SELENIUM AND IODINE STATUS IN THE KURDISTAN REGION OF IRAQ

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Thesis submitted to the University of Nottingham
for the degree of doctor of philosophy

December 2017

I dedicate this work to my parents and my wife,

Without whose love and support, none of this would have been possible

ACKNOWLEDGEMENTS

I would like to thank the Kurdistan Regional Government (KRG) for the financial support, which made this project possible.

I would like to express my sincere gratitude and greatest appreciation to my supervisors Dr. Scott Young and Dr. Liz Bailey for their support, guidance and kind advice throughout my PhD.

Thanks most go to the administrator and technical staff for their support: Emma Hooley, John Corrie, Saul Vazquez Reina.

On personal note, I would like to acknowledge the following institute or people who helped me to achieve this

- Staff of Halabja College of Applied Science for the support and facilities offered during sample collection.
- My friends and colleagues in Halabja College of Applied Science
- My friend Kocher Jamal for his help and support
- My brother and sister Basir and Asmar for their support
- Volunteer families who attributed in questionnaire and biomarker sampling In Sulaimani province of Iraq
- All my friends and colleagues in the division of Agriculture and Environmental Science –University of Nottingham for their support

CONFERENCES, MEETINGS, PUBLICATIONS:

1. 2nd Kurdistan student's conference in Nottingham University on 16 September 2014 –poster presentation "Understanding selenium bioavailability through ^{77}Se biofortification in a pot experiment design in some common vegetables specious using soil and seeds of Kurdistan region of Iraq"

2. Environmental chemistry of water, sediment and soil: early-career researcher meeting-Royal Society of Chemistry on 14th November 2016, London-Oral presentation "An investigation of iodine bioavailability in plants using ^{129}I as a tracer"

3. The 5th International Conference on Selenium in the Environment and Human Health and the 11th International Symposium on Selenium in Biology and Medicine, 13-17 August 2017, Stockholm-Sweden - Oral presentation

4. 1st International WIA (World Iodine Association) Conference 15-17 November 2017, Pisa-Italy, Oral presentation "Iodine status in Iraqi Kurdistan: terrestrial environment, diet and human biomarkers"

5. High dietary salt intake may cause excessive iodine intakes in the Iraqi Kurdish community, IDD Newsletter, February 2018, http://www.ign.org/idd-newsletter-1-2018.htm.

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GLOSSARY OF TERMS

%I_N: proportion of total soil iodine that was extracted by KNO_3

%I_p: proportion of total soil iodine that was extracted by KH2PO4

%Se_N: proportion of total soil Se that was extracted by KNO_3

%Se_P: proportion of total soil Se that was extracted by KH2PO4

%Se_{TMAH}: proportion of total soil Se that was extracted by TMAH

$^{129}I^-$: iodide-129

^{129}I: iodine isotopical -129

$^{129}IO_3^-$: iodate-129

DIC: dissolved inorganic carbon

DOC: dissolved organic carbon

DW: dry waight

FW: fresh weight

HPLC: high performance (anion exchange) liquid chromatography

HPLC-ICP-MS: high performance (anion exchange) liquid chromatography inductively coupled plasma mass spectrometry

ICP-MS: inductively coupled plasma mass spectrometry

$I_{Inorganic}$: inorganic iodine

I_N: soluble iodine (extractaed by KNO_3)

I_{N+P}: soil soluble and adsorbed iodine (labile)

$I_{Organic}$: organic iodine

I_P: adsorbed iodine (extracted by KH_2PO_4)

$I_{Tot\ or}$ I_{TMAH}: total soil iodine

RDA: recommended daily allowance

RDI: recommended daily intake

Se_f: selenium originated from fertilizer

$Se_{Inorganic}$: inorganic Se

Se_N: soluble selenium (extractaed by KNO_3)

Se_{N+P}: soil soluble and adsorbed Se (labile)

$Se_{Organic}$: organic selenium

Se_P: adsorbed selenium (extracted by KH_2PO_4)

Se_S: soil native selenium

Se_{TMAH}: organic-bound selenium (extracted by TMAH)

Se_{Tot} : Total Selenium (digested by HF)

SIC: Soil inorganic carbon

SOC: soil organic carbon

TMAH: tetramethyl ammonium hydroxide

UIC: urinary iodine concentration

UIC_{CRT}: urinary iodine concentration creatinine justified

UIC_{OSM}: urinary iodine concentration osmolality justified

UNaC: urinary sodium concentation

USeC: urinary selenium concentration

ABSTRACT

The primary aims of this project were to: i) provide a survey of selenium and iodine concentrations in the terrestrial environment and locally grown crops of Iraqi-Kurdistan; ii) gain greater understanding of the factors controlling bioavailability of these elements in the calcareous soils of the region; iii) investigate the feasibility of biofortification of selenium and iodine and test the use of isotopically enriched tracers for this purpose; iv) assess iodine and selenium dietary intake and nutritional status of the local population using dietary questionnaires and a survey of a human biomarker.

A survey was conducted covering locally grown crops, matched soil samples and irrigation water including 300 plant, 100 soil and 20 water samples. The potential availability of Se and I for plant uptake was examined by quantifying the soluble and adsorbed fractions of both micronutrients and their species. The influences of soil factors on plant Se and I uptake were examined. Results indicated that total soil Se (Se_{Tot} = 309 µg kg^{-1}) was lower than the global average (400 µg kg^{-1}). Approximately 2.5% of soil Se was present in the soluble and adsorbed fractions with an equal proportion of selenate and selenite in the soluble fraction and mainly selenite in the adsorbed fraction. The organically-bound Se extractability ranged from 20-89% of Se_{Tot}, depending on soil pH. Plant Se content was variable between crop species and different areas within Kurdistan with mean concentration of 113,112, 69 and 49 µg $kg^{-1}{}_{dw}$ for leafy vegetables, tubers, fruit vegetables and wheat grain respectively. Higher plant Se concentrations were observed in plants grown in soils with pH>8. The mean concentration of total soil iodine was 4140 µg kg^{-1}. Almost 10% of this value was present in the soluble and adsorbed fractions. Mean plant iodine concentrations were 439, 368, 140, and 12 µg $kg^{-1}{}_{dw}$ for leafy vegetables, fruit vegetables, tubers and grains respectively. The combined concentrations of soluble and adsorbed iodine were correlated with plant iodine content. The mean concentrations of irrigation water Se and I were 0.495 and 11.9 µg L^{-1} respectively. The amount (%) of soil $CaCO_3$ was strongly correlated with iodine concentration in groundwater used for irrigation and irrigation water iodine concentration was again strongly correlated with plant iodine concentrations.

The feasibility of Se biofortification in calcareous soils using local vegetable genotypes from Kurdistan was examined using 10 g ha^{-1} ^{77}Se as a biofortification treatment (and isotopic tracer). Five commonly used vegetables, including celery, chard, lettuce, radish and spring-onion were planted in soils spiked with the ^{77}Se application and grown for 8 weeks under controlled growth room conditions. Results indicated that, at the end of the growth period approximately 35% of applied ^{77}Se had been transferred to a recalcitrant form in the soil which resisted extraction with 10% TMAH. Only 5% of ^{77}Se was present within the soluble and adsorbed soil fractions combined, at harvest. The amount of ^{77}Se taken up by plant biomass varied according to crop species; 25% for radish and 7-8% for other vegetables from the total ^{77}Se applied. Plant ^{77}Se concentrations varied despite growing in identical soils and, unexpectedly, plants contained more Se originating from the soil rather than the fertilizer. The ratio of $Se_{soil}/Se_{fertilizer}$ also varied between varieties reflecting different growth patterns and uptake rates against a backdrop of decreasing fertilizer Se availability during the growing season.

Biofortification of iodine using a range of vegetable crops grown in calcareous soil was investigated, using soil and vegetable genotypes from Kurdistan, and employing ^{129}I as a tracer. Vegetables were irrigated daily with water containing 5.56 and 6.89 µg L^{-1} $^{129}IO_3^-$ and $^{129}I^-$ respectively for 8 weeks. Total and fractionated iodine (^{127}I and ^{129}I) was conducted on moist soil (c. field capacity) and air dried soil at the end of the growing season. Plant analysis was also undertaken for ^{127}I and ^{129}I, using ICP-MS. Results showed that plant iodine concentrations originating from native soil iodine were variable even when grown in identical soils. Generally, iodine concentrations in roots was greater than in shoots for both ^{127}I and ^{129}I. Vegetables irrigated with $^{129}I^-$ (iodide) had considerably lower iodine concentrations (6.2-12 µg kg^{-1}_{dw}) than those irrigated with $^{129}IO_3^-$ (iodate) (53.3-479 µg kg^{-1}_{dw}). The majority of plant iodine originated from soil iodine rather than fertilizer iodine and varied depending on ^{129}I species applied (iodate or iodide) and the vegetable plant type grown. For vegetable shoots treated with $^{129}I^-$ (iodide) only 3% of the iodine of the three test plants originated from the ^{129}I treatment. By contrast, for $^{129}IO_3^-$ (iodate)

applications 11, 22 and 58% of iodine in the shoots of celery, lettuce and chard were from the ^{129}I additions respectively. The recovery rate of ^{129}I from soil extracted with 10% TMAH ranged from 63-95% of total iodine applied and varied depending on vegetable variety. The ^{129}I recoveries from pots irrigated by $^{129}IO_3^-$ were less than from $^{129}I^-$ irrigated soils suggesting greater loss of iodine from the iodate irrigated system.

To assess the Se and I status of the population in a region of Kurdistan, the food composition data and dietary intake of Se and I was determined for 410 volunteers using a semi- quantitative food questionnaire, including commonly used food items. To directly investigate level of nutritional status of Se and I, urine samples were also collected from each participants as a biomarker. Daily dietary intake and source apportionment of Se and I from each food item was determined using questionnaire survey. The daily intake of I from food excluding salt was 119 $\mu g\ d^{-1}$. Vegetables and fruits supplied 48%, protein sources 25%, cereal and grains 9%, dairy products 8% and water 2% of daily I intake. The majority (>90%) of salt samples collected were iodised with a mean I concentration of 40 mg kg^{-1} and daily intake of salt was estimated as 13.5g d^{-1}. Accounting for salt intake, average daily iodine intakes increased to 668 µg d^{-1}, with salt supplying 82% of daily I intake. The median urinary iodine (corrected for creatinine) was 379 $\mu g\ g^{-1}{}_{CRT}$ and 424 $\mu g\ L^{-1}$ osmolality corrected. More than 90% of school age children and over 55% all participants had excessive I intake according to WHO classification. The salt I concentration consumed by each family was highly correlated with mean urinary iodine of family members. Urinary Na and I were also correlated. The iodine intake estimated according to salt intake (calculated based on urinary Na) was strongly correlated with iodine intake calculated according to urinary iodine. The daily salt intake estimated by urinary Na was 15.3 g d^{-1} considerably higher than WHO recommended. Mean total daily intake of Se according to the questionnaire responses was 72.9 µg d^{-1} with 21% of participants having a daily intake lower than recommended RDA. The mean urinary Se fell in the range of 21.2-24.8 $\mu g\ L^{-1}$ depending on justification methods. The predicted Se intake from urinary Se gave a values of 59 and 42 µg d^{-1} according to the method used which may imply Se deficiency.

To conclude, Kurdistan soils Se content was found insufficient. Despite that, Due to high pH effect in some areas plant Se content seems to be having considerably higher Se content rather than areas with lower pH. Later studies revealed that daily Se intake may not enough to address the Se requirement of population. Biofortification of Se possible but to prevent decreasing availability would be recommend to apply in med season or foliar application. Typically soil and plant of Kurdistan found in a minimum of normal range of iodine and comparable with other areas. Irrigation water was found as a main source of plant iodine uptake. The daily iodine intake from food excluding salt is not enough to meet the recommended iodine level but considering high consumption of iodized salt in that region it can be classified as an excessive iodine intake which could cause high intake iodine consumption disorders such as hyperthyroidism and in turn health issues caused by elevated Na intake such as cardiovascular disease. In current iodine nutritional status of the region plant biofortification would not be recommended.

1 INTRODUCTION

1.1 Selenium geochemistry

Selenium was first identified in 1817 by the Swedish chemist Jöns Jacob Berzelius and was named after *Selene* the Greek moon goddess(Pauling, 1970). Incorporation of Selenium into terrestrial environment can be affected by natural processes such as geological weathering, volcanic activity or anthropogenic activities such as fossil fuel combustion and agricultural practices. Approximately 96% of the rock in the surface section of the Earth's crust (igneous or sedimentary sandstones and limestones) typically contains between 0.05 and 0.09 mg Se kg^{-1} (Johnson et al., 2010). Organic-rich deposits such as US coal can contain elevated Se concentrations (up to 10.7 mg kg^{-1}), whilst some sedimentary rocks such as phosphates and black shales have been reported as having up to 300 and >600 mg Se kg^{-1}, respectively (Fordyce, 2005). Vegetation grown on seleniferous soils can contain >5 mg Se kg^{-1} (Poblaciones et al., 2013) which is classified as toxic for human and animals. Volcanic ash and gases can contain elevated concentrations of Se resulting in high Se levels in surrounding volcanic soils; Se concentrations up to 20 mg Se kg^{-1} have been reported (Oldfield, 1999). The combustion of fossil fuels such as coal and petroleum also has been reported as a source of Se; coal combustion has been recorded to produce Se 1.5-2.5 times greater than natural weathering processes (Andren et al., 1975).

1.2 Selenium in soils

In many regions of the world human Se status generally reflects the Se concentration in soils (Fairweather-Tait et al., 2011, Lyons et al., 2005). Soil Se can vary substantially with some soils being regarded as seleniferous and others as 'Se-deficient' in the sense that the food crops produced from cultivating them provide less Se than the dietary requirements (Bitterli et al., 2010, Hawkesford and Zhao, 2007).

The concentration of Se in most soils lies within the range of 0.01 to 2 mg kg^{-1} (Kabata-Pendias, 2004) with a mean of 0.4 mg kg^{-1}; soils with high concentrations (>10 mg kg^{-1}) are regarded as seleniferous (Fordyce, 2005).

The geological parent material is recognised as the main source of Se in soils although it is likely that prolonged marine inputs are important in some areas. The crustal abundance of Se is low in some areas resulting in very low soil concentrations (De Temmerman et al., 2014). For example in parts of China, Finland and New Zealand soil Se levels in soil are <0.05 mg kg^{-1} and can result in deficiency diseases (Navarro-Alarcon and Cabrera-Vique, 2008). By contrast, Se is found at concentrations much higher than the crustal average in black organic-rich shales such as those in the United States, Wales and Ireland (Davies and Houghton, 1983) and Oldfield (1999) reported concentrations of soil Se up to 1250 mg kg^{-1} in some seleniferous soils in Ireland. Coal, and to a lesser extent petroleum, also have higher Se concentrations. Volcanic sulphur deposits can lead to high Se concentrations in soils of affected regions (De Temmerman et al., 2014). In quaternary parent material, total Se concentrations have been reported to range from 0.2–0.5 mg kg^{-1} in sandy and sandy loam soils and from 0.3–

0.7 mg kg^{-1} in loam and polder clay soils (Gissel-Nielsen, 1984, De Temmerman et al., 2014).

1.3 Selenium forms and behaviour in soils

The chemical form of Se in soil depends mainly on pH and redox potential (Mikkeisen et al., 1987). Selenium is found in both organic and inorganic forms, including insoluble selenides (Se^{2-}), elemental Se (Se^{0}) and the adsorbed ions selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). Elemental Se is formed when conditions become strongly anoxic, as in sediments of water bodies, and selenides are found in highly reducing and basic conditions (Neal and Sposito, 1989). In oxidizing environments such as aerobic soils, selenite and selenate are the dominant inorganic forms (Roussel-Debet et al., 2005). In addition to humic and fulvic bound Se more volatile organic forms of methylated Se compounds including dimethyl-selenide, selenoamino acids, and selenoproteins may exist.

Selenium solubility and consequently its mobility, bioavailability and uptake from soils by plants is highly pH-dependent (Bitterli et al., 2010). Selenate is favoured in alkaline (pH 7.5–8.5) situations and is more soluble and available for uptake in the plants than selenite (Navarro-Alarcon and Cabrera-Vique, 2008, De Temmerman et al., 2014). Plants only partially discriminate between sulphate and selenate which can be taken up in place of sulphate ions (Terry et al., 2000). Selenite is stable in acidic to neutral (pH 4.5–6.5), less oxic conditions and is strongly adsorbed by Fe/Al hydrous oxides so is only poorly available to plants (Navarro-Alarcon and Cabrera-Vique, 2008). In aerated soils, soluble Se concentration is usually controlled by adsorption to mineral and organic surfaces, whereas in reducing

environments, the role of precipitation seems to be more important. Sorption of Se to iron oxides is stronger than to clay minerals, and organic matter also binds Se much more strongly than clay minerals. Organic matter is a significant factor for the retention of Se in the topsoil (Geering et al., 1968, Elrashidi et al., 1987) although the binding mechanism is poorly understood. As the most soluble Se species in soils, selenate is very susceptible to leaching and is readily bioavailable to plants. The mobility of selenite is lower because of its much higher affinity to adsorb to iron oxides and/or hydroxides, clay minerals and form of organic compounds. Thus, bioavailability of soil Se depends on the geochemical environment, pedoclimatic variables (temperature and rain intensity) and is related to fluctuations of soil moisture, pH, redox, and organic matter content (Spadoni et al., 2007).

1.4 Selenium in plants

Plants are the primary source of Se in most human diets (Funes-Collado et al., 2013), either directly or following consumption by animals (Barclay et al., 1995). Selenium uptake by plants is a function of factors including concentration and physicochemical forms in the soil (and hence parent rock), pH and redox potential, soil moisture and salinity, soil sulphate concentration, plant species, soil-management practices, climatic conditions and inputs from wet and dry deposition (Aro et al., 1995, Barclay et al., 1995, Ventura et al., 2009).

Most plants do not accumulate large amounts of Se; concentrations rarely exceed 100 mg kg^{-1}_{dw}. Plants which are rich in sulphur, for example members of the Liliaceae family (onions and garlic) and members of the

Cruciferae family (cabbage and broccoli), typically have higher Se (Brown and Shrift, 1982). Plant species such as chives, Indian mustard, canola, species of the Brassica genus (Brussel sprouts, cauliflower, collards, kohlrabi, mustards and kale) and some mushrooms, accumulate relatively large concentrations of Se and have been described as Se accumulators (Dumont et al., 2006, Kapolna and Fodor, 2007). They have the ability to take up large amounts of Se (up to 1000 mg kg^{-1}) without showing any negative effects (Manjusha et al., 2007, Navarro-Alarcon and Cabrera-Vique, 2008, Dumont et al., 2006). Plants rich in starch and sugars have lower amounts of selenium. In plants that have high levels of protein, such as cereals, Se is able to substitute for S in the amino acids cysteine and cystine making these crops an important dietary source of Se (Ventura et al., 2009).

Fruits generally contain low concentrations of Se because of their low protein content and high water content. Ventura et al. (2006) reported the Se content for fruit and vegetables from Portugal as 1.7 to 24.9 $\mu g\ kg^{-1}$.

1.5 Selenium in humans and animals

Selenium is considered an essential trace element for humans and animals (Munier-Lamy et al., 2007). In the 1970s, it was established that Se has a role as a cofactor in glutathione peroxidase (GPx) family of enzymes which protect against oxidative stress (Zeng and Combs, 2008, Navarro-Alarcon and Cabrera-Vique, 2008). This element is incorporated into the primary structure of selenoproteins as the amino acid selenocysteine (SeCys) (Funes-Collado et al., 2013). Selenium is also believed to play a role in the defence against infection, modulation of growth and development, and

studies have suggested that some organic forms of selenium could have a reductive effect on cancer risk at supra-nutritional levels (Combs et al., 2001, Rayman, 2005). In addition, it has roles in thyroid hormone homeostasis, immunity, and fertility (Reilly, 2006). Several studies have suggested that Se protects animals against toxicity associated with high exposure and/or intake of heavy metals such as mercury, lead, cadmium and silver (Navarro-Alarcon and Cabrera-Vique, 2008).

In farm animals Se deficiency is an important problem. The most common disease associated with Se deficiency is white muscle disease, a nutritional muscular dystrophy. Actively growing animals show symptoms of weakness, have difficulties feeding, and cardiac problems that very often result in death (Navarro-Alarcon and Cabrera-Vique, 2008).

The two diseases most commonly associated with severe selenium deficiency in humans are Keshan and Kaschin-Beck disease. Both occur in rural areas of China and Russia where there is a dependence on locally grown foods that have extremely low selenium contents (Tan et al., 2002, Abrahams, 2002). Dietary intake was as low as 7 μg day^{-1} where the diet consisted mainly of locally-produced cereals (Combs, 2001).

1.6 Selenium dietary intake

Hamilton (2004) reported that there are three Se levels for biological activity: (1) trace concentration required for normal growth and development; (2) moderate concentrations that can be stored and homeostatic functions maintained; and (3) elevated concentrations resulting in toxic effects. Combs et al. (2001) indicated that an adequate adult diet should have at least 40 μg day^{-1} of Se to support the maximum

expression of Se enzymes. Other research has reported that a dietary intake of 300 μg day^{-1} Se could reduce cancer risks (Navarro-Alarcon and Cabrera-Vique, 2008). The US Office of Dietary Supplements suggests a daily recommended Se-intake of 70 μg day^{-1} (Bitterli et al., 2010). Generally a diet comprising foods containing less than 100 μg Se kg^{-1} results in deficiency, while regular consumption of food containing more than 1000 μg Se kg^{-1} results in toxicity (Whanger, 2002).

The European Directive 2008/100/CE recommends a daily allowance (RDA) of Se of 55 μg day^{-1}, and a maximum Se consumption without risk to health of 300 μg day^{-1} in adults. According to the literature, the NOAEL (non-observed adverse effects level) is considered to be about 800 μg Se day^{-1} while the LOAEL (lowest observed adverse effects level) is 1500 μg Se day^{-1} and symptoms of toxicity are observed with an intake of 6300 μg Se day^{-1} (Funes-Collado et al., 2013, Whanger et al., 1996). The Institute of Medicine Academy of Sciences USA (2001) determined the minimum Se requirement to be 55 μg day^{-1} (0.7 μmol/day) based on the required amount to maximize synthesis of the selenoprotein glutathione peroxidase (GPx), and a Tolerable Upper Intake Level (UL) for adults of 400 μg day^{-1} (5.1 μmol/day) based on observation of selenosis at this level.

1.7 Selenium deficiency and toxicity

Serious health outcomes have been reported in low Se regions of China and Eastern Siberia, where Se deficiency results in endemic Keshan disease in the Keshan region of China (Gissel-Nielsen, 1984, Fordyce, 2005). This pathology is an endemic juvenile cardiomiopathy with myocardial insufficiency that primarily affects children aged 2 to 10 years old, and to

some extent women of child-bearing age (Hartikainen, 2005). This disease coincides with low soil Se levels in Keshan (mean Se content 125 μg kg^{-1}). Consequently, a very low Se intake from a diet of locally produced foods was observed, in some cases intakes were lower than 10 μg Se day^{-1}. Selenium deficiency in other regions of China has been linked to a type of osteoarthritis called Kashin–Beck disease (Li et al., 2008).

Despite globally widespread Se deficiency, selenosis (Se toxicity) is a problem in some areas. Selenosis is characterized by hair loss, fingernail changes including brittleness, gastrointestinal disturbances, skin rash, "garlic" breath, and abnormal functioning of the nervous system. Other related toxic effects are a disruption of endocrine function, synthesis of thyroid hormones and growth hormones (Goldhaber, 2003). Other researcher suggests that high Se levels catalyse hydrosulphide oxidation which exerts an inhibitory effect on protein synthesis and an enhanced risk of cancer (Navarro-Alarcon and Cabrera-Vique, 2008). These toxic symptoms are typically associated with Se intakes of 3200 to 6700 μg d^{-1} although milder symptoms such as fingernail changes have been reported for Se intakes of 1260 μg Se d^{-1}. Other researchers have reported selenosis with Se intakes of ≥850 μg Se d^{-1} (Sunde, 2000). Consequently, the US Environmental Protection Agency (USEPA) set a reference dose of 5 μg Se kg^{-1} d^{-1} and defined an intake of 1262 μg Se d^{-1} (resulting in a whole blood Se level of 1350 μg Se L^{-1}) as the concentration at which clinical selenosis appeared (Navarro-Alarcon and Cabrera-Vique, 2008).

1.8 Iodine geochemistry

The iodine concentration of the Earth's crust is typically 0.25 mg kg^{-1} - 0.3 mg kg^{-1} (Fuge, 1988, Muramatsu and Hans Wedepohl, 1998). Marine sediments and continental rocks are the major reservoirs of I in the terrestrial environment, containing more I than igneous and metamorphic rocks (Muramatsu and Hans Wedepohl, 1998). Unlike most of the soil elements which are derived from weathering of parent material rocks, the oceans with I concentrations of 60 mg L^{-1} (Wong, 1991) are the major source of environmental iodine.

