrigation inputs, results in the crop exceeding animal feed thresholds for As or Pb.

Regarding null hypothesis 4, that As or Pb present in soil as a result of medium- to long-term irrigation inputs does not result in edible plant parts exceeding animal feed thresholds, it is accepted for all crops (except barley leaves and garden pea leaves and stems) grown in soils dosed with 43.5 $mg.kg^{-1}$ As; barley grains, barley stems, garden pea pods and peas in soils dosed with 168.9 $mg.kg^{-1}$ As; as well as all crops grown in both soil Pb treatments (except beetroot leaves grown in soils containing 168.9 $mg.kg^{-1}$ Pb).

Finally, hypothesis 4 is accepted for barley leaves, pea leaves and pea stems grown at both As treatment levels, as well as beetroot leaves, beetroot roots and Swiss chard leaves grown in soils dosed with 168.9 $mg.kg^{-1}$ As. In terms of Pb, hypothesis 4 is accepted for beetroot leaves grown in soils containing 168.9 $mg.kg^{-1}$ Pb. None of the crop parts grown under these conditions are suitable for animal feed, according to the animal feed thresholds.

7.3 Limitations

This study investigated the effects of As and Pb in irrigation water on winter crops in a summer rainfall region. Therefore, the results did not account for the potentially positive effects of rainfall rinsing the aboveground biomass when growing summer crops.

Due to the temporal and practical challenges associated with simulating the biophysicochemical soil transformations that would realistically have taken place over 19 or 99 years of As or Pb accumulation through gradual irrigation inputs, soil dosing of As and Pb for this trial effectively simulated a once-off pollution event. Therefore, the results of this trial represent a "worst case scenario" of As and Pb uptake, as the bioavailability of these elements in the soil would be higher than expected over a 19- or 99-year period of accumulation.

Although food safety guidelines are based on peeled beetroot, a more comprehensive understanding of trace element root uptake and biomass accumulation could have been gained if the washed beetroot root peels had been tested for As and Pb as well.

7.4 Future Research

This study has demonstrated that existing irrigation water quality guidelines are not always compatible with the more recently established food and feed safety thresholds for As and Pb. Future research should commence with a review of the impact of irrigating with other potentially hazardous trace elements such as: Cd, Cr and Hg, in terms of the food and feed safety guidelines.

Additionally, the number of crops (with the inclusion of summer crops) should be increased, which will allow for a more extensive evaluation of how irrigation quality guidelines may impact food and feed safety.

Future research also could investigate the effects of potentially hazardous trace elements at a larger range of treatment levels, in order to statistically quantify the crop-specific trace element concentration at which the food safety risk could pose, for example, a 10 % risk of edible parts being unfit for human consumption. This information could assist with crop selection in areas where soil or irrigation water is contaminated.

This study has developed and demonstrated the potential of using a food and feed safety consequence matrix to aid in crop choice on potentially hazardous trace element impacted lands or where water is contaminated with potentially hazardous trace elements. Future research could develop this tool by evaluating the risk associated with the accumulation of other potentially hazardous trace elements in more crops and under a variety of growth media and agricultural systems (field, greenhouse, hydroponic, aeroponic), or by attempting to quantify the factors which influence the foliar absorption pathway.

The potential to integrate the food and feed safety consequence matrix with the decision support system (DSS) developed by du Plessis et al. (2017), in order to incorporate food and feed safety guidelines into decisions regarding irrigation, needs to be investigated.

Finally, as originally proposed in the beginning of this study, these results, and those of similar trials, should be applied to update the irrigation water quality guidelines to ensure that crops comply with food/feed safety standards.

7.5 Academic Contribution

This dissertation adopted a multidisciplinary approach and for the first time, as far as the author is aware, investigated whether irrigating according to the South African (and other international) Irrigation Water Quality Guidelines produces crops which meet the specifications established for human food and animal feed safety of fresh produce.

The results of the trials have demonstrated that the existing irrigation water quality guidelines for As and Pb do pose a human food and animal feed safety risk in certain crops and should therefore be revised to ensure that irrigation thresholds are compliant with food and feed safety standards. Furthermore, these results show that the foliar absorption pathway should not be ignored, as it currently is in irrigation water quality guidelines.

Having established this disconnect between irrigation water quality and food safety guidelines for As and Pb, it is recommended that the irrigation water quality guidelines for all potentially hazardous trace elements that have been allocated food safety thresholds be investigated, namely: As, Pb, Cd, Cr and Hg.