Iodine transfer from the oceans to the atmosphere and subsequently to the terrestrial environment, involves both biological (Saiz-Lopez et al., 2012) and photochemical reactions (Yokouchi et al., 2012). It has been reported that the majority of marine-origin atmospheric I is generated by organisms such as macroalgae, brown algae, red algae, diatoms, phytoplankton and bacteria (Carpenter, 2003, Palmer et al., 2005). This atmospherically transported I is subsequently transferred to the terrestrial environment by wet and dry deposition (Englund et al., 2010, Truesdale and Jones, 1996).

In the terrestrial environment I will react with soils and plants or enter river systems. Typically, surface waters contain <20 μg L^{-1} I with many values reported in the range of 0.5-5 μg L^{-1} (Fuge and Johnson, 1986). Moran et al. (2002) found the median of iodine concentration for North American river waters 5.5 μg L^{-1} with most values <20 mg L^{-1} and some up to 212 mg L^{-1}. Surface waters from Afghanistan and Argentina have been quoted to be in the range of 5.4-22.7 μg L^{-1} and 16-95 μg L^{-1} respectively (Watts

and Mitchell, 2008, Watts et al., 2010). Overall, ground waters are more enriched in I than surface waters (Whitehead, 1984).

1.9 Iodine in soils

Johnson (2003) reported a wide range of soil I concentrations, <0.1-150 mg kg^{-1}, with an average value of 5.1 mg kg^{-1}. Yuita et al. (2005) reported that I-rich oxisols are influenced by ocean deposition in Japan with concentrations ranging from 42 to 71 mg kg^{-1}; they also report average values of 12.5 mg kg^{-1} for rice paddy soils and 2.8 mg kg^{-1} for other top soils. The British Geological Survey database (Fordyce, 2003) reported a wide range of soil iodine concentration for United Kingdom top soils ranging from 0.5-36.9 mg kg^{-1} with a mean of 8.8 mg kg^{-}. The same database reports the mean value of other topsoils to be 2.2 mg kg^{-1} in Germany, 3.8 mg kg^{-1} in Russia and gives a range of 3.65-12.59 for regions of India. Smyth and Johnson (2011) recorded I concentrations of up to 600 mg kg^{-1} in soils from Northern Ireland.

Most soils contain higher I than the rock or parent material from which they originate due to deposition of ocean-derived I via wet and dry precipitation (Hu et al., 2009). Soil I content typically reflects the amount of rainfall they receive (Aldahan et al., 2009, Fuge, 1996) which is influenced by topography and distance from the coast. A significant variation in soil I content has been reported even within areas of marine influence. Johnson (2003) suggesting that the ability of a soil to retain added I is also important. Soil organic matter has an important role in I retention (Shimamoto et al., 2011) with I binding believed to occur via covalent bond

formation with humic macromolecules (Reiller et al., 2006). Iron and Al oxides content also affect I retention (Fuge, 2013). Generally soils containing greater organic content and therefore of low pH, with high aluminium and/or iron oxide content result in high I fixation (Shetaya et al., 2012).

The most common species of soil iodine are iodide (I^-) and iodate (IO_3^-) and elemental iodine (I_2) methyl iodide (CH_3I) may be released under certain conditions (Yang et al., 2007, Yoshida et al., 2007). Speciation is controlled by pH and redox. Under reducing and acidic conditions (low Eh) iodide is the dominant species. Iodate tends to be present in high oxidation situations (high Eh) at higher pH (Fuge, 2013, Johnson, 2003). Sorption of I to soils has been shown to involve transformation to organo-iodine for both IO_3^- and I^- (Muramatsu et al., 2004, Shetaya et al., 2012, Shimamoto et al., 2011).

1.10 Iodine in plants

The plant uptake of iodine via roots from the soil liquid phase is a process by which iodine enters the food chain or converts to volatile forms and is delived to the atmosphere (Ashworth, 2009). Iodine has not been classified as an essential element for plant growth and has been reported to be toxic to plants at high concentration (Zhu et al., 2003) with the I^- ion suggested to be more toxic than the IO_3^- ion (Sheppard and Evenden, 1995). Plant iodine content is generally low; most of the values for samples derived from soils without added iodine were reported to be less than 1 mg kg^{-1} (Fuge and Johnson, 2015).

In many of the soils containing high iodine levels, transfer factors to plants are low due to strongly retained iodine (Uchida and Tagami, 2011); this variation soil iodine behaviour and differing degrees of plant uptake likely reflects the potential of soil for iodine fixation (Sheppard et al., 2010). Despite this low transfer ratios, some studies have demonstrated that plants growing in elevated soils iodine generally contain high iodine levels (Sheppard and Motycka, 1997). Moreover, it has been found that fortification of soils using iodides or iodates, or iodine-rich organic fertilisers such as brown algae increased iodine uptake (Weng et al., 2014). The relative uptake of iodide and iodate has been considered in several studies. For example in some plants such as rice, lettuce and ryegrass in hydroponic system, iodide reported to uptake considerably higher than iodate; whilst in soil, greater uptake occurred for iodate form (Voogt et al., 2010, Zhu et al., 2004).

Whitehead (1984) suggested the aerial parts of plants as an important pathway for iodine uptake through the stomata and absorption from leaf surfaces form rain drops, dust and atmospheric gaseous (Fuge, 2013). Similarly Korobova (2010)realized that mosses and lichens with no root system contained iodine similar to plants with root system.

Several studies have demonstrated iodine volatilization from plants (Weng et al., 2013b). Saini et al. (1995) reported that several higher plants are able to produce CH_3I. They attributed this to a detoxification mechanism of plants by volatilization of CH_3I to reduce likely toxicity of that (Redeker and Cicerone, 2004).

1.11 Iodine in humans and dietary intake

Humans derive their required various elements and compounds via consumed food and inhalation. In the case of iodine, food is generally accepted as a major source. Plant material forms the majority of iodine supply in a normal diet. However, to some extent non-food sources have been studied (Weng et al., 2014). Risher (2009) and Voutchkova et al. (2014) suggested that in some specific areas iodine –enriched water supply a considerable proportion of iodine intake. However, in parts of China the excessive iodine intake has been recorded due to elevated water iodine content causing hyperthyroidism and goitre (Andersen et al., 2008).

Risher (2009) suggested that a very minor proportion of iodine intake supplied by inhalation even in the coastal area with elevated atmospheric iodine inhalation supplied approximately 5 $\mu g\ d^{-1}$.

Marine foods especially fish and shellfish have long been known as one of the richest source of iodine quote values of 0.39 – 6.9 mg kg^{-1} for some of the marine fish, whilst seaweeds have considerably higher iodine contents of up to 4920 mg kg^{-1} (Haldimann et al., 2005, Yeh et al., 2014).

Generally in normal soil and plants very small quantity of iodine can be absorbed by plant; consequently very limited amount of dietary iodine supplied by vegetable materials (Sheppard and Evenden, 1995, Dahl et al., 2004). The general low iodine dietary supplied by vegetables is reported in most literature reviews and databases (Fordyce, 2003, Uchida and Tagami, 2011).

The Institute of Medicine Academy of Sciences USA (2001) determined the recommended daily allowance of 110-130 μg d^{-1} for infants 0-12 months, 90 μg d^{-1} for children 1-8 yr, 120 μg d^{-1} for children 9-13 yr, 150 μg d^{-1} for adults ≥14 , 220 μg d^{-1} for pregnant women and 290 μg d^{-1} for lactation period. In a similar report WHO suggested the recommended nutrient intake of 90 μg d^{-1} for children 0-5 yr, 120 μg d^{-1} for children 6-12 yr, 150 μg d^{-1} for adults ≥12yr. 250 μg d^{-1} for both pregnant and lactating women.

1.12 Deficiency and toxicity of iodine

Iodine is an essential trace element, which plays an irreplaceable role to provide normal growth and development in animals and human. Every healthy human is estimated to have 15–20 mg of iodine which 70–80 % of this amount is in the thyroid gland. The required daily intake of iodine is 100–150 μg d^{-1}, which is increased in pregnancy to 200-250 μg d^{-1} (WHO, 2004). The daily intake of below recommended ranges may result in physiological disorders and biological function abnormalities which are called iodine deficiency disorders (IDD) (Rose et al., 2002, Upadhyay et al., 2002). United Nations International Council for Control of Iodine Deficiency Disorders (ICCIDD), estimated that half of the world's population experienced some degree of dietary iodine deficiency (ICCIDD, 2002). De Benoist et al. (2003) reported that almost 1.9 billion people worldwide were estimated to be at risk of IDD. According to the WHO (1996) approximately 655 million people were estimated to suffer from goitre and about 90 million have some kind of brain damage.

Iodine deficiency will cause insufficient synthesis of thyroid hormones that have many functions such as enhancing protein synthesis, adjusting energy transfer and accelerating growth; also, it is the primary preventable cause of brain damage and mental development retardation (Weng et al., 2013a).

The IDD has accounted one of the most important and common human diseases in the world (WHO, 2007). Iodine deficiency in fetus can cause abortion, stillbirth and perinatal mortality, in neonate period may lead to infant mortality and endemic cretinism , children and adolescent can suffer from impaired mental retardation, delayed physical development ; overall in all ages other health consequences such as goitre, inducing toxic nodular goitre, hypothyroidism can be accounted (Zimmermann, 2008).

Excess iodine intake may very rarely accelerate iodermia, "a skin disorder consisting of acneiform eruptions, pruritic rash, and urticarial" (Parsad and Saini, 1998). Chow et al. (1991) reported that, in the iodine sufficient areas, healthy individuals can tolerate iodine intakes up to 1 mg d^{-1}, as the thyroid is able to regulate a wide range of iodine intakes to adjust the synthesis and release of thyroid hormones. However, high iodine intake in range of mg can lead to hypothyroidism. In children, high intakes of 500 $\mu g\ d^{-1}$ have been reported to correlate with increased thyroid volume which is a sign of thyroid dysfunction (Zimmermann et al., 2005).

Excessive iodine intake in iodine deficient populations may cause iodine-induced hyperthyroidism (IIH) (Delange et al., 1999) which is reported in Zimbabwe and Congo due to excess iodized salt consumption. Symptoms of IIH include "weight loss, tachycardia, muscle weakness, and skin warmth, without the ophthalmopathy of Graves' disease" (Zimmermann,

2008). It has been suggested to monitor salt iodine levels carefully to prevent IIH (Delange et al., 1999).

The European Commission HaCPD-GSCoF. (2002) determined a tolerable upper intake level for iodine in children 4-6 yr of 250 μg d^{-1}, 7-10 yr of 300 μg d^{-1}, 11-14 yr of 450 μg d^{-1}, 15-17yr of 500 μg d^{-1} and adult and pregnant women of 600 μg d^{-1} whilst Institute of Medicine Academy of Sciences USA (2001) suggested 300 μg d^{-1} as a tolerable upper intake for 4-14 yr, 900 μg d^{-1} for 15-17 yr and 1100 μg d^{-1} for adult and pregnant women.

1.13 Selenium and iodine biomarkers

Iodine status of population has been well documented to assess by measuring of urinary iodine concentration (UIC) in random samples due to 90% of ingested iodine is excreted in the urine. This measurement will not determine the iodine status of individuals but can give an overview of iodine nutrition adequacy and recently iodine intake of studied population (Lazarus, 2015). Urinary iodine generally is expressed as a concentration (μg L^{-1}), in association with creatinine excretion (μg iodine g^{-1} creatinine), or as 24-h urine samples (μg d^{-1}).

The thyroid stimulating hormone can be presented as another biomarker as, the level of circulating thyroid hormone determine serum TSH, which can reflect iodine intake, TSH in turn used as an indicator of iodine nutrition. However, in adults and older children TSH is a relatively insensitive indicator of iodine nutrition (Zimmermann, 2008). In contrast, it is used as a sensitive indicator of iodine status in the newborn period (Delange, 1997).

Measuring thyroglobulin (Tg) is another biomarker which can be used in identifying iodine status. It is the only protein which is synthesized only in the thyroid. Knudsen et al. (2001) found a good correlation between serum Tg and the severity of iodine deficiency measured by urinary iodine. It can also be determined on dried blood spots taken by a finger prick (Zimmermann et al., 2003).

The most common biomarkers to assess selenium status are blood plasma and serum (Fairweather-Tait et al., 2011). Plasma and serum selenium are associated with changes in selenium intake and commonly used in supplementation studies (Rayman, 2000). The whole blood selenium content analysed when potential of selenium toxicity wants to be studied, as it continues to increase with rising selenium intake, whilst plasma not increase above approximately 140 $\mu g\ L^{-1}$ despite elevated selenium intake level (Lemire et al., 2012). Urine, toenails and hair also have been used as a biomarker of selenium assessment (Longnecker et al., 1996). Correlations between selenium dietary intake and plasma, serum, whole blood, urine, hair, nail and breast milk concentrations have been showed (Lemire et al., 2012, Longnecker et al., 1996, Yang et al., 1989) as well as correlations between each biomarker. Using of hair and nail selenium as biomarkers of selenium status has been limited (Salbe and Levander, 1990). In general, the most common and reliable biomarkers for selenium appear to be selenium in blood (whole blood and plasma or serum) and urine (Longnecker et al., 1996, Yang et al., 1989).

1.14 Study aims

The main focus of this project is to obtain a general status of Selenium and Iodine in Kurdistan region of Iraq covering background in the environment, bioavailability to the population's diet and nutritional status.

Since the case study of this project is Kurdistan region of Iraq where very little is known about selenium and iodine status of that area. In addition no local or national data are available for these elements in the environment, humans, or local diet of residents. For instance, the last global map of iodine nutrition status showed Iraq as a no-data country (IGN, 2017). The main objectives of this project are summarized in few below:

1. To investigate background concentration of selenium and iodine in terrestrial environment (soil and irrigation water) and locally grown crops of the region in order to better understand factors controlling bioavailability of selenium and iodine in the calcareous soils of that region.
2. To evaluate selenium and iodine biofortification and studying uptake mechanism and rate of uptake by plants using local soil and seeds of the Kurdistan region and applying isotopes of ^{129}I and ^{77}Se as tracers.
3. To determine the daily dietary intake of selenium and iodine by people and dietary source apportionment of these elements using a food amount questionnaire according to local and traditional food of that region.

4. To assess iodine and selenium nutrition status using human biomarkers (urine samples) and understand possibly relations with dietary intake or other sources.

2 MATERIAL AND METHODS

This chapter provides details of the methods and analytical approaches that have been applied throughout this study. Other methods are included within the relevant chapter.

2.1 Soil preparation

Following sampling soils were air-dried and sieved to < 2 mm. A representative sub-sample of c.10 g was finely ground using an agate ball mill (Retsch; Model PM400) and stored in sealed plastic bags pending acid digestion.

2.2 Soil characterization

2.2.1 Soil pH

Soil pH was determined using a pH meter (Model pH 209, HANNA Instruments) with combined glass electrode (Ag/AgCl) after calibration with pH 4.01 and 7.00 buffer solutions at room temperature. Suspensions of 5 g air-dried <2 mm sieved soil in 12.5 mL of Milli-Q water (18.2 MΩ) were shaken for 30 minutes before measurement.

2.2.2 Soil carbon

Total soil carbon (SC) was determined using a CNS analyser (Model Flash EA1112, CE Instruments). Finely ground soil (15-20 mg) was added to tin aluminium capsules. Capsules were crimped using tweezers and the empty capsules used as a blank. Calibration was undertaken using certified soil standard B2176 (peat soil, Elemental Microanalysis) with known carbon

contents of 15.95%. Samples were introduced from a MAS200 auto-sampler into a copper oxide and electrolytic copper combustion tube at 900°C.

Soil inorganic carbon analysis (SIC) was measured using a Shimadzu TOC-VCPH connected to SSM-5000A solids module (Shimadzu UK Ltd). Approximately 100 mg (± 10 mg) of finely ground soil was weighed into a ceramic crucible with 0.7 mL of 25% H_3PO_4 before being heated at 200°C. A soil reference standard (Elemental Microanalysis, chalky soil; product number:B2188) with a known inorganic carbon content of 2.09% and calcium carbonate with an inorganic carbon content of 11.33%, were used as calibration standards. Soil organic carbon (SOC) was determined by subtracting SIC from SC.

2.3 Soil digestion

Finely ground soil (200-250 mg), 2 mL of 70% Trace Analysis Grade (TAG) nitric acid (HNO_3) and 1 mL of 60% perchloric acid ($HClO_4$) were heated on a teflon-coated graphite block digester (Model A3, Analysco, UK) at 80°C for 8 hours and then 100°C for 2 hours. The residue was then digested by addition of 2.5 mL of 70% hydrofluoric acid (HF, Fisher Scientific, UK) and 2.5 mL Mili-Q water (18.2 MΩ) and heating for 1 hour at 120°C followed by 3 hours at 140°C and finally 4 hours at 160°C before addition of a further 2.5 mL HNO_3 and 2.5 ml of Milli-Q water, and heating at 50°C for 30 minutes. The digest was then filtered and diluted to 50 mL with Milli-Q water before analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Wilson et al., 1997).

2.4 Fractionation of soil selenium and iodine

A sequential extraction approach was used to determine soluble, adsorbed and organic fractions of Se and I. Soluble soil Se and I were determined by suspending 4 g of air-dried <2 mm sieved soil in duplicate in 20 mL of 0.01M KNO_3 in 50 mL polypropylene centrifuge tubes (Fisher Scientific) after shaking for 16 hours. Samples were centrifuged at 2500 rpm for 20 minutes and the supernatant filtered (<0.22 µm). A portion (4.8 mL) was preserved with 0.2 mL of 50% HNO_3 for elemental Se analysis and a second portion (4.8 mL) was preserved with 0.2 mL of 25% Tetra Methyl Ammonium Hydroxide (TMAH) for elemental I analysis. A further portion (c. 1.8 mL) of the supernatant was pipetted into a HPLC vial and refrigerated prior to determination of Se and I speciation.

Adsorbed Se and I were extracted using 20 mL of 0.016 M KH_2PO_4 over 16h hours after carry over was accounted for by weight. After centrifugation the supernatant was preserved as described for the soluble fraction (Shetaya et al., 2012).

The final extraction step was undertaken by adding 10 mL of 10% TMAH and heating for 3 hours in an oven (with loosely capped tubes) with shaking after 1.5 hours, before centrifugation at 2500 rpm for 20 minutes (Watts and Mitchell, 2009). Carry over was again accounted for by weight. A 1:10 dilution of the supernatant was undertaken prior to elemental Se and I analysis by ICP-MS.

2.5 Plant preparation

Oven dried (40^{o}C) plant materials were finely ground in an ultra-centrifugal mill fitted with a 0.5mm titanium screen (Retch; Model ZM200, Germany).

Samples were stored in sealed plastic bags prior to acid digestion and TMAH extraction.

2.5.1 Microwave acid digestion of plant material

Approximately 0.2 g of oven-dried finely ground plant material was suspended in 6 mL of HNO_3 (TAG) and microwave digested in pressurised vessels (Anton Paar, Model Multiwave PRO, 24 vessels) for 45 minutes (1500W, 10 min ramp time, 20 min holding time at 140°C, 15 min cooling time at 55°C). For grains (wheat flour) 0.2 g of material was suspended in 3 mL HNO_3, 2 ml Of H_2O_2 (TAG, 30%: VWR) and 3 ml of Milli-Q water and digested as above. Digested samples were made to 20 mL (plants) and 15 mL (grains) using Milli-Q water and stored in universal tubes pending elemental analysis. A 1:10 dilution was undertaken with Milli-Q water immediately prior to analysis by ICP-MS (Wu et al., 1997).

2.5.2 Plant microwave TMAH extraction

Approximately 0.2 g of oven-dried finely ground plant material was suspended in 5 mL of 5% TMAH (25% w/w) and microwave extracted for 40 minutes (1500 W, 5 min ramp time, 20 min holding time at 110°C and 15 min at 50°C). The resulting solution was diluted to 25 mL using Milli-Q water, transferred to a centrifuge tube, and centrifuged at 3000 rpm for 20 minutes before filtration (0.22 μm) filters prior to analysis by ICP-MS.

The method used in this project was modified during this work in the lab according to different plant material using NIST reference material.

2.6 Analysis

2.6.1 Elemental analysis

Elemental analysis was carried out using a quadrupole-based ICP-MS (Model iCAPQ; Thermo Scientific, Bremen, Germany). Samples were introduced from an auto-sampler (Model ASX-520, Cetac, USA). Internal standards including c. 5 ppb ^{72}Ge, ^{185}Re and ^{193}Ir in 4% methanol and 2% nitric acid solution were used as internal standard for multi-element and Se analysis with the ICP-MS operated in KED mode using He as a cell gas (4.25 mL min^{-1}) for all elements other than Se. Selenium analysis utilized hydrogen as a cell gas (3.8 mL min^{-1}). An internal standard of 5ppb ^{185}Re in 1% TMAH was used as an internal standard for iodine analysis in standard mode. Multi-element standards were used (Certi-prep) for major (10, 20, 30 ppm) and trace (20, 40, 100 ppb) elements. Iodine standards were prepared from salts (KI and KIO_3). Standards for ^{77}Se and ^{129}I analysis were prepared from elemental ^{77}Se (isoflex) ^{129}I (high Technology Source Ltd, Oxfordshire).

2.6.2 Speciation analysis

Speciation analysis used chromatographic separation (Dionex; Model ICS-3000, USA) with a Hamilton PRP-x100 anion exchange column (5 μm particle size; 4.1 × 100 mm) operating in isocratic mode with a flow rate of 1.2 mL min^{-1}. The mobile phase was 50 mM NH_4NO_3, 1 × 10^{-5} mM NH_4-EDTA, 0.1 M TRIS and 4% methanol adjusted to pH = 9.4. A standard was analysed repeatedly after every 10-15 samples to enable correction of instrumental drift using linear interpolation across the intervening samples.

Peaks were manually integrated using the manufacturers Qtegra software. Figure 2.1 shows a typical example of peak integration.

2.7 Data processing

Elemental data from ICP-MS analysis were converted to μg kg^{-1}_{dw} of soil or plant according to:

$$C_{(DW)} = \frac{(C_{ICP} - C_{blank}) \times V}{W} \qquad Eq.2.1$$

Where C_{DW} is the element concentration in μg kg^{-1} dry weight, C_{ICP} is the concentration in the digest (μg L^{-1}), V is volume of the digests and W is weight of sample digested (g).

Fresh weight elemental concentrations in plants were calculated according to:

$$C_{(FW)} = C_{(DW)} \times {}^{Dry}/_{Fresh}\, Ratio \qquad Eq.2.2$$

Where C_{FW} is concentration in μg kg^{-1} fresh weight, C_{DW} is the concentration (μg kg^{-1}) dry weight and dry/fresh ratio is the plant dry weight divided by the fresh weight.

The concentrations of Se and I in the sequential extraction of the soils was determined using the following approach. For the soluble fraction:

$$C_{(F1)} = \frac{\left(C_N - C_{B(N)}\right) \times V_N}{W} \qquad Eq.2.3$$

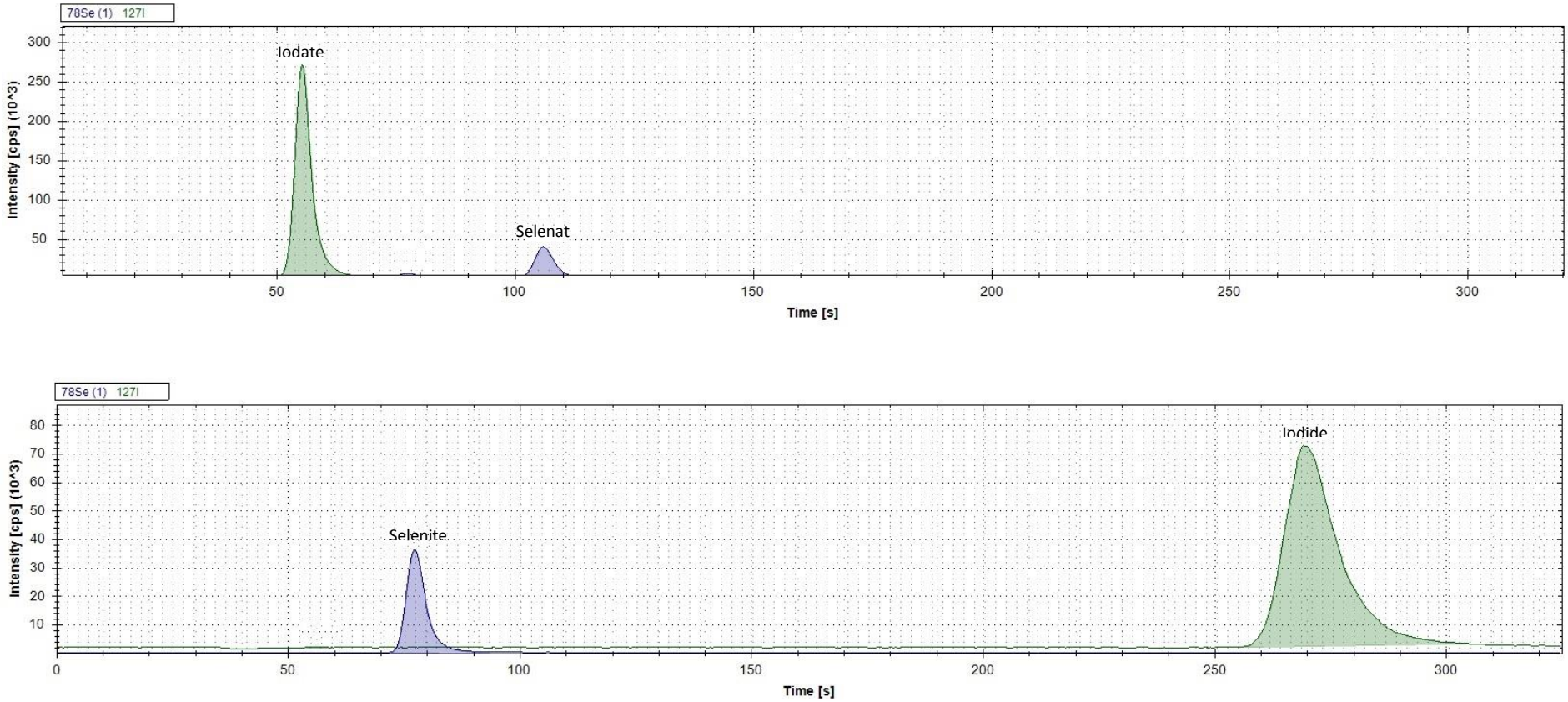


Figure 2-1. Iodine and selenium species chromatograph for standards

Where $C_{(F1)}$ is the Se or I concentration in the soluble fraction (Se_N or I_N; μg kg^{-1}_{dw} soil), C_N is total concentration of Se or I in the KNO_3 extract (μg L^{-1}), $C_{B(N)}$ is the element concentration in the blank 0.01M KNO_3 (μg L^{-1}), V_N is digest volume (mL) and W is the weight of dry soil (g).

For the adsorbed fraction:

$$C_{(F2)} = \frac{[(C_P - C_{B(P)}) \times V_P] - [(C_N - C_{B(N)}) \times V_{R(N)}]}{W} \qquad Eq.\,2.4$$

Where $C_{(F2)}$ is element concentration in the adsorbed fraction (Se_P or I_P; μg kg^{-1}_{dw} soil), C_P is the total element concentration in the KH_2PO_4 extract (μg L^{-1}), $C_{B(P)}$ is element concentration in the blank 0.016M KH_2PO_4 (μg L^{-1}), V_P is the digest volume for the adsorbed fraction added to the carryover from the soluble fraction ($V_{R(N)}$).

For the TMAH fraction:

$$C_{(F3)} = \frac{[(C_{TMAH} - C_{B(TMAH)}) \times V_{TMAH}] - [(C_P - C_{B(P)}) \times V_{R(P)}]}{W} \qquad Eq.\,2.5$$

Where $C_{(F3)}$ is element concentration in the organic-bound fraction (Se_{TMAH} or I_{TMAH}; μg kg^{-1}_{dw} soil), C_{TMAH} is the total element concentration in the TMAH extract (μg L^{-1}), $C_{B(TMAH)}$ is the element concentration in the blank 1% TMAH (μg L^{-1}), V_{TMAH} is digest volume for third fraction added to the carryover ($V_{R(p)}$). The total concentration of iodine was determined by combining all fractions (F_1+F_2+F_3) whereas for Se the total of three fraction is the TMAH extractable Se which may be less than the total soil Se concentration determined by HF digestion.

2.8 Quality control

Reference materials; Montana soil (NIST 2711), tomato leaves (NIST 1573a) and wheat flour (NIST 1567b) were used as a certified standards and digested alongside soil, plant and grain samples. Selenium recovery was 101, 98.9 and 104% for soil, tomato and wheat flour reference materials respectively. The iodine concentration in the reference materials was not certified but the mean recovery was 95% for plant materials.

All sample analysis was carried out in duplicate and in some cases triplicate. In addition, three blank was applied in each batch of samples.

2.9 Statistical analysis

Microsoft Excel 2013 and 2016 was used for basic statistical calculations such as mean, median, standard error and standard deviation. Minitab 17.2.1 and Graph Pad Prism 7.02 was used for analysis of variance; two way and one way ANOVA, Pearson correlation coefficient and other statistical analysis. A P value of <0.05 was considered as a statistically significant difference.

3 A SURVEY OF SELENIUM AND IODINE IN SOIL AND CROP PLANTS OF THE KURDISTAN REGION OF IRAQ

3.1 Introduction

Selenium and iodine are important trace elements whose deficiency or toxicity can have severe consequences for human health, as documented in many studies. Most strategies to address this issue have concentrated on direct food fortification, but crop biofortification through soil application can also be effective (Zhu et al., 2009, Hong et al., 2008, Landini et al., 2011). It is generally accepted that the soil and plant selenium concentrations are partially determined by that of the underlying parent material (Johnson et al., 2010). However, the transfer of Se from soil to the human diet is mainly determined by the availability of this element in soil and the affinity of the crop for Se (Hartikainen, 2005). Normally, soil Se is geogenic, inherited from parent material, but it can also be affected by anthropogenic inputs, such as atmospheric deposition, the use of organic and non-organic fertilizers and flooding (He et al., 2010). Iodine concentration in soil is also affected by parent material. However, because of the high concentration of iodine in marine water (c. 60 μg L^{-1}) (Linhares et al., 2015), soil I is also affected by geographic location in relation to coastal areas and prevailing wind direction. As a result, areas far away from the ocean tend to be more vulnerable to iodine deficiency if they only rely on locally produced food (Allaway, 1986). The soil properties that affect the availability of soil selenium and iodine include organic matter concentration, pH and the presence of interacting elements such as sulphur for selenium and selenium for iodine (Bevis, 2015).

A survey of soil, crop plants and water is the essential preliminary approach to investigate areas where there is little data on trace elements. In this study the Kurdistan region of Iraq has been investigated to determine the prevailing selenium and iodine status in soils, water and crop plants.

Study objectives.

(i) To obtain background information on the Se and iodine concentrations in agricultural soil, water sources, field crops and vegetables grown in Kurdistan soils and thereby assess their contribution to the Se and iodine intake of the local populations.

(ii) To investigate whether there is a relationship between Se and iodine concentration in crop plants and the concentration of these elements in their corresponding soil and water sources considering the possible role of soil parameters such as pH, soil organic carbon and other factors.

3.2 Abridged view of the Kurdistan region of Iraq

The autonomous Kurdistan Region of Iraq (40,643 km^2) consists of the three governorates of Erbil, Sulaimani and Duhok with a total population of 5.2 million (Fig 3.1). It borders Syria to the west, Iran to the east, and Turkey to the north; the capital city is Erbil. Kurdistan's topography is

dominated by plains with an altitude of less than 300 m in Erbil and the southwestern part of the Sulaimani province. From the south of Dahuk in the north to the central part of Sulaimani, the landscape consists of low hills (AAI, 2013). The average height of mountains is about 2400 m, rising to 3000-3300 m near to the border with Iran. The climate of the Kurdistan Region is semi-arid continental: very hot and dry in summer, and cold and wet in winter. Precipitation varies across the region, from 400 to 1000 mm per year in the arid south and mountainous areas in the north respectively.

Rainfall normally falls in the range of 375-724 mm. Spring season starts with mean temperatures in the range 13-18°C and season ends with 27-32°C . The summer months from June to September are very hot and dry and the hottest months have mean high temperatures of 39-43°C but can reach 50 °C in the south part of Kurdistan. Mean winter high temperatures are 7-13 °C, and mean lows are 2-7 °C.



Figure 3-1. Map of Iraqi Kurdistan and its provinces

Iraq is located in the border area between the two main Phanerozoic units of the Middle-East between the Arabian part of the African Platform and the Asian branches of the Alpine Geosyncline.

The boundary is placed on the north-eastern (Kurdistan) border of the Folded Zone. It is divided by three main thrust zones, highly folded zone and low folded zone (Fig 3.2). These areas (Kurdistan) are identified as a non-stable shelf whereas the south and south-west of Iraq is a stable shelf. The Imbricated zone is considered as being the outer miogeosynclinal unit of the Alpides. The Folded Zones are the areas where the platform cover had been folded and orogenically uplifted during the Alpine orogenesis and it is essentially an intromontane Tertiary basin. The outer unit, filled in by Cretaceous up to Miocene sediments is marked by thrust-folded structures. The northern Thrust Zone, is composed of sediments of the miogeosynclinal ridge thrust over the miogeosynclinal trough of northern Iraq. The formation is composed of dark calcareous shales with interbeds of silty marls, lentils of organic detrital limestones and of fine-grained limestones.

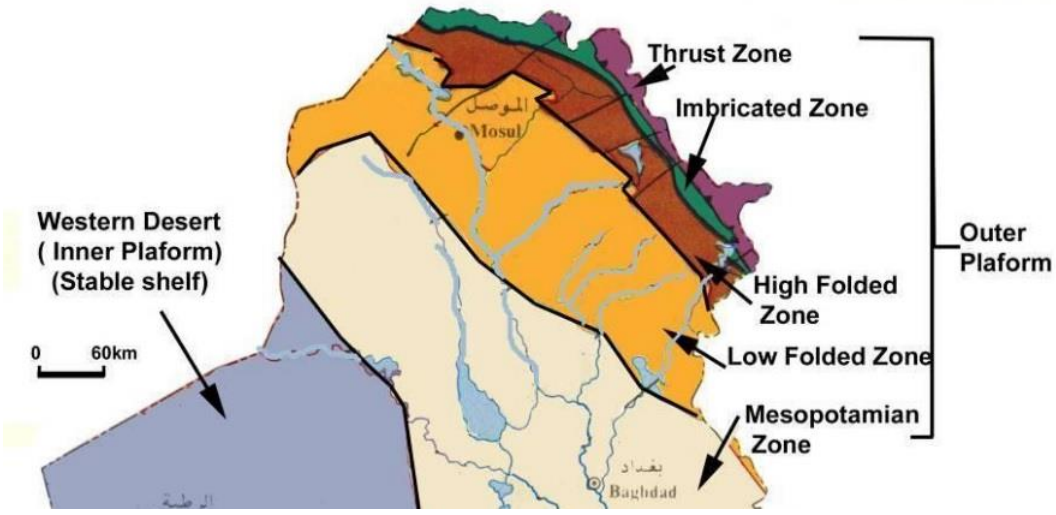


Figure 3-2. Geological Iraq map showing Kurdistan tectonic subdivision

Predominant geological formations are sedimentary in origin with the exception of some igneous rock in the thrust zone (Buday, 1980).

Kurdistan and Iraq water sources are predominantly dependent on rainfall and snow and on a system of rivers and dams, consequently water quantity is variable both within and between years (Grego, 2004). The common sources of irrigation water are spring and well and, in the urban areas, waste water is applied as an alternative due to lack of other sources (Ormizyari, 2009).

The Kurdistan agricultural situation has been changed significantly in parallel with the current political situation and its role has been severely influenced by decades of war and poor government policies. In 1975, Iraqi Kurdistan produced 45% of Iraqi wheat. After the Iraq-Iran war (1980-1988) and the Iraq-Kuwait war (1991) the 'Oil-for-Food' programme emerged which caused a reduction in indigenous agricultural activity and encouraged farmers to look for other sources of income. After 2009 the government supported the farmers by providing glasshouses and fertilizers for a short period of time (Walliser, 2010). In the last few years, because of a new war starting in 2014 and decreasing incomes, farmers re-started field-based agriculture and the production of food for local consumption. Farmers have been cultivating vegetables both in open field cultivation and also glasshouses. The main crops produced are tomato, cucumber, pepper, water melon, melon, onion, cabbage, eggplant, and most of the leafy vegetables. However current production is insufficient to meet demand and Kurdistan still depends on the importation of 40-50% of its vegetables and fruits from Jordan, Syria, Iran and Turkey.

3.3 Material and methods

3.3.1 Description of the sampling areas

A survey was conducted in Sulaimani province in April and May 2014. Four main areas were chosen in Sulaimani province shown in Fig. 3.3. The areas including Sulaimani and the surrounding area, Sharazoor plain, Halabja and Kalar. Some areas included sub areas which have been listed in Table 3.1. The choice of sampling areas and fields was based on history of vegetable planting, geographical diversity, differences in source of irrigation water and soil type and relative importance as a supplier of food crops for markets. For example, the Kalar area is known to be a major supplier of leafy vegetables in the region. The fields in each area are used to cultivate local leafy and fruit vegetables for the farmers' own use and also for commercial sale. The details of each area are specified below.

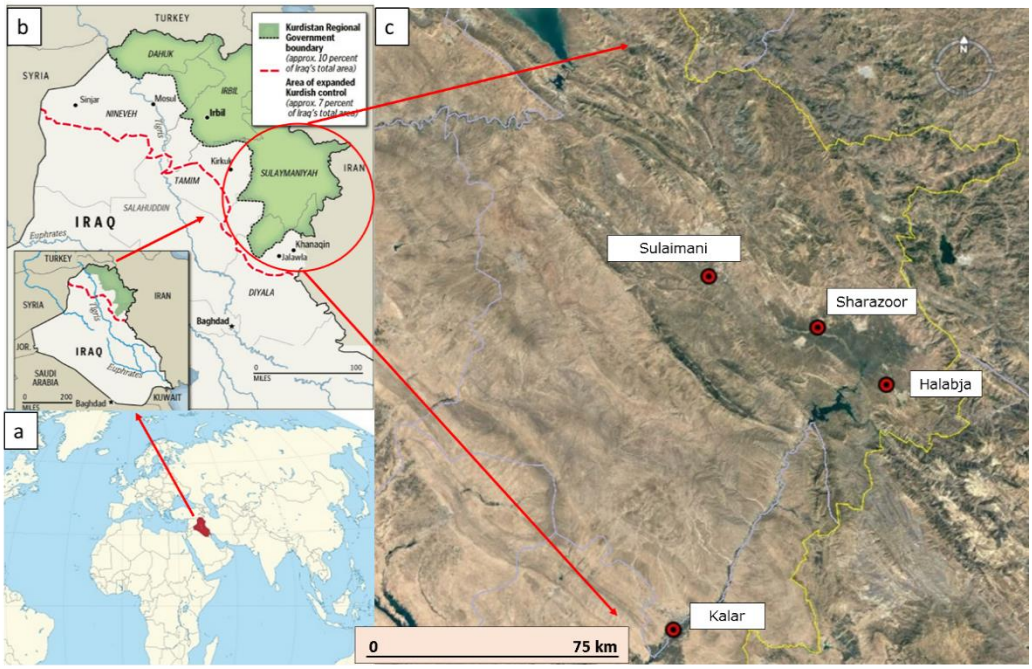


Figure 3-3. Map of Kurdistan region showing the main sampling areas

Table 3.1. The list of areas and sub-areas showing soil, plant and water samples taken from each site

Area	Sub area	Code	Soil (n)	Plant (n)	Water (n)
Sulaimani	Tanjaro	STA	15	33	2
	Baziyan	SBA	2	2	2
	Zhalla	SZH	2	2	1
	Dukan	SDU	2	6	2
	west area	SWA	3	7	
	Industrial	SID	2	10	
	Kana Sura	SKS	9	12	
	Kani Goma	SKG	6	17	
Kalar	Kalar	KAL	13	33	4
Sharazoor	Sharazoor	SHA	7	7	
	New Halabja	N-HAL	1	7	1
Halabja	Kani Kolka	HKK	13	31	
	Anab	HAN	8	24	
	Kani Spi	HKS	5	12	
	Bakrawa	HBA	4	11	3
	Zamaqi	HZA	2	8	2
	Ka Grdal	HKG	1	8	1
	Bia Wella	HBI	1	7	1
	Khormal	HKH	1	27	1

3.3.1.1 Sulaimani

The soil parent material of this area is late cretaceous. The bedrocks are silty marl, siltstone, sandstone, conglomerate and sandy or silty organic detrital limestone with thicknesses of 500 m (Karim, 2000). Based on the information provided in the FAO soil map (Berding, 2001), the land form is that of a valley plain with low hills including small alluvial plains and depression areas. The parent material is non-gravelly and gravelly deposits in tertiary and quaternary terraces with conglomerate layers in more recent deposits (Holocene) in alluvial plains. The dominant soils of this area are deep, well drained, lime-rich non-gravely and gravely silty clay to clay with surface rocks, lime-rich silty clay loam to clay. The soil types are mainly Kastanozems, Vertisols, Chronixc and Calcic soils (FAO classification).

The sub-areas of Sulaimani including Tanjaro (STA), the Industrial area (SID), the West disposal area (SWA), Kana sura (SKS), Kani goma (SKG), Bazian (SBA) and Zhalla (SZH) are located from 3-15 km distance from the

city center (Fig 3.4). The Tanjaro site is located in the south of the city. There are some vegetable fields close to the Tanjaro river where river water is utilized as irrigation water. The Tanjaro river conveys a large proportion of household waste water to remote areas. In the spring season, leached fertilizer from farms upstream is transported by the river and deposited within downstream alluvial soils. The sites of Bazian (SBA) and Zhalla (SZH) are located approximately 15 km west and 10 km east of the city respectively. These sites are predominantly used to cultivate fruit vegetables such as cucumber, tomato and peppers in soil-based glasshouses.

Kana sura (SKS) and Kani goma (SKG) are rural areas near the city in which farmers cultivate leafy and fruit vegetables to meet local market requirements. The last site was Dukan which is located 75 km to the north west of the city. As there were not enough vegetable farms in this location a few samples were taken from local farms.

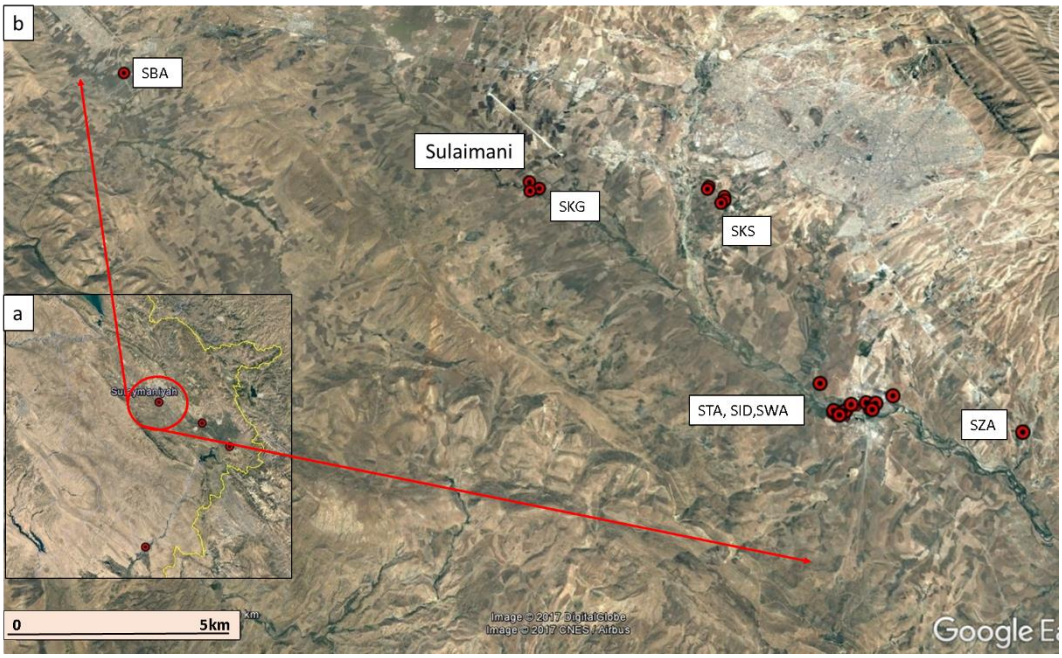


Figure 3-4. Sulaimani area showing subareas and sampling fields

3.3.1.2 Kalar

The Kalar area is in the south-east part of Iraqi Kurdistan, located 150 km south of Sulaimani. The geological characteristics of this area indicates that the age of this area is quaternary (Holocene) and most of the bedrock is conglomerate with flood plain deposits in some areas and gypsum (Barwary, 1993). Dominant soils are well drained lime-rich non-gravely silty-clay loam to silty-clay with surface cracks and gravelly to very gravelly silty loam. Soils are generally Gypsiferous and are classified as Calcisols, Leptosolsl, Gypsosols and Vertisols (Berding, 2001). The rural farms belonging to this city are in the Grda Gozinah area which is familiar to all people in the region as a major source of a wide variety of leafy and fruit vegetables during the spring and summer. This area provides a high proportion of vegetables consumed in the entire Sulaimani province. Fourteen farms were selected

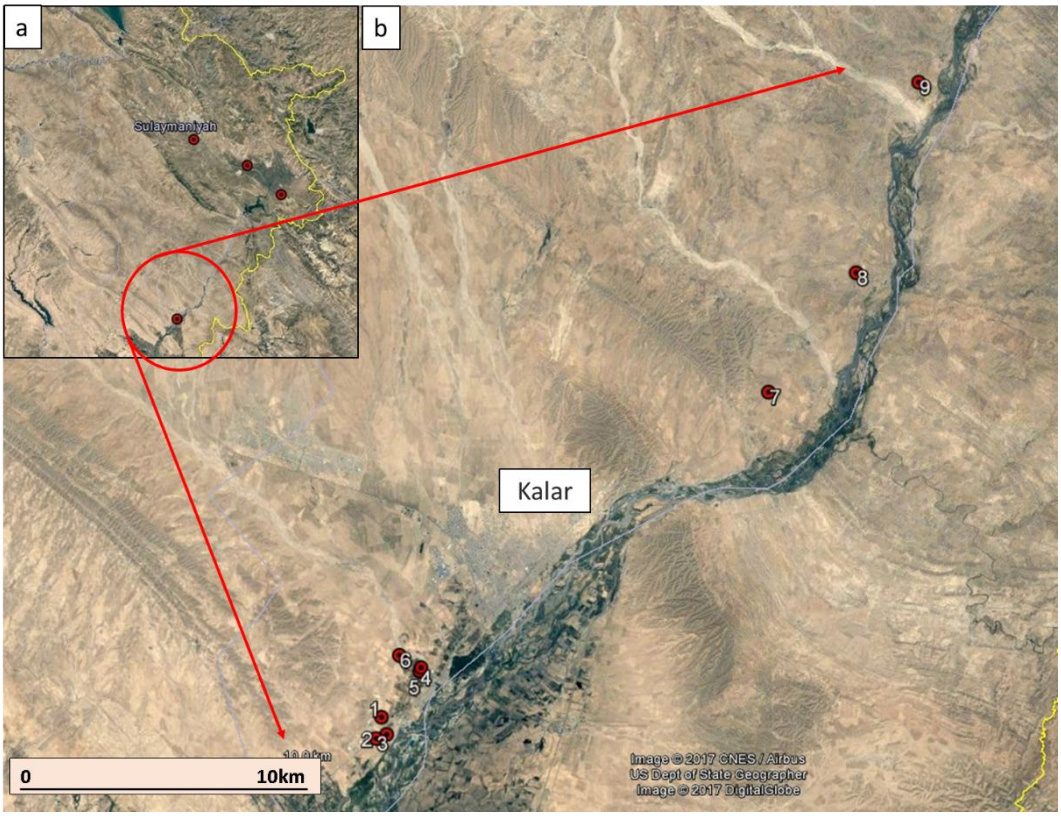


Figure 3-5. Kalar area showing vegetable farms (No. 1-6) and wheat farms (No. 7-9)

in this area, including six vegetable fields and eight fields for wheat sampling as indicated in (Fig. 3.5).

3.3.1.3 Sharazoor

This area is an extensive plain located between the Halabja and Sulaimani areas (Fig. 3.3). The parent material of this area is varied and includes river terraces and a mixture of clay and sand in south of the area. The north is more similar to the Sulaimani area, containing silty marl, siltstone, sandstone, conglomerate and limestone deposits and, in the northern part, there is black shale although some parts of these areas are not arable (Karim, 2000). Dominant soils are Calcisols, Vertisols, Leptosols, Rendzinas, Kastanozems and possibly Luvic soils (Berding, 2001).

In rural areas, vegetables are planted but this is less common than in the Kalar area and production is insufficient to supply all local markets as most of the farms are utilized for wheat under rain-fed farming. The vegetable

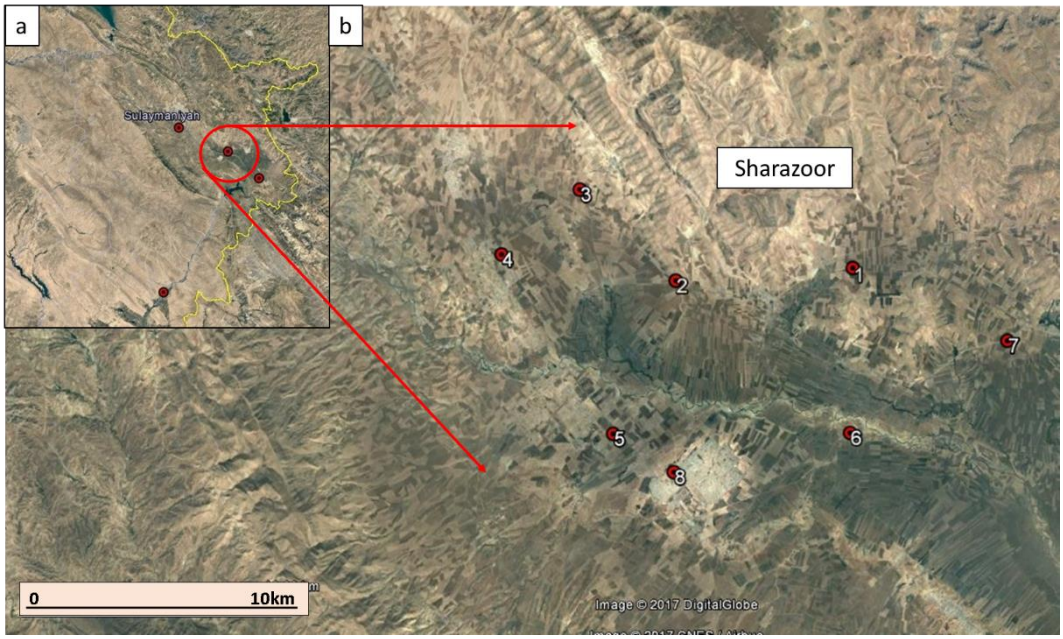


Figure 3-6. a) Sulaimani province map showing b) Sharazoor area

samples were collected from New Halabja city (No. 8 in Fig. 3.6) in this area and the wheat samples were collected from other fields.