7.6 Conclusion

In conclusion, this dissertation contributed new knowledge to the field of agriculture by demonstrating that certain crops irrigated with As or Pb loaded water or grown in soil impacted by As or Pb as a result of irrigation inputs, at concentrations acceptable to both South African and international irrigation water quality guidelines, were deemed unfit for human and/or animal consumption.

Therefore, if international food safety standards for fresh produce are to be adhered to, the irrigation water quality guidelines for As and Pb should be critically reviewed so as to negate all possible future contamination of fresh produce as a result of irrigation inputs.

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APPENDIX A1:

Yield response of crops following irrigation with As or Pb loaded irrigation water.

Crop	Plant part	Average yield ± SD, dry mass (g.pot^{-1})				
		Control	**As [0.1]**	**As [2.0]**	**Pb [0.2]**	**Pb [2.0]**
Barley	Grain	9.29 ± 1.54	9.68 ± 1.99	9.71 ± 2.11	10.47 ± 1.08	9.99 ± 1.09
	Leaf	5.41 ± 0.61	4.55 ± 0.54	5.63 ± 1.05	4.65 ± 1.06	4.69 ± 0.69
	Stem	8.61 ± 1.15	7.63 ± 1.52	6.57 ± 0.69	7.55 ± 1.09	7.74 ± 0.41
Garden pea	Pea	2.56 ± 0.21	2.61 ± 0.74	2.47 ± 0.19	1.97 ± 0.14	2.08 ± 0.39
	Pod	0.69 ± 0.05	0.82 ± 0.17	0.87 ± 0.08	0.70 ± 0.05	0.84 ± 0.05
	Leaf	1.05 ± 0.20	1.18 ± 0.29	1.07 ± 0.23	1.15 ± 0.01	1.05 ± 0.11
	Stem	1.78 ± 0.27	1.42 ± 0.16	1.45 ± 0.36	1.47 ± 0.47	1.64 ± 0.08
Swiss chard	Leaf	3.37 ± 0.51	4.25 ± 0.55	4.00 ± 0.35	3.23 ± 0.63	3.29 ± 0.47
Beetroot	Root	2.63 ± 0.68	3.80 ± 1.06	2.91 ± 0.17	3.86 ± 1.19	4.06 ± 0.12
	Leaf	2.96 ± 1.81	2.57 ± 0.36	2.35 ± 0.31	2.02 ± 0.19	2.78 ± 0.51

APPENDIX A2:

Yield response of crops grown in As or Pb loaded soil.

Crop	Plant part	Average yield ± SD, dry mass (g.pot^{-1})				
		Control	**As [43.5]**	**As [168.9]**	**Pb [88.1]**	**Pb [168.9]**
Barley	Grain	9.89 ± 1.06	9.65 ± 0.24	9.66 ± 0.79	9.68 ± 2.37	8.82 ± 2.50
	Leaf	4.90 ± 0.73	3.62 ± 0.40	4.08 ± 0.93	6.42 ± 0.56	5.08 ± 0.26
	Stem	7.65 ± 0.96	7.59 ± 0.64	7.86 ± 1.40	10.37 ± 1.25	9.59 ± 2.78
Garden pea	Pea	2.01 ± 0.41	2.68 ± 0.69	3.12 ± 0.51	3.76 ± 0.67	4.64 ± 0.50
	Pod	0.82 ± 0.10	1.34 ± 0.15	1.19 ± 0.21	1.19 ± 0.03	1.43 ± 0.16
	Leaf	1.22 ± 0.26	1.77 ± 0.09	1.30 ± 0.09	1.53 ± 0.15	2.09 ± 0.11
	Stem	1.52 ± 0.33	2.35 ± 0.40	2.08 ± 0.15	2.08 ± 0.11	2.33 ± 0.09
Swiss chard	Leaf	3.79 ± 0.61	2.75 ± 0.75	2.15 ± 0.10	3.99 ± 0.92	2.59 ± 0.71
Beetroot	Root	3.13 ± 0.70	2.52 ± 0.63	3.30 ± 0.59	2.31 ± 0.09	3.01 ± 0.41
	Leaf	2.48 ± 0.88	2.06 ± 0.17	1.91 ± 0.91	2.26 ± 0.42	1.72 ± 0.53

APPENDIX B1:

Mean As or Pb concentration (fresh mass) of crops following irrigation with As or Pb loaded irrigation water.