3.3.1.4 Halabja

The Halabja area is located near to the border of Iran and ranges from the Zagros mountains to the south east of Iran. The bedrock and parent material are different from those of Halabja city and the surrounding area is predominantly river terraces and recent alluvial flood plain deposits. The north and north-west areas contain conglomerate, clay, sandstone and, in some parts, dolomite, shale and marl are found. The soils of these area vary from shallow to moderately deep, excessively to moderately well-drained soils with variable texture and gravel content, loamy to clayey soils, lime-rich non gravelly and gravelly silty clay with surface cracks and self-mulching Vertisols can be found in Northern areas (Berding, 2001).

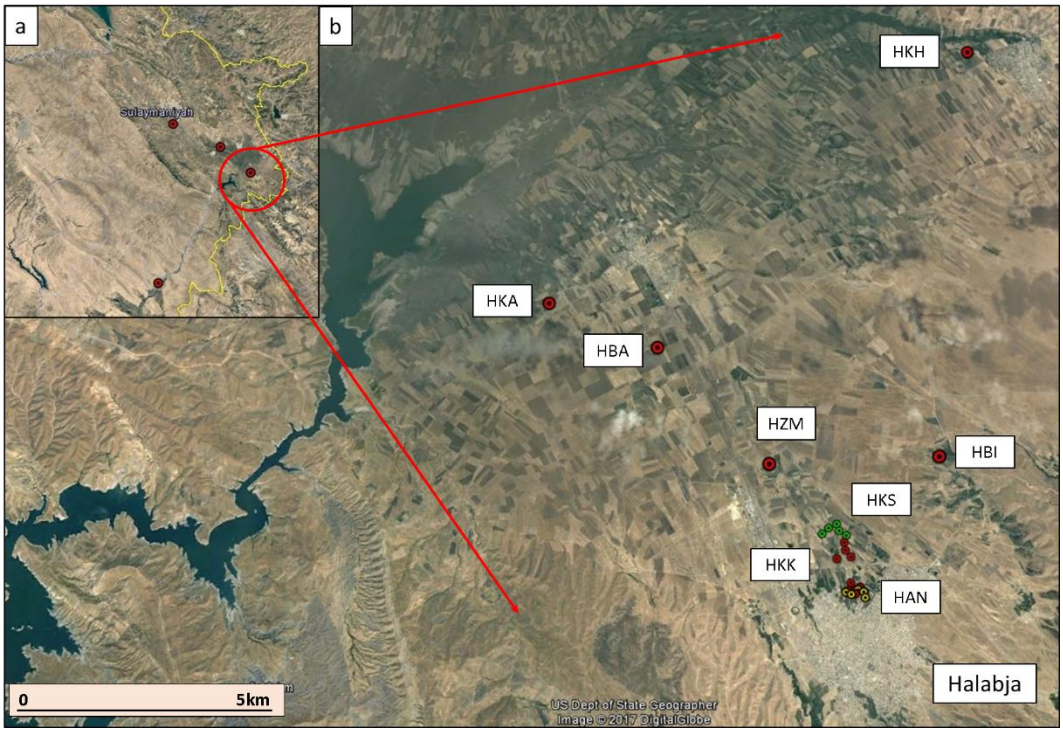


Figure 3-7. a) Sulaimani province map b) Halabja area and sub areas

Sampling was carried out in the eight sub-areas, identified in (Fig. 3.7). The sub-areas of Kani Kolka (HKK) and Anab (HAN) can be designated as peri-urban areas. The other sub-areas, Kani spi (HKS), Biawella (HBI), Zamaqi (HZM), Bakrawa (HBA), Ka Grdal (HKA), Khormal (HKH) are in rural locations. All the vegetables grown in these sub-areas were used to provide a part of the local population's dietary requirement. However, production is insufficient to meet demand and leafy and fruit vegetables are still supplied from other areas, such as Kalar.

3.3.2 Soil sampling

In most of the chosen sites (97), matching soil and plant samples were collected and, where possible, water sampling was carried out also. In each field selected, nine small soil samples (0-20 cm) were taken along a 'W-transect', using a clean stainless steel trowel, and mixed to make a composite sample. In the laboratory a 1 kg representative sample was then air-dried in aluminium trays at room temperature, disaggregated gently using a plastic hammer, sieved to <2mm, homogenized and stored in labelled bags. A representative sample was then taken and stored in a universal tube (30 g), double labelled and placed in double plastic bags (with other samples) for transport to the University of Nottingham, UK. A second set of labelled samples were retained in Kurdistan in case they were required later.

3.3.3 Plant sampling

Plant samples were classed as 'leafy vegetables' and 'non-leafy vegetables' (fruit vegetables), tubers and grains. After harvesting each vegetable, samples were placed in clean, labelled paper bags and delivered to the laboratory on the day of sampling. The edible parts were then separated, cut up in the case of large leaves and fruit vegetables such as chard and cucumber, peeled in the case of bulbs such as garlic and onion, and placed in aluminium trays with paper underneath. Fresh weight was recorded and the samples were oven dried (45-50˚C to avoid iodine volatilization) and weighed again before being stored in labelled bags. All plant samples were placed in separate labelled paper bags for transport to the University of Nottingham for further sample processing and analysis. The wheat grain samples were taken following the same pattern as for soil sampling: 9 samples in a W-transect with each sample comprising 2-3 heads of wheat. The straw was separated (already air dry), mixed, and a representative sample taken. Finally, a subsample of 20 grams was stored in tubes, wrapped in plastic bags for transport to the UK.

3.3.4 Water sampling

Two water samples were taken and labelled according to the location of the vegetables and soils which received water from that irrigation source. Both water samples were immediately filtered in the field using 0.22 µm cellulose acetate syringe filters into polyethylene 25 ml capacity tubes: one containing 0.5 mL of 50% HNO_3 and another one with no added acid. Samples were stored in a fridge pending delivery to the UK. All processes are shown in brief in (Fig. 3.8) as a schematic diagram.

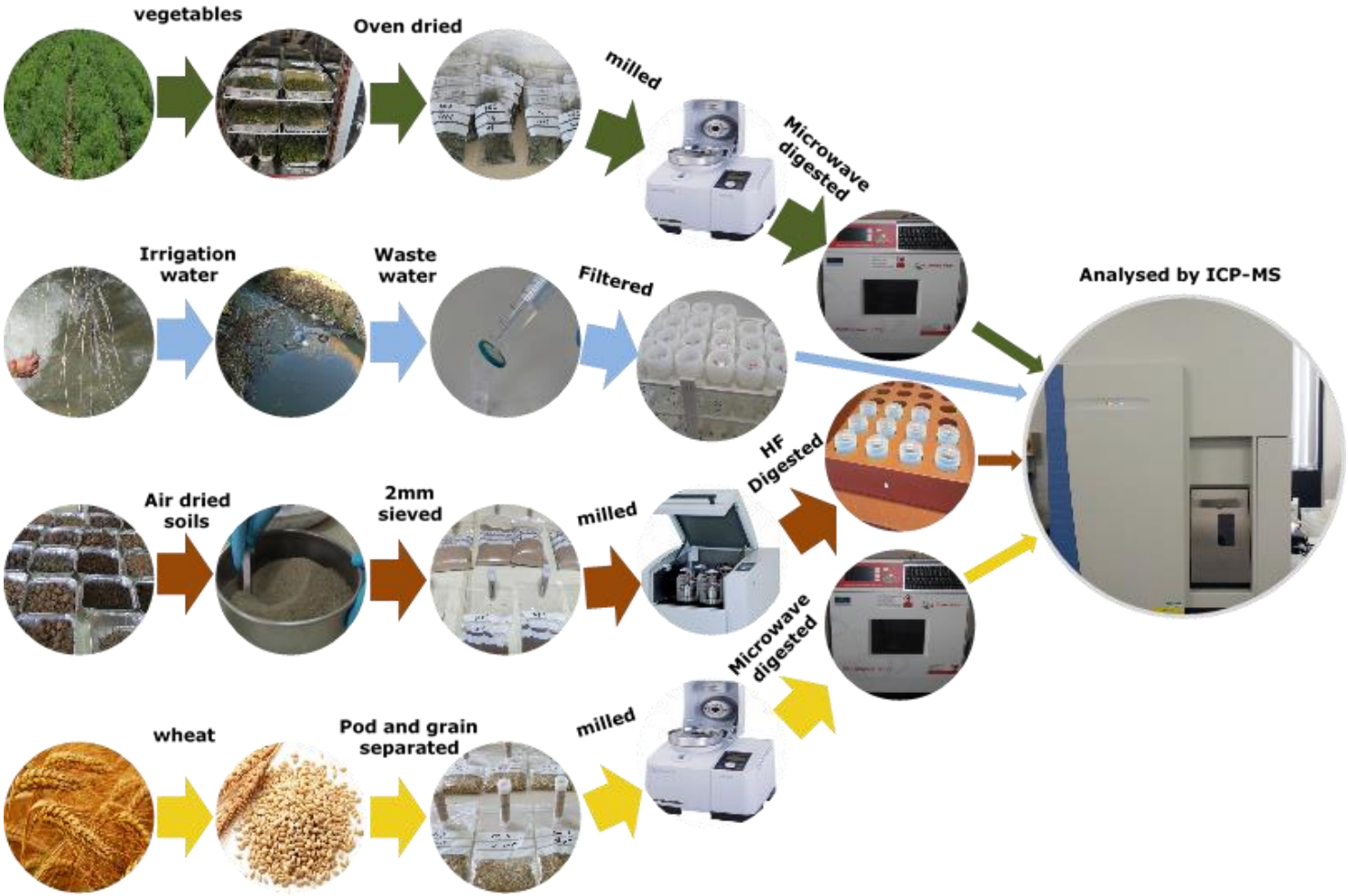


Figure 3-8. Schematic diagram showing sample preparation pending analysis

3.4 Results and Discussion

3.4.1 Soil characteristics

Table 3.2 provides details of key soil characteristics, including soil pH, concentrations of inorganic and organic carbon and calcium carbonate and the proportion (%) of total soil Ca presented as a calcium carbonate in all areas and sub-area sites. Kurdistan is known to have mainly calcareous soils and there was no significant difference in soil pH between the four studied areas ($P > 0.05$). Soil pH ranged from 7.10 to 8.35 across all the sites. The average of sub-areas' soil pH within the four main areas demonstrates that they have relatively uniform soil pH (7.82-7.95 n=97) (Fig. 3.9a).

Soil organic carbon concentrations fell in the range of 0.37% to 4.69% in Sulaimani-Zhalla (SZH site) and Halabja-Kanikolka (HKK site) respectively; the mean value for all sites was 1.85% (n=97). The SZH site is located in Sulaimani where limestone and sandstones are the main parent materials but this particular site is also near the main road which has been affected by anthropogenic activities. The HKK site is located in the Halabja area and is irrigated with wastewater which may explain the higher soil organic carbon content (Table 3.2). The Halabja area had highest values of OC% compared to other three areas (mean= 2.32%; n=35). The Sharazoor and Sulaimani areas had the lowest OC% values of 1.50 and 1.57% respectively (Fig. 3.9b).

The HKH and SWA sites had the lowest and highest inorganic carbon contents 0.15% and 4.17% respectively. The Sulaimani and Kalar areas had higher inorganic carbon concentrations (3.05%, n=41 and 3.08%, n=13) compared to Halabja and Sharazoor (1.93%, n=35 and 1.85%, n=8).

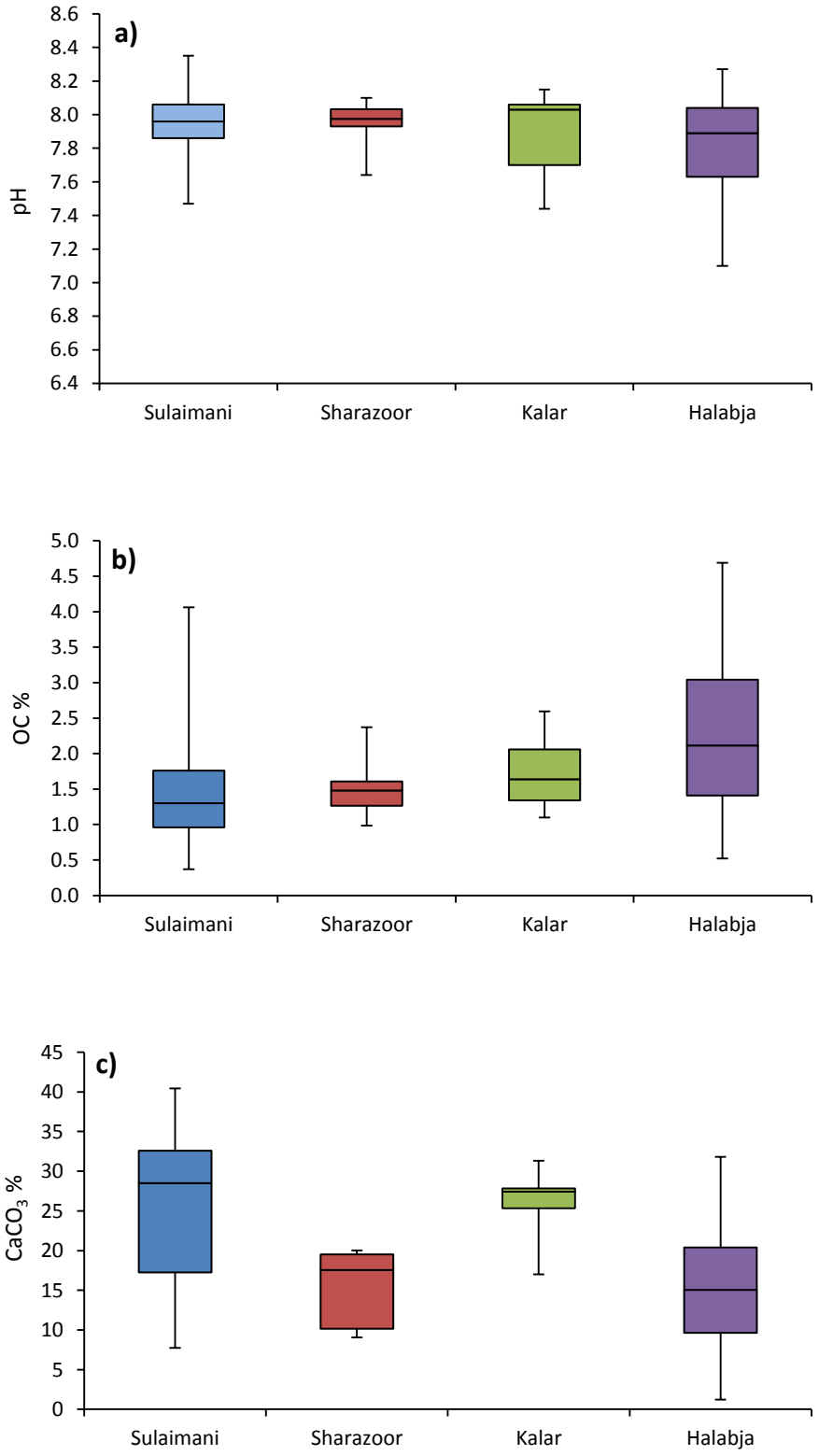


Figure 3-9 . Distribution of soil characteristics in different areas a) pH b) oganic carbon, c) carbonate calcium content (%)

Table 3.2. Basic soil characteristics of the four main areas: a)Sulaimani, b) Sharazoor, c) Kalar, d) Halabja including subareas and sample locations.

a) Sulaimani

Sub areas	Area Code	pH	SIC	SOC	$CaCO_3$[a]	Ca_{CaCo3}[b]	Soil Ca[c]	Ca[d]
			%	%	%	%	%	%
	STA 1	8.12	1.76	3.39	14.7	5.87	11.4	51.3
	STA 2	8.03	1.77	3.28	14.8	5.900	11.2	52.6
	STA 3	7.96	0.929	4.061	7.74	3.097	10.96	28.3
	STA 4	7.96	1.17	3.79	9.75	3.900	10.6	36.9
	STA 5	8.16	1.048	3.89	8.73	3.49	10.1	34.5
	STA 6	7.9	3.32	1.59	27.7	11.1	11.02	100
	STA 7	8.12	4.85	1.17	40.4	16.2	15.6	103
Tanjaro	STA 8	8.21	4.48	1.39	37.3	14.9	14.6	102
	STA 9	8.12	3.23	1.29	26.9	10.8	10.6	102
	STA 10	8.12	3.42	1.103	28.5	11.4	10.9	105
	STA 11	7.98	3.56	1.34	29.7	11.9	11.7	101
	STA 12	7.5	3.53	0.963	29.4	11.8	12.7	92.4
	STA 13	7.86	2.95	1.68	24.6	9.83	10.7	91.6
	STA 14	7.65	3.89	1.078	32.4	12.97	12.5	104
	STA 15	8.05	3.91	1.204	32.6	13.03	12.9	101
Baziyan	SBY 1	7.54	2.03	1.59	16.9	6.77	12.7	53.1
	SBY 2	7.47	1.57	1.89	13.1	5.23	10.7	48.7
Zhalla	SZH 1G	7.5	2.902	0.371	24.2	9.67	12.5	77.2
	SZH 2	8.06	3.701	0.758	30.8	12.3	12.9	95.6
Dukan	DUK 1	8.01	2.58	1.025	21.5	8.60	7.77	111
	DUK 2G	7.49	2.47	2.58	20.6	8.23	6.56	125
	SWA 1	8.01	3.88	0.894	32.3	12.9	11.8	110
SUL -west area	SWA 2	7.7	4.097	0.925	34.1	13.7	11.9	115
	SWA 3	7.97	4.54	1.032	37.8	15.1	13.5	112
	SID 1	8.05	3.99	0.662	33.3	13.3	11.6	114
Industrial	SID 2	7.86	3.78	1.011	31.5	12.6	11.1	113
	SKS 1	7.87	2.82	1.301	23.5	9.40	8.78	107
	SKS 2	7.94	1.026	1.78	8.55	3.42	6.64	51.5
	SKS 3	7.77	1.36	1.52	11.3	4.53	5.02	90.4
	SKS 4	7.55	1.74	1.92	14.5	5.80	6.00	96.7
Kana Sura	SKS 5	7.88	2.068	1.14	17.2	6.89	6.96	99.1
	SKS 6	7.91	4.13	0.674	34.4	13.8	12.7	108
	SKS 7	7.96	2.57	1.702	21.4	8.57	8.58	100
	SKS 8	7.94	2.65	1.51	22.1	8.83	8.48	104
	SKS 9	7.92	3.58	1.77	29.8	11.9	13.7	86.8
	SKG 1	8.02	4.052	0.848	33.8	13.5	11.9	114
	SKG 2	8.01	3.79	0.927	31.6	12.6	11.5	110
Kani Goma	SKG 3	8.27	3.96	1.76	33.0	13.2	11.9	111
	SKG 4	8.17	3.83	0.763	31.9	12.8	11.8	109
	SKG 5	8.35	4.025	1.72	33.5	13.4	11.9	113
	SKG 6	8.23	4.065	0.872	33.9	13.6	11.5	118

a) Calculated based on soil in-organic carbon b) soil Ca present as calcium carbonate c) total soil calcium analysed by ICP-MS d) percentage of soil Ca present in calcium carbonate

b) Sharazoor

Sub areas	Area Code	pH	SIC	SOC	$CaCO_3$	Ca_{CaCo3}	Soil Ca	Ca
			%	%	%	%	%	%
	SHA 1	8.03	1.09	1.72	9.064	3.63	5.24	69.2
	SHA 2	7.97	2.25	1.57	18.72	7.49	10.25	73.0
	SHA 3	7.96	1.97	1.39	16.39	6.55	9.13	71.8
Sharazoor	SHA 4	7.64	1.14	1.095	9.49	3.796	6.45	58.9
	SHA 5	7.98	2.38	1.32	19.83	7.93	10.36	76.5
	SHA 6	8.04	2.40	1.57	20.02	8.01	10.06	79.6
	SHA 7	8.10	1.25	0.986	10.38	4.15	6.32	65.6
New Halabja	SHA 8	7.84	2.33	2.37	19.42	7.77	9.81	79.1

c) Kalar

Sub areas	Area Code	pH	SIC	SOC	$CaCO_3$	Ca_{CaCo3}	Soil Ca	Ca
			%	%	%	%	%	%
	KAL 1	7.44	3.49	2.59	29.07	11.63	13.16	88.4
	KAL 2	7.70	3.76	1.34	31.31	12.52	13.66	91.7
	KAL 3 W	8.06	3.29	1.198	27.42	10.97	13.38	82.0
	KAL 3	8.06	3.307	1.48	27.56	11.02	13.82	79.7
	KAL4a	7.88	3.33	1.64	27.74	11.10	12.32	90.1
	KAL4b	7.67	3.26	2.06	27.20	10.88	12.38	87.9
Kalar	KAL4c	7.87	3.59	1.94	29.92	11.97	13.13	91.2
	KAL 5G	7.70	3.040	2.089	25.33	10.13	11.76	86.2
	Kal 6	8.03	3.16	2.44	26.34	10.54	13.03	80.8
	KAL 6W	8.03	3.34	1.64	27.83	11.13	14.27	78.0
	KAL 7	8.06	2.040	1.35	17.00	6.801	9.602	70.8
	KAL 8	8.10	2.18	1.32	18.13	7.25	9.900	73.2
	KAL 9	8.15	2.31	1.098	19.25	7.70	9.826	78.4

d) Halabja

Sub areas	Area Code	pH	SIC	SOC	$CaCO_3$	Ca_{CaCo3}	Soil Ca	Ca
			%	%	%	%	%	%
	HKK 1	7.38	1.22	4.69	10.17	4.068	5.27	77.3
	HKK 2	7.38	1.87	4.09	15.6	6.23	5.043	124
	HKK 3	7.74	1.88	1.87	15.6	6.26	3.18	197
	HKK 4	7.42	1.002	3.75	8.35	3.34	3.72	89.9
	HKK 5	8.01	1.17	2.11	9.73	3.89	3.44	113
	HKK 6	7.52	1.40	3.397	11.6	4.65	3.78	123
Kani Kolka	HKK 7	8.05	1.14	1.398	9.54	3.82	3.29	116
	HKK 8	8	2.34	2.45	19.5	7.81	5.97	131
	HKK 9	8.02	2.02	1.91	16.8	6.73	5.28	127
	HKK 10	7.6	0.930	3.07	7.75	3.100	2.82	110
	HKK 11	7.1	0.817	2.90	6.81	2.72	2.76	98.6
	HKK 12	8.02	2.39	1.28	19.95	7.98	5.98	133
	HKK 13	7.52	2.33	3.22	19.5	7.78	9.101	85.5
	HAN 1	8.13	3.82	1.93	31.8	12.7	10.10	126
	HAN 2	8.27	3.69	2.28	30.73	12.3	9.200	134
	HAN 3	8.13	3.81	3.502	31.7	12.7	9.69	131
Anab	HAN 4	8.06	3.75	1.81	31.2	12.5	9.502	131
	HAN 5	8.09	1.70	4.19	14.2	5.67	5.26	108
	HAN 6	8.07	3.13	1.99	26.05	10.4	7.11	147
	HAN 7	8.08	3.30	0.588	27.5	10.98	9.98	110
	HAN 8	8.03	2.82	2.58	23.5	9.41	9.046	104
	HKS 1	7.89	1.81	3.15	15.06	6.023	6.93	86.9
	HKS 2	7.71	1.79	2.91	14.9	5.95	6.040	98.5
Kani Spi	HKS 3	7.43	1.87	3.015	15.6	6.23	6.36	97.9
	HKS 4	7.90	1.78	1.046	14.8	5.94	6.196	95.8
	HKS 5	7.54	1.45	2.28	12.08	4.83	5.37	89.98
	HBK 1	8.01	0.452	1.42	3.77	1.51	3.15	47.8
Bakrawa	HBK 2	7.73	0.905	1.39	7.54	3.018	4.76	63.3
	HBK 3	7.94	0.680	1.15	5.67	2.27	3.95	57.4
	HBK 4	7.75	0.782	0.521	6.52	2.61	4.33	60.2
Zamaqi	HZM 1	7.74	1.79	2.037	14.9	5.95	8.59	69.3
	HZM 2	7.86	1.99	1.84	16.6	6.63	8.84	75.02
Ka Grdal	HKG	7.66	2.50	2.98	20.84	8.34	10.55	79.03
Bia Wella	HBW1	8.09	3.12	1.205	25.97	10.39	12.5	83.1
Khormal	HKH	7.80	0.148	1.25	1.23	0.493	1.79	27.6

The amount of soil calcium carbonate was estimated by multiplying soil inorganic carbon (SIC) by 100/12 ratio assuming all inorganic carbon was present as calcium carbonate in these calcareous soils. Soil $CaCO_3$ content varied from 1.2 to 40% for HKH and STA sites respectively (Table 3.2). Soil calcium carbonate contents up to 40% is consistent with the limestone parent material and the incidence of Sulaimani Calcisols. Figure 3.9c demonstrates that Kalar and Sulaimani have higher calcium carbonate contents (25.7%, n=13 and 25.4, n=41) compared to the Halabja and Sharazoor areas (16.09%, n=35 and 15.4%, n=8).

The proportion of soil calcium present as soil calcium carbonate (Ca_{CaCO3}) was calculated by multiplying the estimated soil calcium carbonate content by 0.4 and dividing by the total soil calcium concentration. The value ranged from 27% for the HKH site to 100% in many of sites with mean values of 93%, 72%, 83% and 99% in Sulaimani, Sharazoor, Kalar and Halabja respectively.

3.4.2 Soil multi-element analysis

The concentrations of major and trace elements are presented in appendix 1. Calcium was the dominant cation, reflecting the calcareous nature of the soils, with a range of 17900 to 156000 mg kg^{-1} (mean=92200 mg kg^{-1}, n=97). Concentrations of other major elements, including Al, Mg and K, fell in the range of calcareous soils reported for other areas (Iqbal and Shah, 2011; Nazif et al., 2015)

3.4.3 Soil selenium

3.4.3.1 Total soil selenium concentration

Soil selenium concentrations (Se_{Tot}) fell in the range of 165 to 617 µg kg^{-1} across all the studied sites and the mean concentration was 309 µg kg^{-1} (n=97). The Halabja area had the highest selenium concentrations, which ranged from 0.267 to 0.617 µg kg^{-1} (mean 419 µg kg^{-1}, n=35), broadly in line with the global selenium average of 400 µg kg^{-1} (Fordyce, 2005). The average concentrations of the other three areas were lower, and not significantly different, with 235, 245 and 269 µg kg^{-1} for Kalar, Sharazoor and Sulaimani areas respectively (Fig 3.10). Based on a lithostratigraphic map of the Kurdistan region the samples collected from the Halabja area were distributed between 5 different types of parent material including black shale. The presence of the latter parent material may be the reason for a higher selenium concentration in this area (Karim, 2000). Soil Se concentrations of each site are presented in appendix 1.

The average of Kurdistan Se_{Tot} (309 µg kg^{-1}) is less than the world Se average. In content of reported Se concentrationsw from other countries, Kurdistan can be classified as a Se deficient area in terms of soil Se_{Tot} content. For instance, in China, Se deficient areas are reported to have Se concentrations ranging from 4 to 480 µg kg^{-1} while the 'adequate areas' for the same country ranged from 730 to 5660 µg kg^{-1}. Similar values for India and Sri lanka Se-deficient areas were reported in the range of 25 to 710 and 112 to 5240 µg kg^{-1} respectively (Fordyce, 2005). De Temmerman et al.

(2014) reported low Se concentrations in Belgian soils (n = 539) ranging from 140 to 610 µg kg^{-1} which is very similar to the range for Kurdistan soils. Similarly, Pérez-Sirvent et al. (2010) investigated the Murcia region of Spain which has similar soil properties to those of Kurdistan. The region was characterised partly by limestones and marl and so contained carbonate-rich soils. They found an average Se concentration of 370 µg kg^{-1} in their survey of 490 soils. In most cases there is a strong correlation between the Se concentration of geological parent material and overlying soils (Fordyce, 2005).

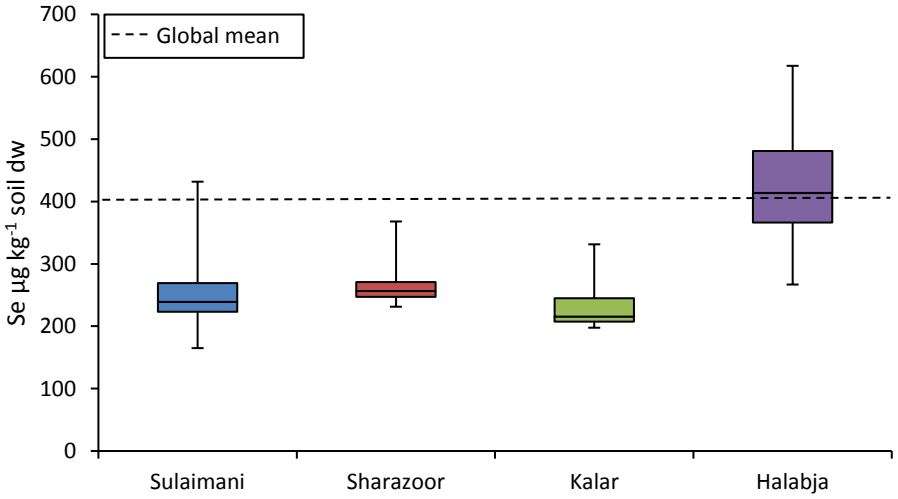


Figure 3-10. Distribution of soil total selenium in each area compared to the global range of soil Se concentration Sulaimani; n=41, Sharazoor; n=8, Kalar; n=13, Halabja; n= 35.

3.4.3.2 Fractionation and extractability of soil Se

In common with most trace elements, Se in soil can be 'fractionated' using a sequential fractionation approach. Typically, such schemes differentiate

between soluble, adsorbed, and organic-bound and residual fractions of Se (Zhang and Frankenberger, 2003, Tolu et al., 2011).

The sequential extraction was carried out for all soil samples (n=97) using 0.01M potassium nitrate (soluble Se, Se_N), 0.016M potassium dihydrogen phosphate (adsorbed Se, Se_P) and 10% TMAH (organic-bound Se, Se_{TMAH}).

Soluble Se concentrations across all sites ranged from 0.42 to 8.34 μg kg^{-1}. The highest value of Se_N was found in the Kalar area which ranged from 1.02 to 5.22 μg kg^{-1} with a mean concentration of 2.57 μg kg^{-1} (n=13). The lowest values were found in the Sharazoor area with 0.65 to 3.12 μg kg^{-1} (mean value = 1.18 μg kg^{-1}; n=8). The Sulaimani and Halabja areas had mean Se_N values of 2.09 and 1.59 μg kg^{-1} respectively (Fig. 3.11a).

The concentration of Se_N accounted for 0.096-2.18% of soil Se_{Tot} content (%Se_N extractable) with an average value of 0.69% (n=97); approximately 90% of the soils had <1% soluble Se as a proportion of soil Se_{Tot} content. The Sulaimani and Kalar soils had higher extractability (average values 1.09 and 0.86%) compared to those from Halabja and Sharazoor (0.4 and 0.45%) (Table 3.3). Similar recoveries of soluble Se have been observed: Keskinen et al. (2009) studied the efficiency of different methods of extracting Se and calculated a typical recovery of 1% for soluble Se. Wang et al. (2012) undertook similar extractions for 20 soils in China and reported recovery of 0.3% for soluble Se.

Selenium in the adsorbed fraction extractable by phosphate (Se_P), possibly originating from Se bound to Al and Fe oxides, showing slightly greater values than Se_N; values ranged from 1.51 to 12.1 μg kg^{-1}. The Halabja area had the greatest mean value (7.32 μg kg^{-1}) with a range from 2.30 to 12.1 μg kg^{-1}

whereas the other areas had fairly similar average values of 4.50, 4.64 and 4.77 µg kg^{-1} for Sharazoor,Kalar and Sulaimani areas respectively (Fig. 3.11b). Values of $\%Se_P$ ranged from 0.69 to 3.46% with average values that were relatively constant across the four areas (overall mean=1.88%, n=97) Table (3.3). In a study of UK agricultural soils, Stroud et al. (2010) found recoveries of <1-6% for Se_P. Similarly, Chilimba et al. (2011) determined Se_P for 81 Malawi soils and reported <1-8% for (Se_P). By contrast, Schilling et al. (2014) reported much higher recovery rates of 12-26% for Indian high Se content soils using potassium phosphate extraction.

The Se extracted by 10% TMAH (Se_{TMAH}), representing organic-bound Se, showed a wide range of Se concentrations from 33.4 to 340 µg kg^{-1} with a mean value of 108 µg kg^{-1} (n=97). The greatest average concentration was found in the Halabja area (161 µg kg^{-1}, n=35) while the other three areas had similar mean concentrations of 108, 103 and 101 µg kg^{-1} for Sulaimani, Sharazoor and Kalar respectively (Fig. 3.11c).

The extractability of Se_{TMAH}, as a proportion of Se_{Tot}, covered a relatively wide range of 20.3 to 89% (mean value = 41%; n=97). The average values of $\%Se_{TMAH}$ for Sharazoor and Halabja (38.9%; n=8 and 39.1 %; n=35) were slightly lower than those of Kalar and Sulaimani (42.3%; n=13 and 43.4%; n=41) respectively (Table 3.3). The majority of soils (90%) had a Se_{TMAH} recovery less than 50% of Se_{Tot}. In a study by Supriatin et al. (2015) they applied an alkaline and oxidising extraction (NaOH and NaOCl) to recover 'recalcitrant' Se pools from arable Dutch soils (pH≤6) and observed 55-78% recovery of Se_{Tot}.

Table 3.3. Selenium extractability as percentage of total soil Se

Area	Sub area	n	Se_N extractable			Se_P extractable			Se_{TMAH} extractable		
			Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
			%								
Sulaimani	*Tanjaro*	15	0.291	1.16	0.681	1.00	3.46	2.43	35.7	58.2	45.1
	Baziyan	2	0.980	1.070	1.025	1.55	2.29	1.92	35.2	50.6	42.9
	Zhalla	2	0.546	2.12	1.33	0.915	2.17	1.54	20.3	43.5	31.9
	Dukan	2	1.62	2.18	1.901	1.21	2.26	1.73	67.7	89.0	78.4
	west area	3	0.430	1.13	0.709	2.39	2.49	2.43	38.6	42.9	40.3
	Industrial	2	0.870	1.24	1.056	2.42	2.16	2.29	38.2	35.8	36.9
	KanaSura	9	0.122	1.87	0.775	0.689	1.81	1.22	22.1	52.4	41.5
	KaniGoma	6	0.599	1.041	0.853	1.51	2.00	1.67	27.9	44.7	38.0
Halabja	*Kani Kolka*	13	0.096	0.428	0.226	0.436	1.81	1.15	20.9	43.8	32.5
	Anab	8	0.206	0.845	0.446	2.007	3.31	2.77	37.3	49.7	43.3
	Kani Spi	5	0.145	0.659	0.341	1.61	2.94	2.086	29.7	34.9	33.3
	Bakrawa	4	0.317	0.830	0.515	1.38	1.57	1.49	34.3	45.9	38.8
	Zamaqi	2	0.579	1.009	0.794	1.59	1.64	1.61	49.0	52.6	50.8
	Ka Grdal	1			1.11			1.79			60.0
	Bia Wella	1			0.447			2.58			63.8
	Khormal	1			0.455			1.79			52.3
Sharazoor	*Sharazoor*	7	0.223	0.560	0.345	0.924	3.15	1.74	29.4	56.9	36.6
	NewHalabja	1			1.202			1.74			55.6
Kalar	*Kalar*	13	0.445	1.93	1.086	1.22	3.28	1.99	27.3	62.9	42.3

Keskinen et al. (2009) studied extracting Se in soils of Finland and reported 50% Se organically associated, applying (0.1 M NaOH). Comparable recoveries using alkali extraction have been reported in other studies of soils from India 75-89% (Schilling et al., 2014), and from Finland 70% (Keskinen et al., 2009).

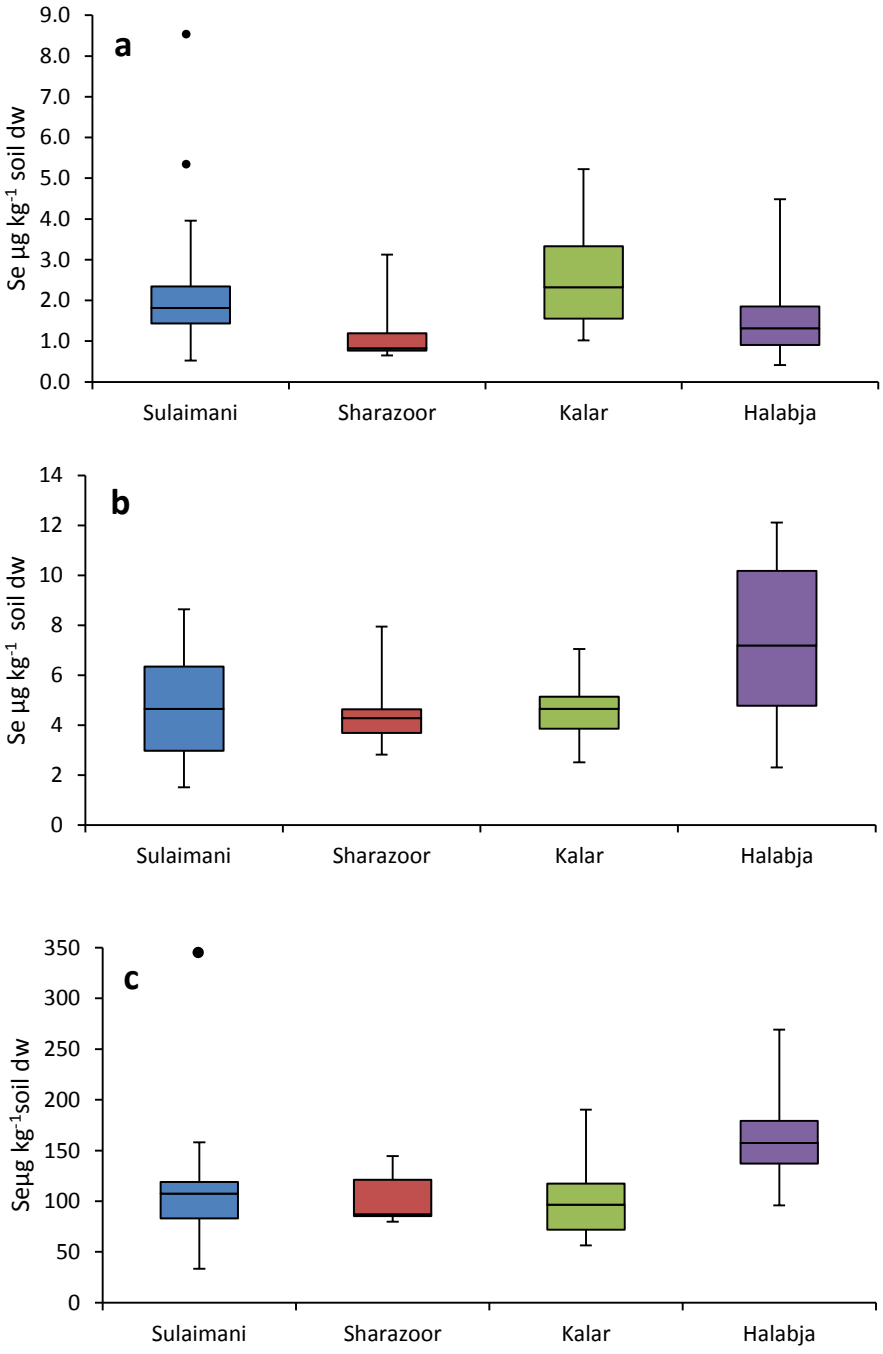


Figure 3-11. Soil Se content in fractions a) soluble Se extracted by 0.01M KNO_3 b) adsorbed Se extracted by 0.016M KH_2PO_4 c) total organic Se extracted by 10% TMAH. Sulaimani; n=41, Sharazoor; n=8, Kalar; n=13, Halabja; n= 35.

• outlier data

Figure 3.12 demonstrates the relationship between pH and %Se $_{TMAH}$ in a wide range of the soil types from previous projects (Mathers, 2015). It shows %Se $_{TMAH}$ can change from 100% at very low soil pH (mainly organic soils) to <10% in highly alkaline soils. There is a marked decrease in soil extractability at pH >6. Including data from this study shows that they fit the general trend shown by the larger dataset and, based on Kurdistan's soil pH values, the extractability of Se was around 40%.

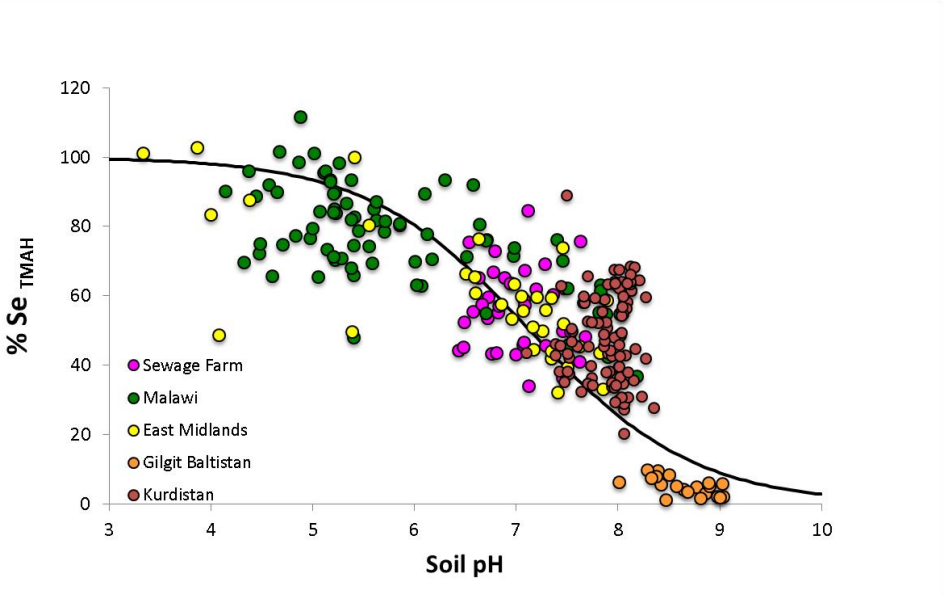


Figure 3-12. Relationship between %Se TMAH extractable and pH as a model in different areas with a wide range of pH

3.4.3.3 Soil selenium speciation

Selenium speciation was carried out for the soil Se_N fraction (extracted with 0.01M KNO_3) (table 304) and it was found that $Se_{N\text{-organic}}$ generally varied from 0 – 77% of the Se_N in this fraction with a mean of 49%. The highest

$Se_{N\text{-}inorganic}$ was found in the Sulaimani area (range = 36-113%; mean = 71.6%; n=18) and the lowest was for the Kalar area (range = 23-61%; mean = 37%; n=13). Halabja and Sharazoor had an average of 51 and 56% $Se_{N\text{-}inorganic}$ in proportion of Se_N. There were few sites within the Sulaimani subareas which had 100% inorganic Se; these were mainly urban and industrial areas and were affected by anthropogenic activities and variety of unknown sources imports to this soils or could be because of low organic matter content of this area.

The average proportions of selenate (Se^{6+}) and selenite (Se^{4+}) in the $Se_{N\text{-}inorganic}$ fraction were 65 and 35% respectively across all areas. However, distribution of these values differed between sub-areas. For example, the subareas Dukan, Kagrdal and new Halabja had 100% and Tanjaro had 86% Se^{4+} while the proportion of Se^{4+} for the Sulaimani industrial area was only 5% and 95% of $Se_{N\text{-}inorganic}$ was Se^{6+} (Table 3.4). Interpretation of the soluble speciation data is hampered by the very small concentrations of soluble Se (90% were <2 μg kg^{-1}) and even smaller concentrations of the inorganic species Se^{6+} and Se^{4+} (>90% were < 1 μg kg^{-1}).

Speciation of adsorbed or ligand-exchangeable Se_P (extracted by 0.016 M KH_2PO_4) indicated that 78% of Se_P was inorganic. The mean proportions of $Se_{P\text{-}inorganic}$ were 96, 88, 77 and 67% for the Sharazoor, Halabja, Kalar and Sulaimani areas. The majority of inorganic exchangeable Se (96%) was Se^{4+}; in most of the sites Se^{4+} was the only inorganic species present in the Se_P. This result was predictable, as selenite tends to bond more strongly than selenate with amorphous Fe and Al oxides. Other studies have found similar results: Supriatin et al. (2015) carried out ammonium oxalate extraction (extraction amorphous Fe and Al oxides) and found that inorganic Se was

Table 3.4. Speciation of selenium in the soluble fraction (KNO_3) and proportion of organic and inorganic Se

Area	Sub area	Code	Se(N)	SeIV(N)	SeVI(N)	In-org	Org	SeIV(N)	SeVI(N)
			µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	%	%	%	%
		STA 1	0.699	0.287	0.345	90.4	9.64	45.4	54.6
		STA 2	1.16	0.488	0.549	89.3	10.7	47.0	53.0
		STA 3	1.26	0.519	0.594	88.0	12.0	46.6	53.4
		STA 4	1.38	0.558	0.256	58.9	41.1	68.6	31.4
		STA 5	2.62	0.383	2.292	102	0	14.3	85.7
		STA 6	1.63	0.901	0.476	84.4	15.6	65.5	34.5
		STA 7	1.30	0.231	0.881	85.7	14.3	20.8	79.2
	Tanjaro	STA 8	1.61	0.994	0.170	72.4	27.6	85.4	14.6
		STA 9	2.10	0.561	1.55	101	0	26.5	73.5
		STA 10	2.24	0.992	0.783	79.2	20.8	55.9	44.1
		STA 11	1.95	0.443	1.41	95.3	4.66	23.9	76.1
		STA 12	1.62	0.776	0.127	55.8	44.2	86.0	14.0
		STA 13	1.73	0.678	0.117	46.0	54.0	85.3	14.7
		STA 14	3.15	1.45	0.238	53.4	46.6	85.9	14.1
Sulaimani		STA 15	2.31	0.939	0.159	47.5	52.5	85.5	14.5
	Baziyan	SBY 1	2.42	0.951	0.245	49.4	50.6	79.5	20.5
		SBY 2	2.34	0.562	0.520	46.2	53.8	51.9	48.1
	Zhalla	SZH	3.96	0.921	0.514	36.3	63.7	64.2	35.8
		SZH 2	0.900	0.199	0.216	46.1	53.9	48.0	52.0
	Dukan	DUK 1	2.75	1.18	0.000	42.9	57.1	100	0.0
		DUK	8.34	3.40	0.378	45.3	54.7	90.0	10.0
		SWA 1	2.79	0.871	0.922	64.3	35.7	48.6	51.4
	west area	SWA 2	1.43	0.549	0.391	65.5	34.5	58.4	41.6
		SWA 3	1.016	0.354	0.325	66.9	33.1	52.1	47.9
	Industrial	SID 1	1.89	0.158	1.83	105	0.0	7.95	92.0
		SID 2	2.81	0.059	2.99	109	0.0	1.95	98.1
		SKS 1	1.44	0.220	1.10	91.9	8.1	16.6	83.4
	Kana Sura	SKS 2	0.525	0.0800	0.166	46.8	53.2	32.5	67.5
		SKS 3	5.18	0.324	5.52	113	0.0	5.55	94.5
		SHA 1	0.832	0.395	0.095	58.9	41.1	80.6	19.4
		SHA 2	0.648	0.126	0.145	41.8	58.2	46.3	53.7
		SHA 3	0.671	0.367	0.111	71.3	28.7	76.8	23.2
Sharazoor	***Sharazoor***	SHA 4	0.802	0.384	0.149	66.5	33.5	72.0	28.0
		SHA 5	1.12	0.584	0.204	70.4	29.6	74.1	25.9
		SHA 6	1.41	0.0589	0.356	29.4	70.6	14.2	85.8
		SHA 7	0.818	0.349	0.289	78.0	22.0	54.7	45.3
	New Halabja	SHA 8	3.12	0.917	0.083	32.0	68.0	91.7	8.25
		KAL 1	3.88	1.18	0.044	31.5	68.5	96.4	3.62
		KAL 2	2.15	0.631	0.226	39.9	60.1	73.6	26.4
		KAL 3	1.20	0.282	0.034	26.5	73.5	89.1	10.9
		KAL 3	1.20	0.216	0.186	33.5	66.5	53.8	46.2
		KAL4a	2.32	0.852	0.063	39.4	60.6	93.1	6.92
		KAL4b	5.22	1.25	1.42	51.0	49.0	46.8	53.2
Kalar	***Kalar***	KAL4c	3.068	1.013	0.148	37.8	62.2	87.2	12.8
		KAL 5G	4.084	1.13	0.111	30.3	69.7	91.0	8.98
		Kal 6	2.42	0.819	0.143	39.8	60.2	85.2	14.8
		KAL	3.33	1.22	0.250	44.2	55.8	83.0	17.0
		KAL 7	1.019	0.477	0.140	60.6	39.4	77.3	22.7
		KAL 8	2.00	0.158	0.301	22.9	77.1	34.5	65.5
		KAL 9	1.56	0.136	0.250	24.8	75.2	35.2	64.8
		HKK 1	2.26	0.826	0.134	42.5	57.5	86.0	14.0
		HKK 2	1.47	0.555	0.114	45.3	54.7	83.0	17.0
		HKK 3	0.830	0.372	0.0748	53.8	46.2	83.2	16.8
		HKK 4	0.968	0.273	0.0919	37.7	62.3	74.8	25.2
		HKK 5	0.415	0.108	0.0944	48.8	51.2	53.4	46.6
		HKK 6	0.819	0.181	0.0875	32.7	67.3	67.4	32.6
	Kani Kolka	HKK 7	0.507	0.221	0.0946	62.2	37.8	70.0	30.0
		HKK 8	0.778	0.216	0.0698	36.7	63.3	75.6	24.4
		HKK 9	0.724	0.156	0.102	35.6	64.4	60.6	39.4
		HKK 10	1.42	0.605	0.0861	48.7	51.3	87.5	12.5
		HKK 11	1.25	0.668	0.188	68.3	31.7	78.0	22.0
		HKK 12	1.42	0.887	0.252	80.4	19.6	77.9	22.1
		HKK 13	1.95	0.541	0.138	34.9	65.1	79.7	20.3
Halabja		HAN 1	1.54	0.800	0.157	62.0	38.0	83.6	16.4
		HAN 2	1.90	0.688	0.123	42.7	57.3	84.8	15.2
		HAN 3	3.98	1.47	0.128	40.0	60.0	92.0	8.04
	Anab	HAN 4	1.29	0.395	0.100	38.5	61.5	79.8	20.2
		HAN 5	1.47	0.610	0.193	54.7	45.3	76.0	24.0
		HAN 6	3.28	1.17	0.0751	38.1	61.9	94.0	6.02
		HAN 7	0.755	0.268	0.185	60.1	39.9	59.2	40.8
		HAN 8	0.912	0.399	0.260	72.2	27.8	60.5	39.5
		HKS 1	1.12	0.217	0.0776	26.4	73.6	73.7	26.3
		HKS 2	2.85	0.799	1.48	80.2	19.8	35.0	65.0
	Kani Spi	HKS 3	0.701	0.380	0.0927	67.4	32.6	80.4	19.6
		HKS 4	1.53	0.719	0.345	69.7	30.3	67.6	32.4
		HKS 5	1.25	0.556	0.115	53.8	46.2	82.9	17.1
		HBK 1	1.37	0.524	0.172	50.9	49.1	75.2	24.8