Crop	Plant part	Mean As or Pb concentration, fresh mass (mg.kg^{-1})				
		Control	**As [0.1]**	**As [2.0]**	**Pb [0.2]**	**Pb [2.0]**
Barley	Grain	0.01	0.11	0.26	0.14	0.16
	Leaf	0.10	9.28	19.32	7.01	41.64
	Stem	0.03	0.46	0.48	15.67	2.28
Beetroot	Leaf	0.01	0.31	2.05	6.93	4.85
	Root	<0.01	<0.01	<0.01	<0.01	<0.01
Garden pea	Leaf	0.04	1.41	6.83	9.17	12.13
	Pea	<0.01	<0.01	0.01	<0.01	<0.01
	Pod	0.01	0.02	0.16	0.02	0.08
	Stem	0.01	0.14	0.57	0.11	0.50
Swiss chard	Leaf	0.01	0.25	5.34	5.19	9.99

APPENDIX B2:

Mean As or Pb concentration (fresh mass) of crops grown in As or Pb loaded soil.

Crop	Plant part	Mean As or Pb concentration, fresh mass (mg.kg^{-1})				
		Control	**As [43.5]**	**As [168.9]**	**Pb [88.1]**	**Pb [168.9]**
Barley	Grain	0.01	0.87	0.75	0.13	0.34
	Leaf	0.10	11.38	31.77	2.17	4.93
	Stem	0.03	1.12	2.24	1.97	2.97
Beetroot	Leaf	0.01	0.58	3.17	0.48	10.88
	Root	<0.01	0.45	1.02	0.40	1.58
Garden pea	Leaf	0.04	3.61	6.09	1.42	11.54
	Pea	<0.01	0.03	0.05	0.01	0.03
	Pod	0.01	0.25	0.34	0.06	0.99
	Stem	0.01	1.21	1.96	0.90	3.67
Swiss chard	Leaf	0.01	0.62	1.97	0.37	1.95

APPENDIX C:

Fertilizer programme for barley:

Nutrient	Straight fertilizer	Application rate (kg/ha)	Timing
N	CAN (27%)	185	At planting
		115	6 weeks after emergence
		75	At 100 % ear
P_2O_5	SSP (16%)	310	At planting
K_2O	MOP (60%)	140	At planting
Lime	Calcitic	2000	4 weeks before planting

Fertilizer programme for garden pea:

Nutrient	Straight fertilizer	Application rate (kg/ha)	Timing
N	CAN (27%)	150	At planting
P_2O_5	SSP (16%)	310	At planting
K_2O	MOP (60%)	250	At planting
Lime	Calcitic	2000	4 weeks before planting

Fertilizer programme for Swiss chard:

Nutrient	Straight fertilizer	Application rate (kg/ha)	Timing
N	CAN (27%)	225	At planting
		115	4 weeks after emergence
		130	8 weeks after emergence
P_2O_5	SSP (16%)	160	At planting
K_2O	MOP (60%)	210	At planting
Lime	Calcitic	2000	4 weeks before planting

Fertilizer programme for beetroot:

Nutrient	Straight fertilizer	Application rate (kg/ha)	Timing
N	CAN (27%)	150	At planting
		40	10 days after emergence
		40	20 days after emergence
		40	30 days after emergence
		40	40 days after emergence
P_2O_5	SSP (16%)	470	At planting
K_2O	MOP (60%)	210	At planting
Lime	Calcitic	2000	4 weeks before planting

APPENDIX D:

Water analysis:

Date	Borehole water		Treatment Spray Bottles			
			As [0.1]	As [2.0]	Pb [0.2]	Pb [2.0]
	As ($mg.L^{-1}$)	Pb ($mg.L^{-1}$)	As ($mg.L^{-1}$)		Pb ($mg.L^{-1}$)	
07/06/2017	-	-	0.15	2.02	0.22	1.99
11/07/2017	BDL	BDL	0.14	2.05	0.19	1.98
16/08/2017	-	-	0.08	1.99	0.16	2.00
05/09/2017	BDL	BDL	0.08	1.98	0.22	1.96
03/10/2017	-	-	0.11	2.06	0.24	2.05
AVERAGE:	BDL	BDL	0.11	2.02	0.21	2.00

BDL: Below method detection limit of 0.04 mg.kg-1 for As or 0.07 mg.kg-1 for Pb