	Bakrawa	HBK 2	2.50	0.260	1.17	57.5	42.5	18.1	81.9
		HBK 3	0.902	0.244	0.335	64.2	35.8	42.2	57.8
		HBK 4	1.32	0.191	0.513	53.5	46.5	27.2	72.8
	Zamaqi	HZM 1	3.40	1.71	0.104	53.3	46.7	94.3	5.74
		HZM 2	1.80	0.449	0.073	28.9	71.1	86.1	13.9
	Ka Grdal	HKG	4.48	1.71	0.000	38.1	61.9	100.0	0.0
	Bia Wella	HBW1	1.19	0.648	0.118	64.3	35.7	84.5	15.5
	Khormal	HKH	1.29	0.370	0.249	48.1	51.9	59.8	40.2

Table 3.5. Speciation of selenium in the absorbed fraction (KH_2PO_4) and proportion of organic and inorganic Se

Area	Sub area	Code	Se(P) µg kg^{-1}	SeIV(P) µg kg^{-1}	SeVI(P) µg kg^{-1}	In-org %	Org %	SeIV(N) %	SeVI(N) %
Sulaimani	***Tanjaro***	STA 12	2.86	1.24	0	43.4	56.6	100	0
		STA 13	2.81	1.52	0	53.9	46.1	100	0
		STA 14	7.71	7.42	0	96.3	3.73	100	0
		STA 15	4.87	4.16	0	85.3	14.7	100	0
	Baziyan	SBY 1	3.84	2.51	0	65.3	34.7	100	0
		SBY 2	5.022	4.96	0	99	0.00	100	0
	Zhalla	SZH 1G	4.044	2.86	0	70.8	29.2	100	0
		SZH 2	1.51	0.06	0.0264	5.79	94.2	69.7	30.3
	Dukan	DUK 2G	8.65	3.15	0.0484	37.0	63.04	98.5	1.52
	Kana Sura	SKS 4	2.29	2.13	0.0110	93.5	6.48	99.5	0.514
		SKS 5	2.67	1.71	0.0190	64.9	35.1	98.9	1.10
		SKS 6	3.81	2.25	0	58.9	41.1	100	0
		SKS 7	2.52	2.93	0	116	0.00	100	0
		SKS 8	2.89	2.10	0	72.5	27.5	100	0
		SKS 9	2.15	2.01	0	93.1	6.86	100	0
	Kani Goma	SKG 1	3.36	0.63	0	18.8	81.2	100	0
		SKG 2	4.65	0.02	0.285	6.46	93.5	5.3	94.7
		SKG 3	3.65	2.70	0	74.0	26.0	100	0
		SKG 4	4.51	5.36	0	119	0.00	100	0
		SKG 5	2.98	3.32	0	112	0.00	100	0
		SKG 6	2.94	0.51	0.463	33.0	67.0	52.3	47.7
Sharazoor	***Sharazoor***	SHA 1	3.40	3.46	0	102	0	100	0
		SHA 2	2.82	2.30	0	81.7	18.3	100	0
		SHA 3	3.78	4.44	0	117	0	100	0
		SHA 4	4.13	4.02	0	97	0	100	0
		SHA 5	4.94	5.37	0	109	0	100	0
		SHA 6	7.95	8.99	0.0336	114	0	99.6	0.372
	New	SHA 7	4.43	3.68	0	83.0	17.0	100	0
		SHA 8	4.53	3.04	0	67.0	33.0	100	0
Kalar	***Kalar***	KAL 1	5.33	1.22	0	22.9	77.1	100	0
		KAL 2	5.052	3.54	0	70.1	29.9	100	0
		KAL 3 W	3.90	4.16	0	107	0	100	0
		KAL 3	4.43	4.76	0	108	0	100	0
		KAL4a	3.27	2.58	0	79.1	20.9	100	0
		KAL4b	4.69	2.98	0.0560	64.8	35.2	98.2	1.84
		KAL4c	2.50	1.91	0	76.4	23.6	100	0
		KAL 5G	3.60	0.74	0	20.6	79.4	100	0
		Kal 6	3.86	3.37	0	87.5	12.5	100	0
		KAL 6W	5.14	5.33	0	104	0	100	0
		KAL 7	4.65	5.22	0	112	0	100	0
		KAL 8	7.05	2.60	0	36.9	63.1	100	0
		KAL 9	6.86	7.85	0	114	0	100	0
Halabja	***Bakrawa***	HBK 1	4.82	4.58	0	94.9	5.091	100	0
		HBK 2	4.73	4.36	0.0458	93.1	6.93	99.0	1.039
		HBK 3	4.12	4.30	0	104	0	100	0
		HBK 4	3.87	2.35	0.0154	61.2	38.8	99.4	0.648
	Zamaqi	HZM 1	5.36	5.26	0	98.1	1.91	100	0
		HZM 2	5.11	5.20	0	102	0	100	0
	Ka Grdal	HKG	7.19	6.29	0	87.4	12.6	100	0
	Bia Wella	HBW1	6.89	3.54	0	51.4	48.6	100	0
	Khormal	HKH	5.07	5.29	0	104	0	100	0

mainly present in Se^{4+} while Se^{6+} was hardly measurable. In a similar study. Wang et al. (2012) reported that Se^{4+} was the only chemical species that could be detected in the KH_2PO_4 extracts.

Figure (3.13a,b) shows the correlation of Se^{4+} in Se_N and Se_P fraction with total Se in each fraction; there was no correlation between Se^{6+} and total Se in those fractions.

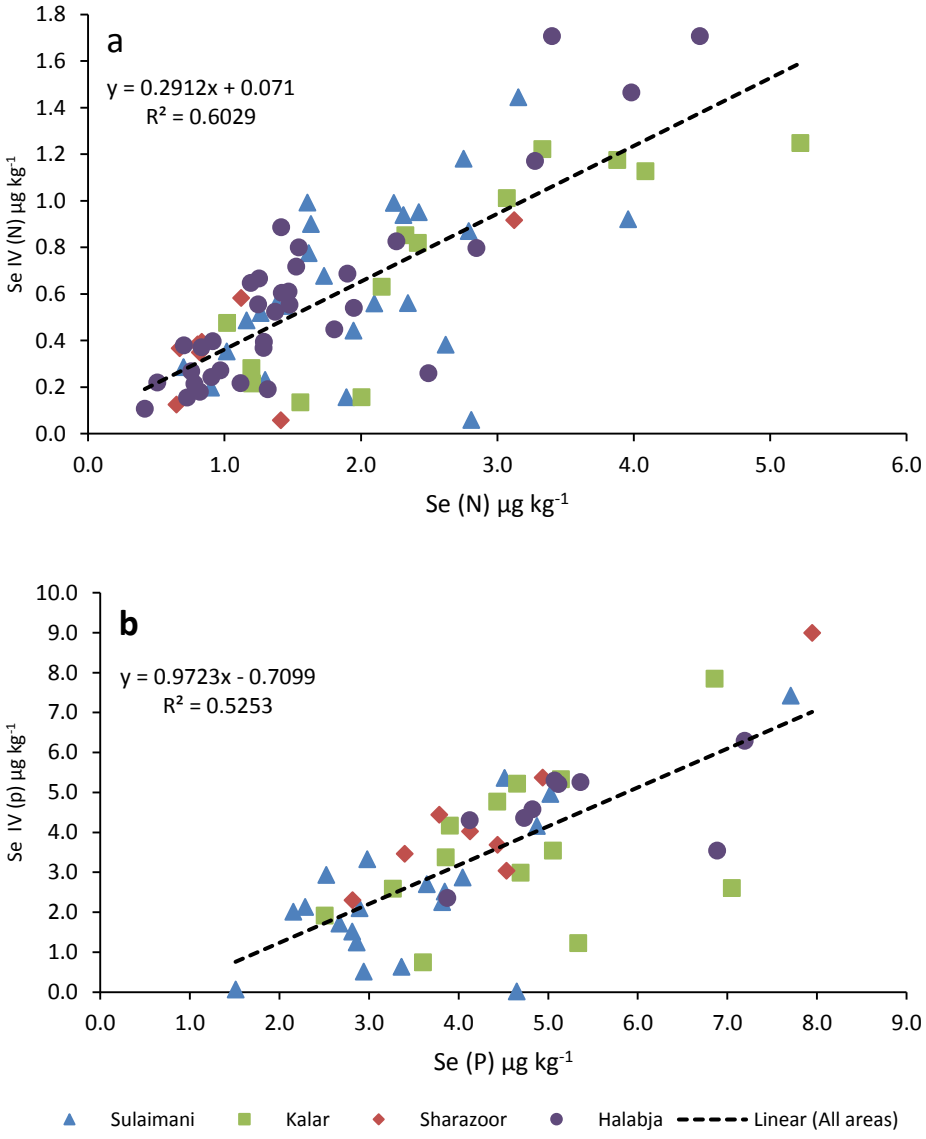


Figure 3-13. Relationship between selenite and total Se in the (a) soluble and (b) absorbed fractions

3.4.3.4 Relationship of soil selenium in fractions and soil properties

A positive correlation was observed between Se_{TMAH} and soil organic carbon (OC%) when all areas were considered (Fig 3.14a). By separating the areas, this relationship was not found in Sulaimani and Sharazoor sub-areas compared to Kalar and Halabja. The reason with respect to the Sharzoor area could be that soil OC% values were all very similar and so presented a narrow

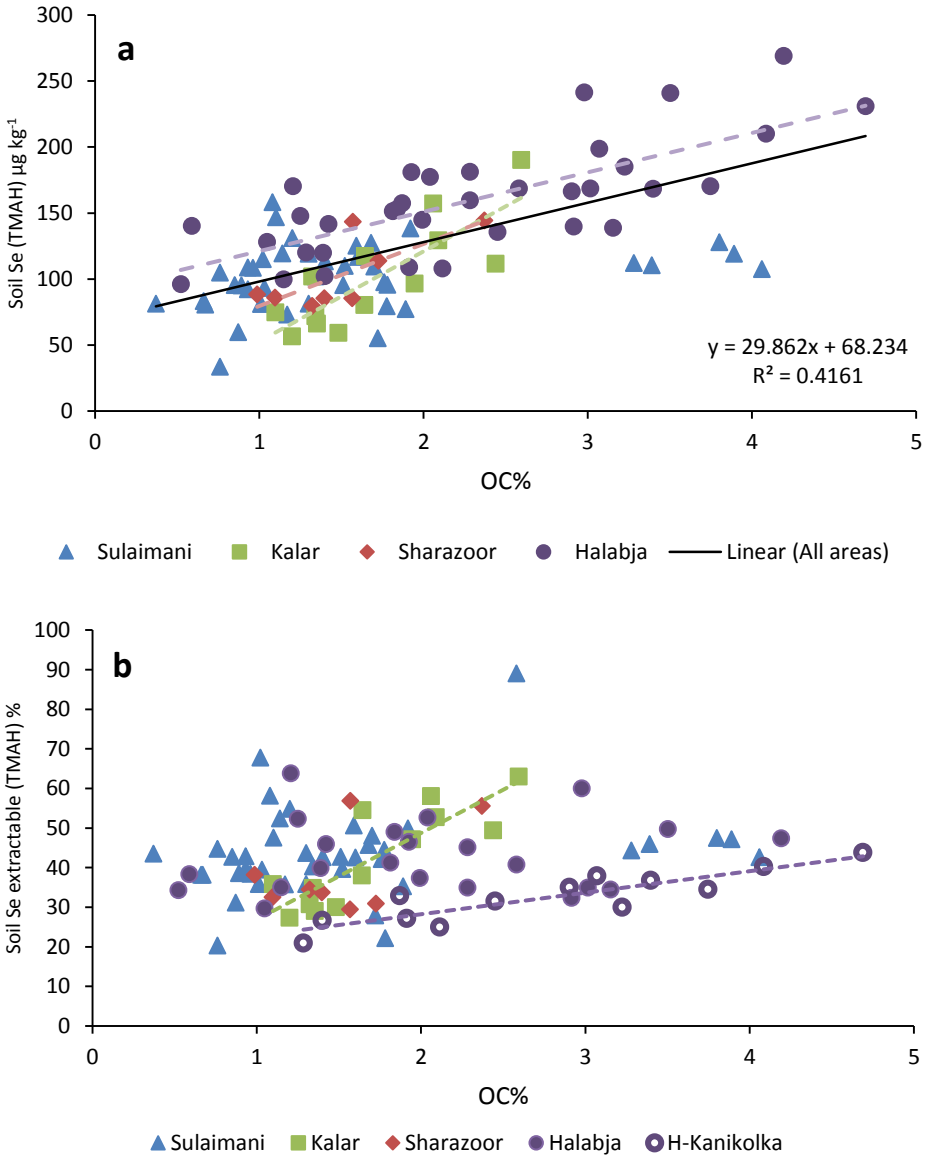


Figure 3-14. Relationship of soil organic carbon with (a) soil Se in 10% TMAH extract and (b) Se TMAH extractable in proportion of total Se consider to areas or regional effects (blank circles are subareas of filled circles.

range of around 1% OC. However, all the areas aggregated together produced a correlation. This supports the suggestion that the majority of Se_{TMAH} is present in organic form. Figure 3.14 demonstrates that the percentage of Se_{TMAH} extractability as a proportion of Se_{Tot} was also followed the increasing trend in Kalar area and Kanikolka subarea from Halabja area.

Figure 3.15 demonstrates that selenium concentration in adsorbed fraction seems to be not controlled by organic carbon variation. There was no

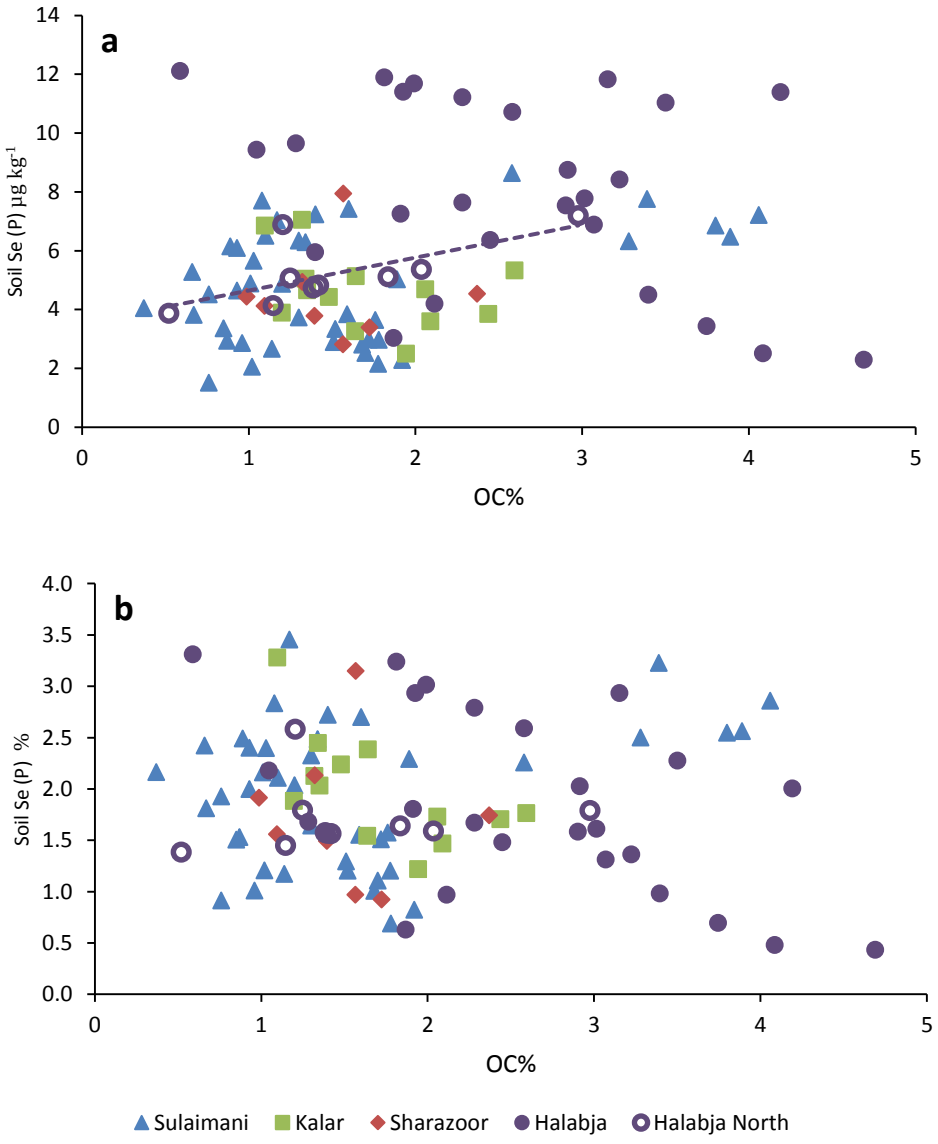


Figure 3-15. Relationship of soil organic carbon with (a) soil Se in 0.016M KH_2PO_4 extract and (b) soil iodine extractable in this fraction in proportion with total iodine consider to areas or regional effects (Halabja North is Anab+Kanikolka)

correlation for the main areas except a weak association for one of the Halabja sub-areas (Halabja north; Anab+ Kanikolka subareas). This could be because selenium in this fraction is not bound to organic matter as it is more associated with aluminium and iron oxides. In addition, speciation results showed that the majority of Se in this fraction was inorganic. Figure 3.15 b shows no relationship between the amount of soil organic carbon and extractability of $\%Se_p$ as a proportion of Se_{Tot}.

As found for $Se_{p,}$ soil organic carbon was not correlated with Se_N when aggregating the data for all areas together. However, considering each area or sub-area separately showed a relationship in some instances. For example, considering the sub-areas there was a correlation for Kanikolka and Hal-Northern areas within the sub-areas in those regions. There was no association between %OC and Se_N in the Sulaimani area even at the level of the sub-areas (Fig. 3.16a). A similar trend was followed for $\%Se_N$ and soil OC% (Fig. 3.16b). Sulaimani subareas are urban or semi-urban and anthropogenic effects may have altered soil organic matter content or provided alternative Se sources; Se present in the soluble fraction was predominantly inorganic (71%).

The Se_{TMAH} concentration was negatively correlated with soil pH (Figure 3.17a) in the Kalar area, Sulaimani and Halabja sub-areas but not in the Sharazoor area. For the Sharazoor area there was a very narrow range of pH values. The proportion of Se_{Tot} that was extractable with TMAH ($\%Se_{TMAH}$) also decreased with increasing pH in Kalar and the Kanikolka sub-area of Halabja (3.17b). This suggests the presence of recalcitrant forms of Se at high pH which cannot be easily liberated by TMAH. Martens and Suarez (1997) carried out a sequential extraction using phosphate buffer, $K_2S_2O_8$

and HNO_3 on soils with pH >7 and found an intractable pool of Se not extracted with phosphate buffer or $K_2S_2O_8$ representing 15-84% of Se_{Tot}. Mathers (2015) fitted a simple logistical equation to a wide range of soils from the UK and Malawi with pH ranging from 3 to 8 and estimated that $\%Se_{TMAH}$ decreased from 100 to 40% with increasing pH from 3 to 8. In most

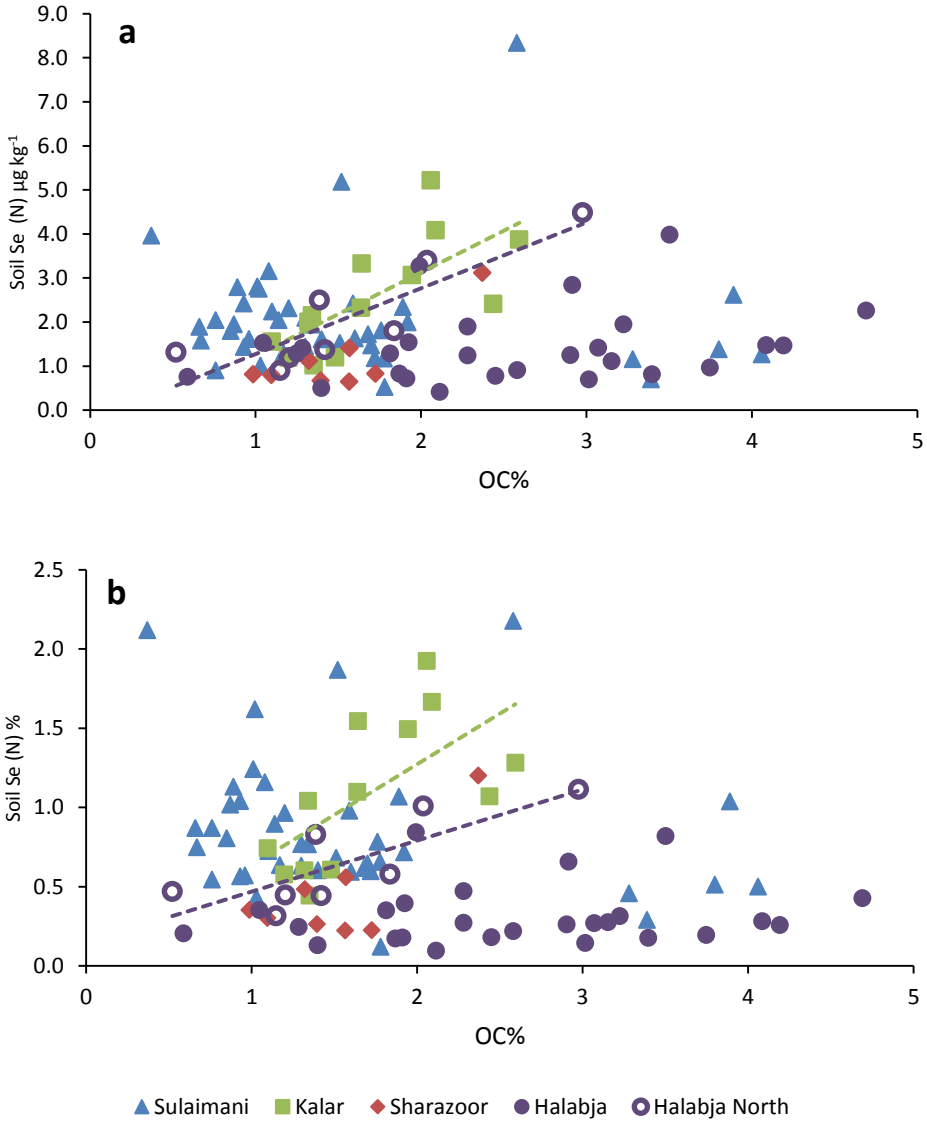


Figure 3-16. Relationship of soil organic carbon with (a) Se_N and (b) soil Se_N as proportion of total Se consider to areas or regional effects

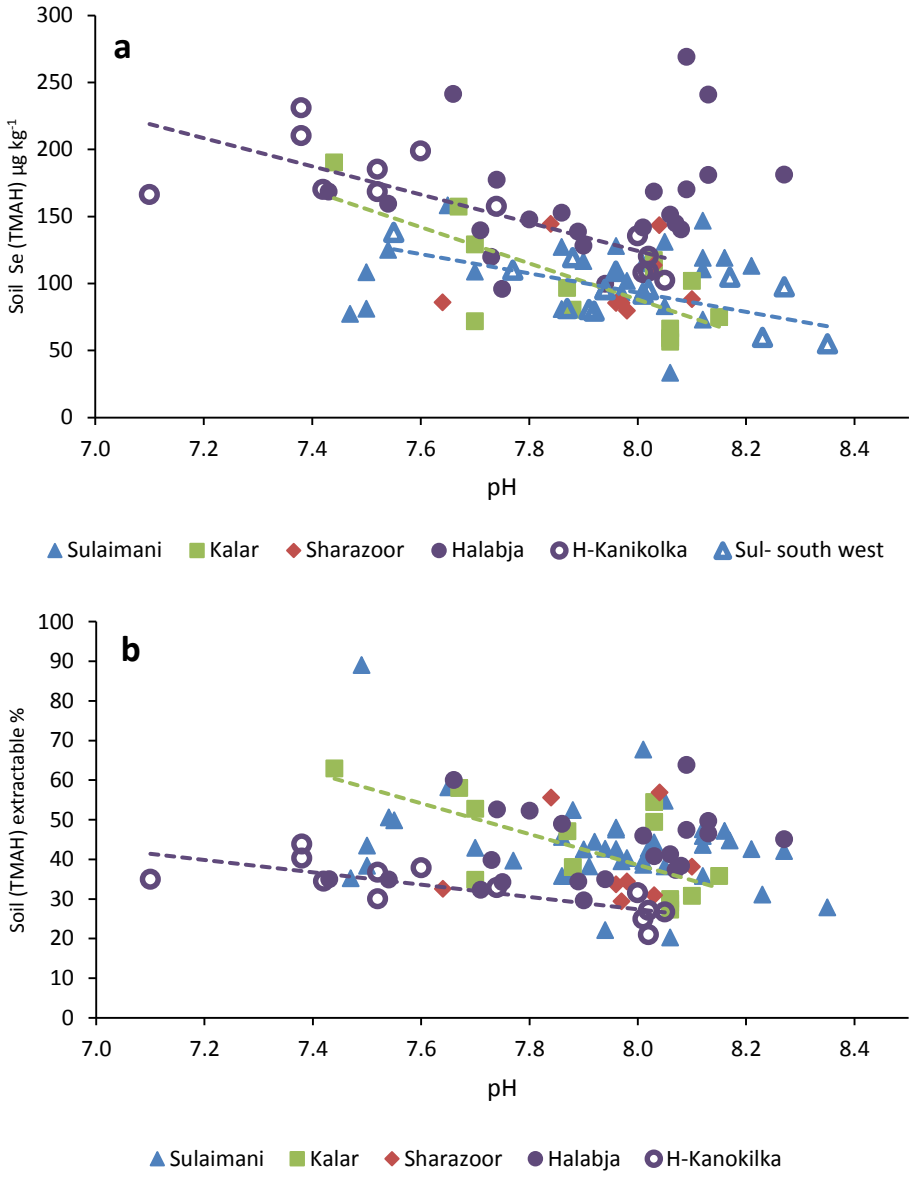


Figure 3-17. Relationship of soil pH with (a) soil Se in 10% TMAH extract and (b) Se $_{TMAH}$ extractable in proportion of total Se consider to areas or regional effects

of the relationships derived for Se fractions and soil properties it was observed that relationships emerged between sub-areas but were not apparent between areas. This could be because of the geogenic similarity of soils present within sub-areas in contrast to major geological differences between areas. There was no association between soil pH and Se_P or $\%Se_p$

when all areas considered together. However there was a positive relationship in part of the Halabja area; soils with pH≥8 in each area had higher values of Se_p and $\%Se_p$ (Fig 3.18 a & b). The point of zero net charge (pznc) for Al and Fe hydrous oxides is about pH=8 (Pulford, 2006) as a result,

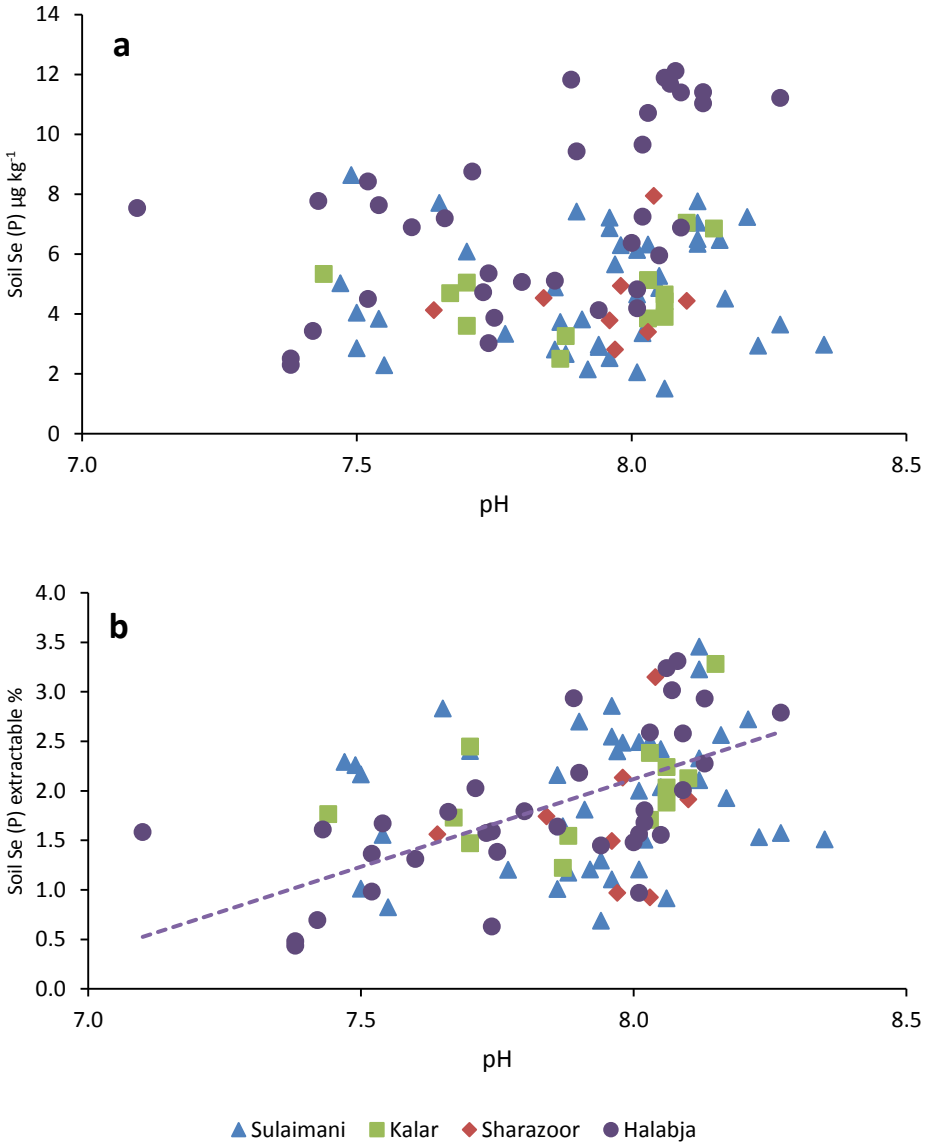


Figure 3-18. Relationship of soil pH with (a) soil Se in 0.016M KH_2PO_4 extract and (b) soil $\%Se_P$ as proportion of total selenium consider to areas or regional effects

the pH above that has a negative charge and selenite anions do not have affinity to adsorb. Based on speciation result the majority of Se in this fraction was inorganic and Se^{+4}. This could be a possible reason for higher Se_p in higher pH and in this study. Mathers (2015) found a strong positive correlation between $\%Se_p$ and soil pH in UK and Malawian soils. Again, the narrow range of pH in the calcareous soils of this study (1 unit) could be the reason that this correlation was not strong in all the areas.

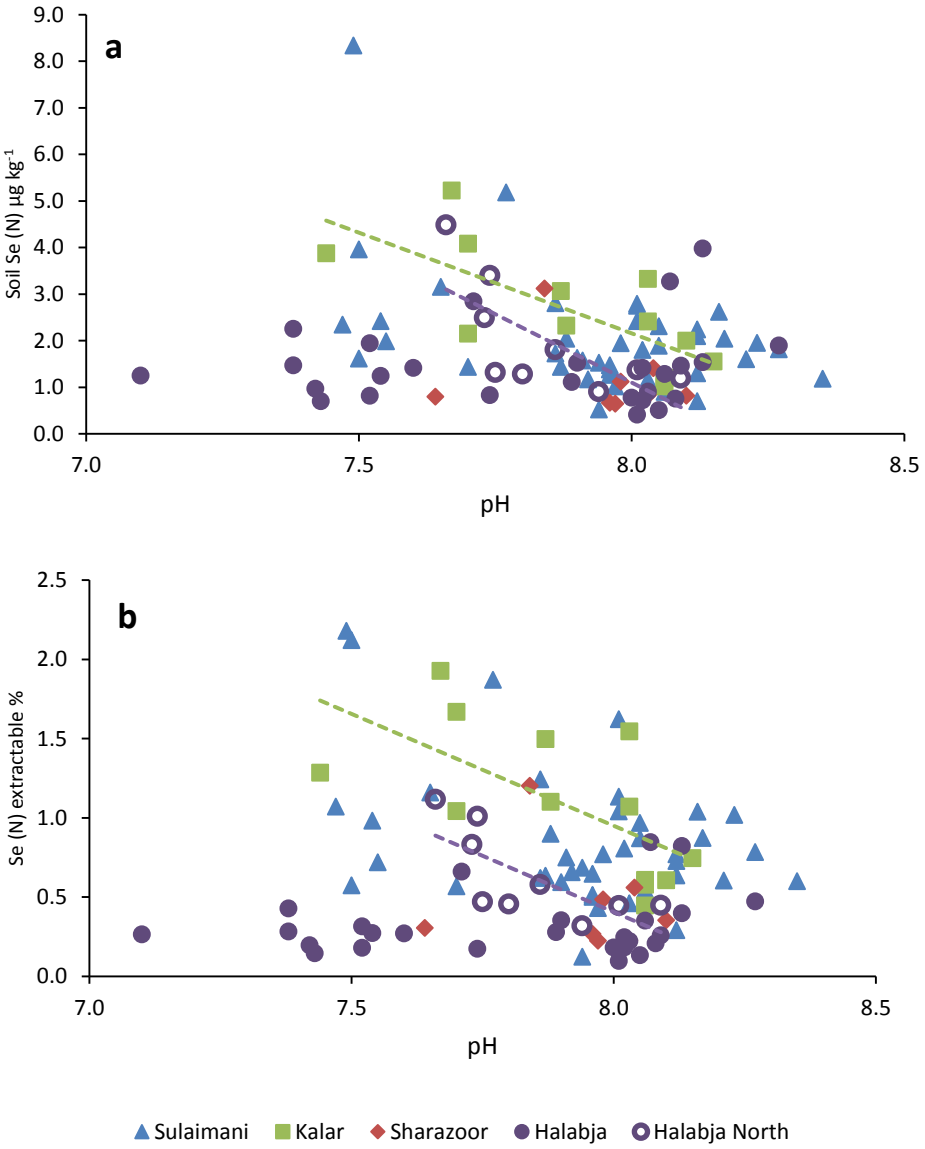


Figure 3-19. Relationship of soil pH with (a) soil Se in 0.01M KNO_3^- extract and (b) and Se_N as proportion of total Selenium consider to areas or regional effects

There was no correlation between soluble selenium (Se_N) and soil pH when considering all the areas together. A negative relationship (r =0.72) was found for the Kalar area and Halabja north sub-areas but this relationship did not apply to Sulaimani and Sharazoor (Fig 3-19 a). The same pattern was followed for $\%Se_N$ and soil pH.

A negative correlation (r =0.71) was observed between soil %OC and soil calcium carbonate content in the Sulaimani area (Fig 3.20) but this trend was not followed in the other three areas – again probably due to the narrow range of %OC values (1-2%) in the Kalar and Sharazoor areas.

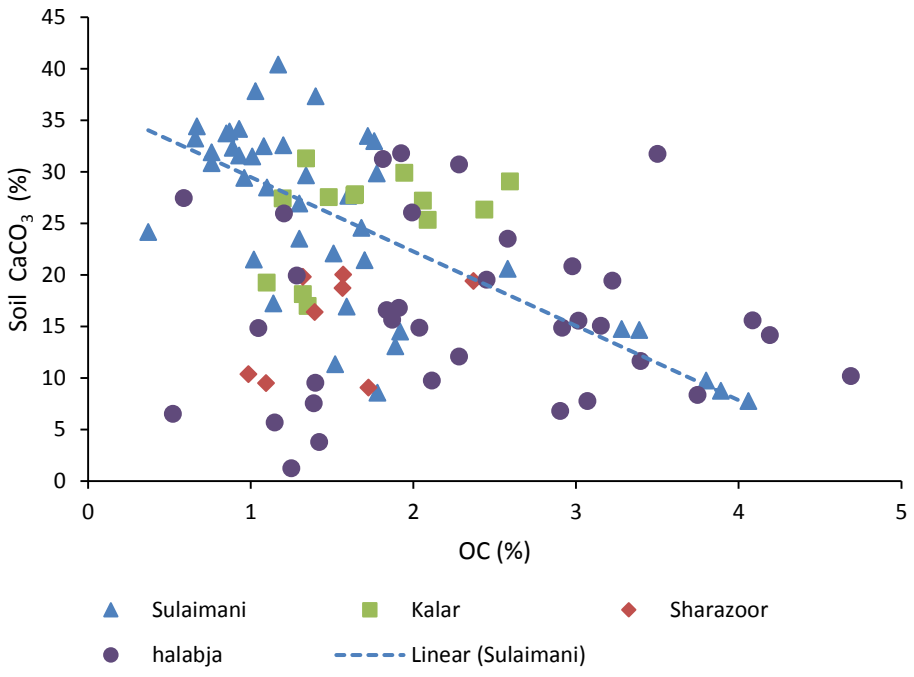


Figure 3-20. Relationship between soil organic carbon and calcium carbonate content

3.4.4 Plant uptake of selenium

3.4.4.1 Plant selenium content

The Se contents of 300 plants (30 species), including leafy and crop vegetables, tubers and cereal grains, were determined (Table 3.6 and Fig. 3.21). The Se concentration across all plant samples ranged from 4.34 to 678 μg kg^{-1} on a dry weight basis (dw). Celery had the highest Se concentration for leafy vegetables, with an average of 215 μg kg^{-1} dw and a range of 25.4-678 μg kg^{-1} dw (n=24); purslane had the lowest Se content, with a mean value of 36.4 μg kg^{-1} and a range from 12.6-85.9 μg kg^{-1} dw (n=17). The Se concentration in celery was higher than a value reported for Belgium (106 μg kg^{-1} dw n=7) (De Temmerman et al., 2014). Selenium concentration in celery on a fresh weight basis ranged from 7.0-192 μg kg^{-1} fw with a mean value of 59.8 μg kg^{-1} fw. This value was much higher than those reported from (Thailand[b], Belgium[d] ,Greece[f], Croatia[h], Australia[i] and USDA food composition database, Table 3.7). The Se contents of other leafy vegetables such as Dill, Spring onion, Leek, Chard, Parsley and Basil were also higher than data reported for other (Table 3.7) By contrast, values for the other leafy vegetables studied, including cress, mallow, mint and purslane were in agreement with those reported from USDA[o] and Saudi Arabia[c] (Table 3.7). Overall the Se content of the leafy vegetables studied were generally higher than those reported from other countries (Table 3.7).

Table 3.6. Plant Selenium content including leafy and crop vegetables, tubers and grains

Plant species	n	Se µg kg^{-1} (DW)			Se µg kg^{-1} (FW)		
		Average	Median	Range	Average	Median	Range
Leafy Vegetables							
Celery	24	215	199	25.4-678	59.8	47.7	7.0-192
Dill	18	188	171	66.5-492	36.08	32.9	11.1-106
Radish Leaves	8	185	176	59.7-343	37.4	24.0	7.70-103
Sp-Onion	16	167	143	36.4-424	20.3	18.6	3.55-41.2
Leek	22	152	119	51.7-386	31.02	17.6	7.12-83.7
Spinach	1	118			13.4		
Chard	28	112	90.4	11.8-538	23.02	15.0	8.69-189
Parsley	12	104	96.2	18.2-194	35.3	28.6	4.41-131
Cress	5	96.3	62.9	41.4-162	13.3	11.6	5.93-19.9
Taragon	6	96.3	84.2	58.2-159	43.01	38.7	5.29-90.7
Thyme	2	88.6		84.8-92.4	12.1		
Basil	4	68.1	67.5	47.6-89.9	11.0	11.6	8.03-12.7
Mallow	1	63.4			15.3		
Pennyroyal	1	60.0			12.05		
Mint	2	56.3		52.3-60.4	16.7		
Purslane	17	36.4	27.3	12.6-85.9	6.34	4.48	1.62-16.8
Fruit vegetables							
Cow pea	18	109	99.3	7.9-314	44.9	45.1	3.11-129
Tomato	13	108	93.0	13.5-265	13.08	10.7	1.77-30.4
Cucumber	13	95.1	68.9	24.4-228	11.4	4.53	2.27-37.1
Water melon	4	75.4	78.5	5.4-139	6.89	7.1	0.41-13.0
Pepper	16	59.2	34.8	10.4-217	10.4	6.91	1.78-35.9
Egg plant	11	55.7	25.9	4.34-216	10.1	3.9	0.56-40.3
Fava. Bean	6	54.8	60.5	26.1-72.0	13.8	10.5	4.47-26.9
Courgette	6	49.8	53.1	18.8-76.5	4.43	3.96	1.29-7.99
Melon	2	47.5		5.70-89.2	3.26		
Okra	18	37.6	22.8	7.20-116.1	13.05	8.17	1.903-42.6
Tubers							
Garlic	1	187			67.7		
Radish	7	95.9	99.0	50.6-157.5	19.7	7.68	3.39-64.7
Onion	5	55.3	60.4	14.1-90.4	8.92	8.43	1.59-15.8
Cereals							
Wheat	14	49.4	40.8	18.6-125	49.4	40.8	18.6-125

The mean Se concentration for all leafy vegetables together, accounted for 113 and 24.1 µg kg^{-1} on a dry and fresh weight basis respectively. Similar studies in Thailand[b], S-Arabia[c], Belgium[d], Greece[f] and Korea[K] calculated mean Se concentrations for a range of leafy vegetables of 12, 9.4, 9.6, 6.35 and 19 µg kg^{-1}(fw) which are less than the value found in this study.

Among fruit vegetables cow pea had the highest Se concentration, ranging from 7.9-314 with a mean of concentration was 109 μg kg^{-1} dw (n=18) and okra had the lowest Se concentration ranging from 7.20-116 with a mean of 37.6 μg kg^{-1} dw (n=18) (Fig 3.21 and Table 3.6). On a fresh weight basis the Se content of cow pea was 44.9 μg kg^{-1} (fw) and all of other fruit vegetables had Se contents <15 μg kg^{-1} (fw). Courgette and melon had the lowest Se content: 4.43 and 3.26 μg kg^{-1} (fw) respectively. Compared to other studies, on a fresh weight basis, cowpea Se content (44.9 μg kg^{-1} fw) was less than values from Thailand 58 μg kg^{-1} fw (Sirichakwal et al., 2005). Tomato (13.1 μg kg^{-1} fw) was in agreement with values reported from Slovenia[a] and Thailand[b] but less than Saudi Arabia[c] and higher than data from other countries suggests. For example, 1.5 times higher than values from Croatia[h] and 26 time higher than values from Australia[j] (Table 3.7). Pepper (10.4 μg kg^{-1} fw) corresponded to data from Slovenia[a] but was less than examples from Korea[k] and higher than data from other countries.

The average Se concentrations for all fruit vegetables on a dry and fresh weight basis were 69.2 and 13.1 μg kg^{-1}. The latter value was higher than data reported from Thailand[b], Belgium[d], and Greece[f], (1.2, 1.6. 4.2 μg kg^{-1} fw).

Within the group of tuber and root vegetables there were only three examples: garlic and onion had the highest and lowest Se concentration (187 and 55.3 μg kg^{-1} dw respectively) and radish had a Se content of 95.9 μg kg^{-1}. On a fresh weight basis garlic and radish had a higher Se content (67.7 and 19.7 μg kg^{-1} fw) than values quoted for all other countries but Se content

of onions was less than values from Thailand[b], Croatia[h], Korea[K] (11, 15.3 and 52 µg kg^{-1} fw) (Table 3.7 and Fig. 3.21).

Wheat, which was the only cereal in this study and known to be an important constituent of the diet in Kurdistan region, had a Se concentration of (49.4 µg kg^{-1} dw, n=14). Compared to other countries this was only higher than the value reported from Spain[e] while it was lower than all other countries. For example Egypt[m] and Pakistan[n] reported Se concentrations of 193 and 220 µg kg^{-1} (dw) (Table 3.7).

In general, leafy vegetables had a higher Se concentration compared to the other plant groups studied; in general the order of Se concentrations was leafy vegetables > tubers >fruit vegetables >grains (Fig. 3.21).

Overall, the plant Se concentrations in this study appear to be higher than in most other published studies, possibly suggesting greater Se availability in calcareous Kurdistan soils; this will be discussed in the following section. It should also be considered that values based on fresh weight are subject to variation depending on temperature, humidity, irrigation time and stage of plant maturity as all of these factors can influence plant water content.

Plant samples could be contaminated by soil due to dust or small soil particles during the sample collection or preparing the samples. The possibility of contamination was assessed by measuring several candidate elements in soils and plant samples, including aluminium, vanadium, chromium and iron. These elements have a high correlation in soils but would not be expected to be correlated in plants unless they originate from superficial contamination with soil dust, rather than systemic uptake through roots. So, for example,

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a,b,c and …. are the references demonstrated in Table 3.7

the proportion of Se in plant originating from contamination with soil dust was calculated by the below equation which assumed that all vanadium in the plant originates from soil dust (Shetaya et al., 2012):

$$\frac{Plant(V)\times Soil(Se)}{Plant(Se)\times Soil(V)}$$ Eq 3.1

Plant (V)= vanadium concentration in plant (mg kg^{-1})

Plant (Se)= Se concentration in soil (mg kg^{-1})

Soil (V)= vanadium concentration in soil (mg kg^{-1})

Plant Se= Se concentration in plant (mg kg^{-1})

This proportion was also calculated based on the assumption that the concentrations of Al, Cr and Fe all originated from soil dust. The average proportion of Se when all plants were considered was 0.014 (1.4%) and values ranged from 0.0002 to 0.078 (90% of plants < 0.01 (1%)).

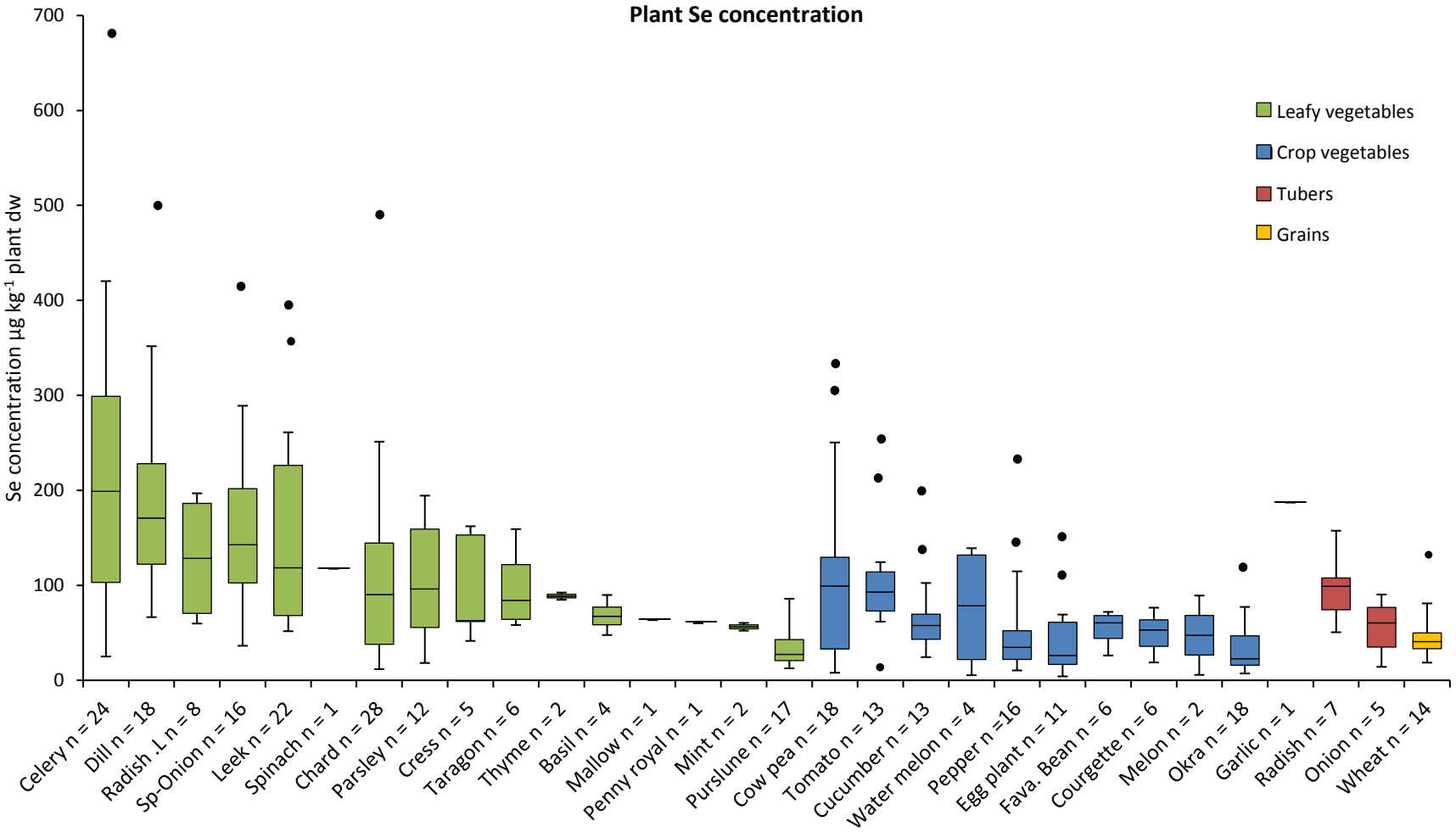


Figure 3-21. Selenium concentration of plants including leafy vegetables, crop vegetables, tubers and grains and the distribution of each vegetable selenium concentration in different locations

Table 3.7. Vegetable selenium content compared to other studies which are carried out in different countries

	plants	n	This study	Other authors	This study	Other authors
			Se µg Kg^{-1} (dw)	Se µg Kg^{-1} (dw)	Se µg Kg^{-1} (fw)	Se µg Kg^{-1} (fw)
Leafy vegetables	Celery	24	215	106[d]	59.8	3[b]; 14.8[d]; 2.4[f]; 14.7[h]; 11.8[i]; 4[o]
	Dill	18	188	121[o]	36.1	13.4[f];
	Radish Leaves	8	185		37.4	
	Sp-Onion	16	167		20.3	6[b]; 5[o]
	Leek	22	152	82.9[d]	31.0	8.2[d]; 2.1[g]; 10[o]
	Spinach	1	117	169[d]; 16[n];	13.4	6[b]; 7[c]; 12.4[d]; 24[k]; 10[o]
	Chard	28	112	72[d];	23.0	7.3[d]; 9[o]
	Parsley	12	104	63[d]; 141[o]	35.3	[1.4-24.2][a]; 7[c]; 7.2[d]; 7.2[f]; 17.6[h]; 9.0[h]; 1[o]
	Cress	5	96.3		13.2	9.0[o]
	Taragon	6	96.3	44[o]	43.0	
	Thyme	2	88.6	46[o]	12.1	
	Basil	4	68.1	30[o]	11.0	3[b]; 3[o]
	Mallow	1	63.4		15.3	11[c];
	Pennyroyal	1	59.9		12.0	
	Mint	2	56.3		16.7	14[c]
	Purslune	17	36.4		6.34	9[o]
Crop vegetables	Cow pea	18	108	45[c];	44.9	58[b]; 9[o]
	Tomato	13	108	36.2[d]; 39[n]; 55[o]	13.1	[1.1-29.1][a]; 12[b]; 15[c]; 1.8[d]; 2.3[f]; 0.5[g]; 7.9[h]; 10.2[h]; 0.5[i]; 0.3[l]; 1[m]; 5[o]
	Cucumber	13	95.1	72.2[d]; 21[c]	11.4	14.7[a]; 4[b]; 1.8[d]; 11.5[h]; 6.3[h]; 3[o]
	Water melon	4	75.4		6.89	1[b]; 6[k]; 4[o]
	Pepper	16	59.2	52[c]; 30[o]	10.4	11[a]; 1[b]; 4.2[f]; 0.7[g]; 9.1[h]; 32[k]; 3[o]
	Egg plant	10	60.3	67[c]	10.1	3[b]; 4[k]; 3[o]
	Fava. Bean	6	54.8	31[c]	13.8	8[o]
	Courgette	6	49.8	32[c]	4.43	2[o]
	Melon	2	47.5		3.26	4[c]; 4[o]
	Okra	18	37.6		13.1	27[c]; 7[o]
tubers	Garlic	1	187	142[o]	67.7	34[b]; 13.7[f]; 57.9[g]; 34.2[h]; 57.6[h]; 21[k];
	Radish	7	95.9	33[c];18[d];13[n];	19.7	4.5[a]; 2[b]; 87[d]; 0.7[g]; 1[k]; 7[o]
	Onion	5	55.3	43[c]; 40.9[d]; 137[n];	8.92	[1.1-10.5][a]; 11[b]; 5.4[d]; 7.3[f]; 5.8[g]; 0.7[g]; 15.3[h]; 12.4[h];52[k]; 5[o]
cereal	Wheat	14	49.4	165[c]; 54.8[d]; 35.5[e]; 114[k]; 193[m]; 220[n]; 61[o]	49.4	11[a]; 48.7[d]; 70[f]; 25.1[g]; 28[j];

a (Smrkolj et al., 2005) , b(Sirichakwal et al., 2005) , c(Al-Ahmary, 2009) ,d (De Temmerman et al., 2014), e(Díaz-Alarcón et al., 1996), f(Pappa et al., 2006) , g(Kadrabova et al., 1997) , h(Klapec et al., 2004) ,i(McNaughton and Marks, 2002) , j(Sigrist et al., 2012) ,k(Choi et al., 2009) , l(Ventura et al., 2009) , m(Hussein and Bruggeman, 1999), n(Waheed et al., 2002), o(USDA, 2017)

3.4.4.3 Plant Se content in relation to soil properties

Figure 3.22 shows the relationship between soil pH and Se concentration in plants. Plants grown in soils with pH>8 generally had higher Se concentrations compared to those grown in soils with lower pH values. The relationship between plant Se concentration and soil pH appears to be an exponential association, especially evident in pepper, cowpea and eggplant. However, this pattern was only found for fruit vegetables and was not followed by leafy vegetables (Fig 3.23). A possible reason for this could be that fruit vegetables need a longer growth period than leafy vegetables; alternatively internal Se mobilization and plant transfer mechanisms may be the cause.

The Se uptake of plants can be mediated by sulphate transporters due to the chemical similarity between sulphate and selenate. These transporters have different selectivity for selenate and sulphate in various plant species (White et al., 2004).

Selenate is known to be the predominant form of inorganic Se in alkaline aerobic soils (Elrashidi et al., 1987). Chilimba et al. (2011) studied maize grain Se content in relation to soil properties in Malawi. They found higher Se concentrations in maize grown on calcareous vertisols than on a range of acidic soil types. They concluded that the pH-dependence of selenite and selenate adsorption on Fe oxides could be one of the reasons for this trend. Peak and Sparks (2002) also reported that the specific bonding (co-ordination) of selenate on Fe oxides decreased between pH 3.5 and 6 and selenite was adsorbed specifically in pH higher than the 'point of zero charge' (7-8) of oxide

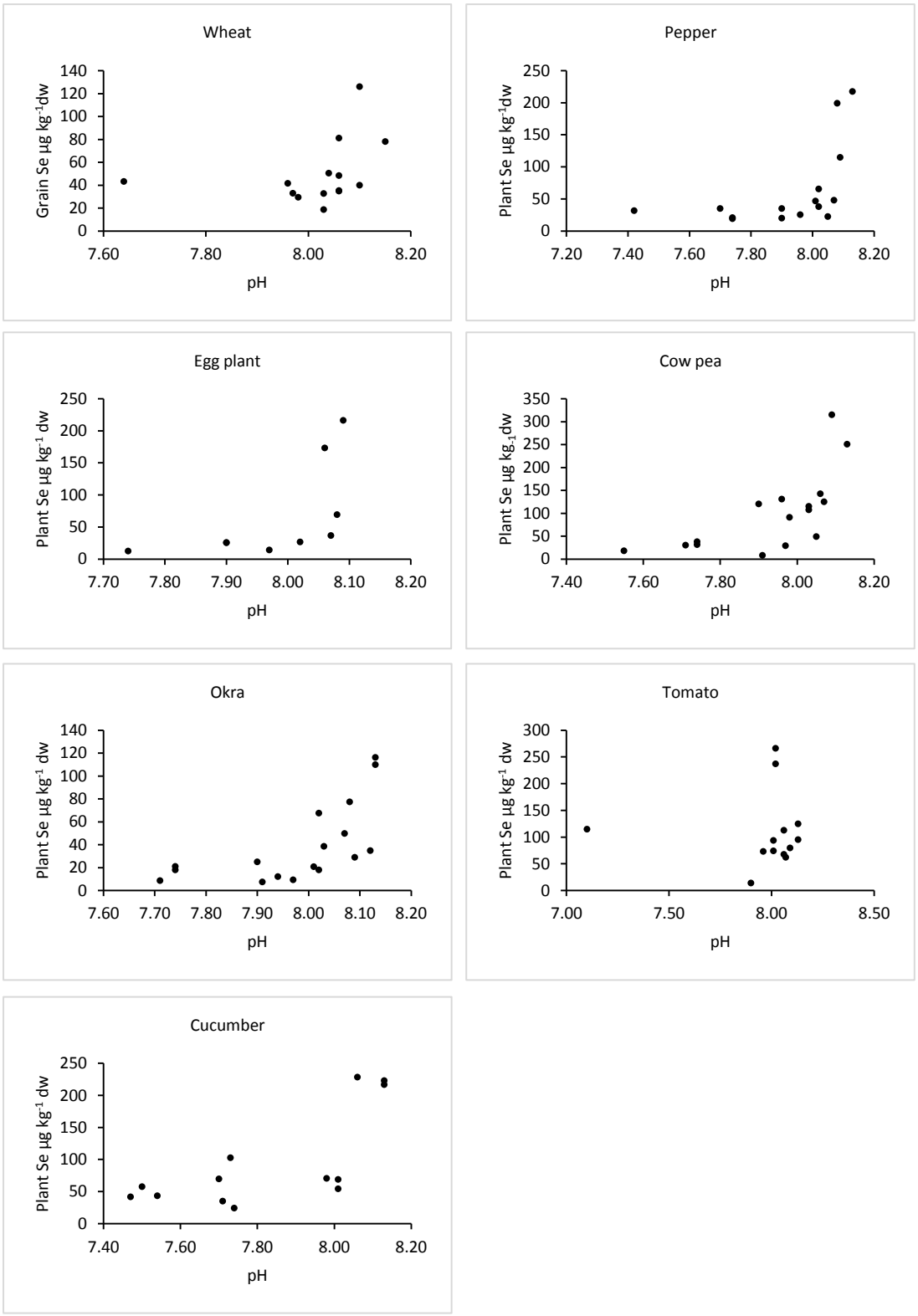


Figure 3-22. Selenium concentration in crop vegetables and grains as a function of soil pH

minerals. Other studies of Se bioavailability and solubility have also pointed out the role of phosphate ($H_2PO_4^-$, HPO_4^{2-}) competition for soil adsorption sites and sulphate (SO_4^{2-}) for root uptake. Considering the calcareous Kurdistan soils of this study it is likely that availability of Se would be relatively high due to low Se adsorption strength and the formation of relatively insoluble calcium phosphates.

Gissel-Nielsen (1975) found that the Se content of plants decreased during the growing season, suggesting a gradual depletion of

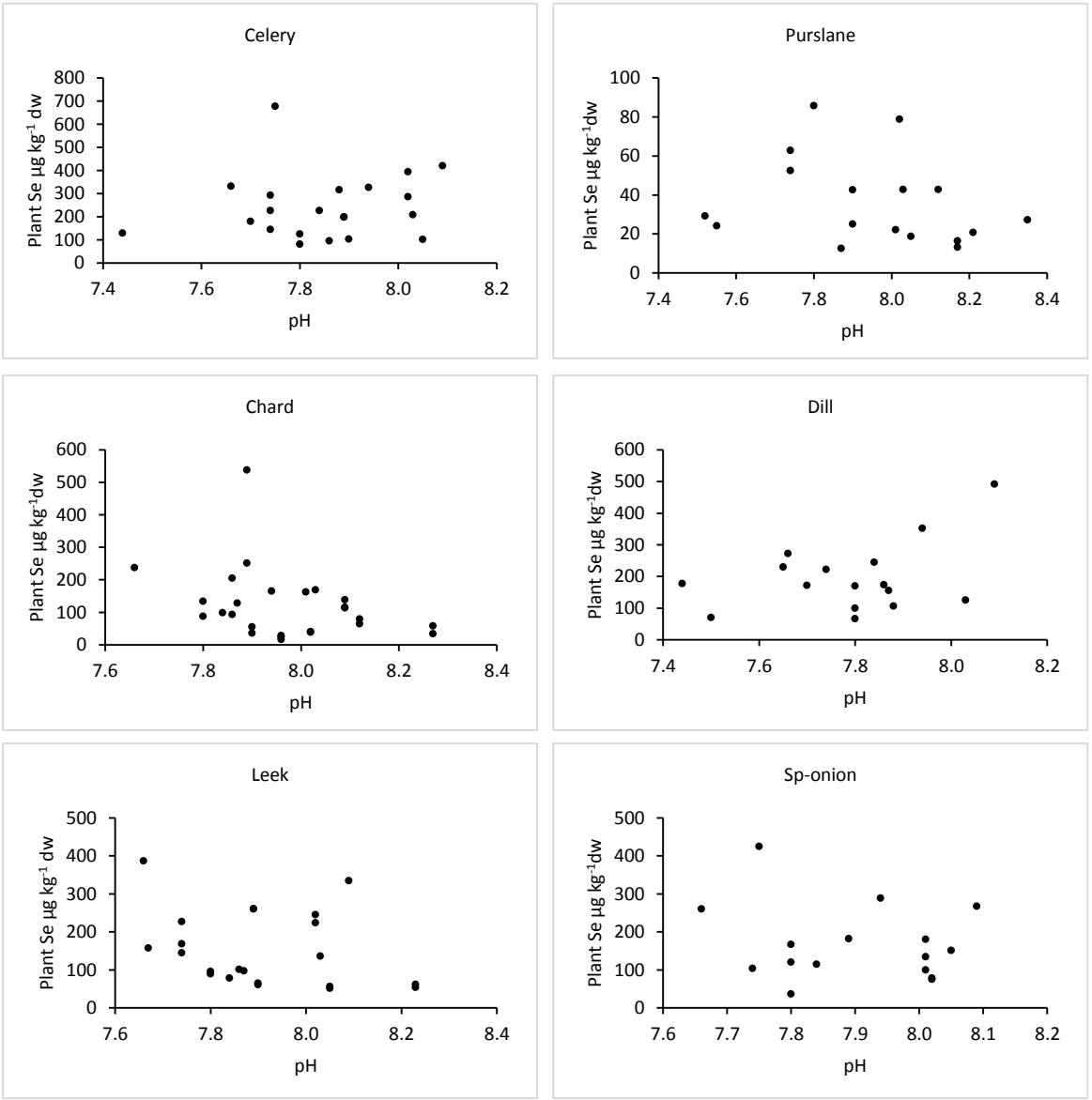


Figure 3-23. Selenium concentration in leafy vegetables as a function of soil pH

bioavailable soil Se. During the winter when no Se is taken up by plants, organic decomposition and weathering processes may replenish the available Se pool, allowing high Se uptake in the beginning of the growing season. It was suggested that as plants develop rapidly during the growing season Se depletion will exceed its rate of replenishment. They also found that the Se content of plants during June, July and August was 6-7 times lower compared to March. This may be one of the reasons that leafy vegetables did not seem to be affected by higher Se availability in soils with pH>8. The leafy vegetables were collected in April, which is the start of the growing season and had a smaller biomass compared to fruit vegetables. By contrast, the fruit vegetables were collected in July and August, at which time available Se may be depleted, and had a much greater biomass compared to the leafy vegetables.

A similar relationship could not be found for the rest of the plants due to insufficient samples. Figure 3.24 shows all plants together based on both a fresh and dry weight concentration of Selenium.

Only a very small proportion of total soil Se was present in a soluble form (Se_N <1%) but higher Se concentrations in some plants were associated with higher concentrations of soluble Se (Se_N) (Fig. 3.25).

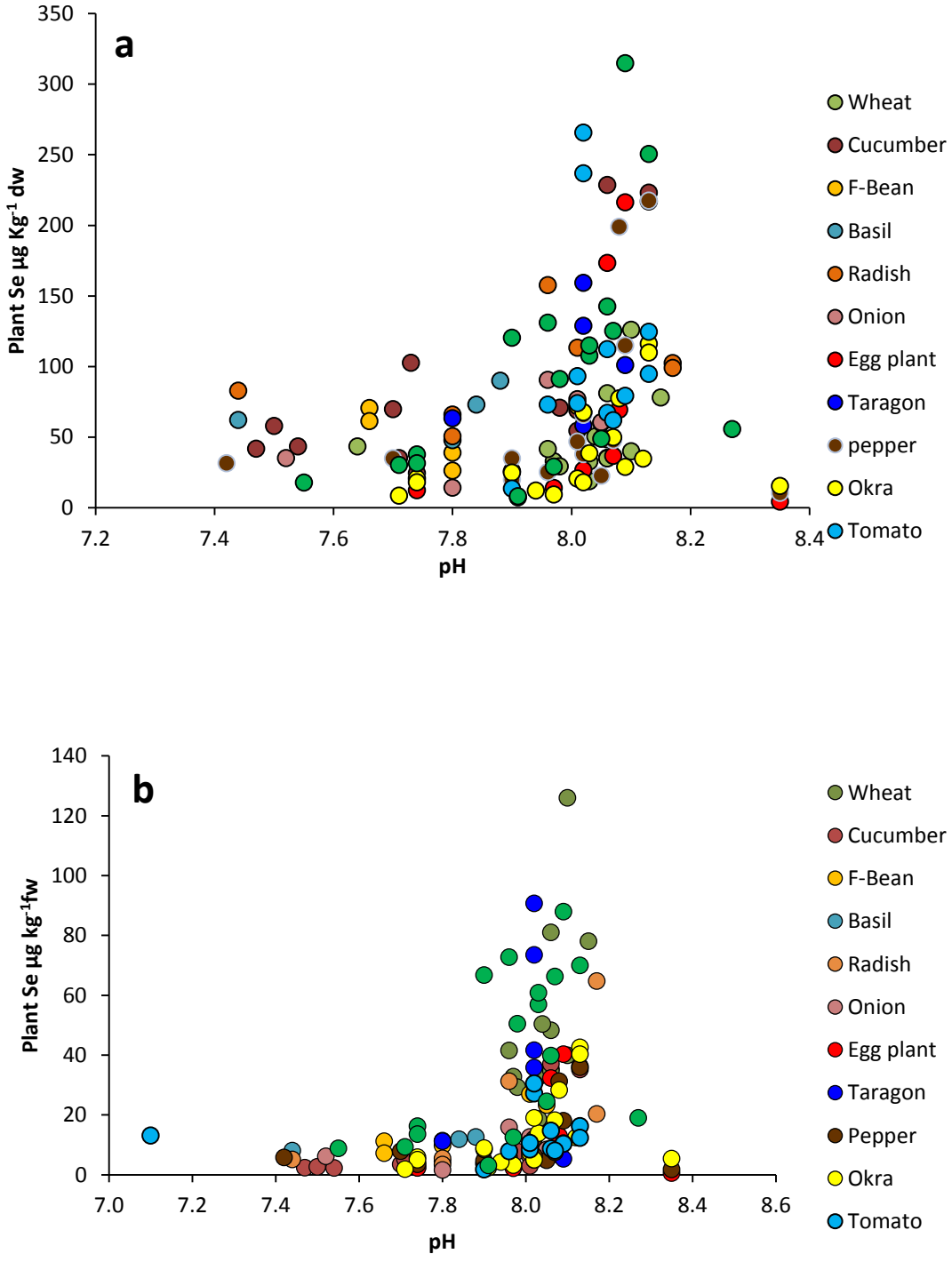


Figure 3-24. Selenium concentration in plants as a function of soil pH on a dw(a) and fw(b) basis

The scatter in Fig. 3.26 partly arises because the soil samples were aggregated across individual farms and not directly associated with particular plant samples. For several vegetables there were insufficient samples to form a convincing correlation.

Figures 3.26 and 3.27 illustrates a similar trend between plant Se content for both leafy and fruit vegetables and phosphate-extractable Se (Se_P). A general trend in Figures 3.27 and 3.28 reveals that the Se

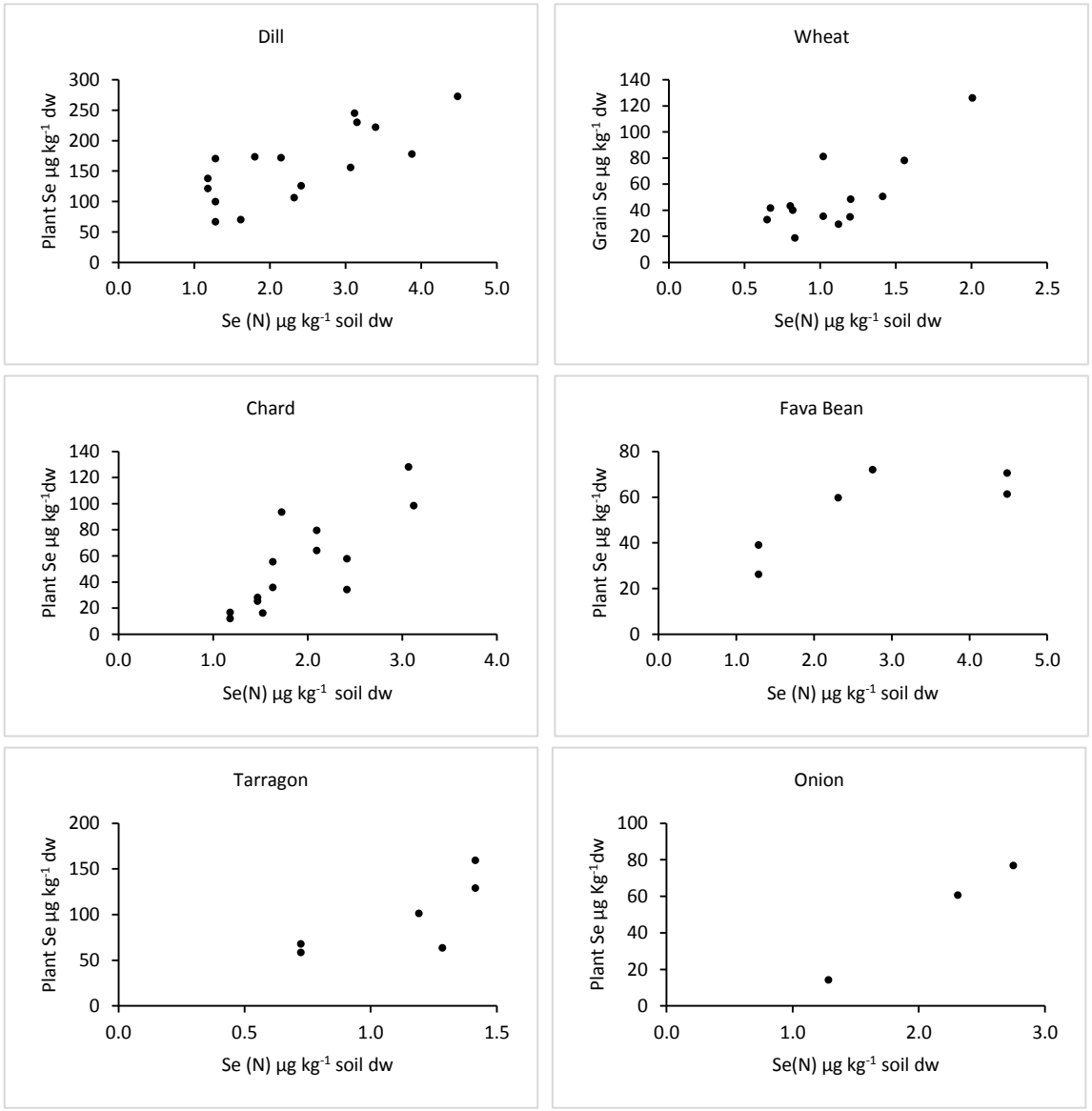


Figure 3-25. Plant selenium uptake from se soluble and available (extracted by 0.01M KNO_3)

concentrations in fruit vegetables increased substantially when Se_P exceeded 15 μg kg^{-1}. However, those soils were also the most alkaline with soil pH>8 and some soils with low Se_P concentrations also had pH values > 8.

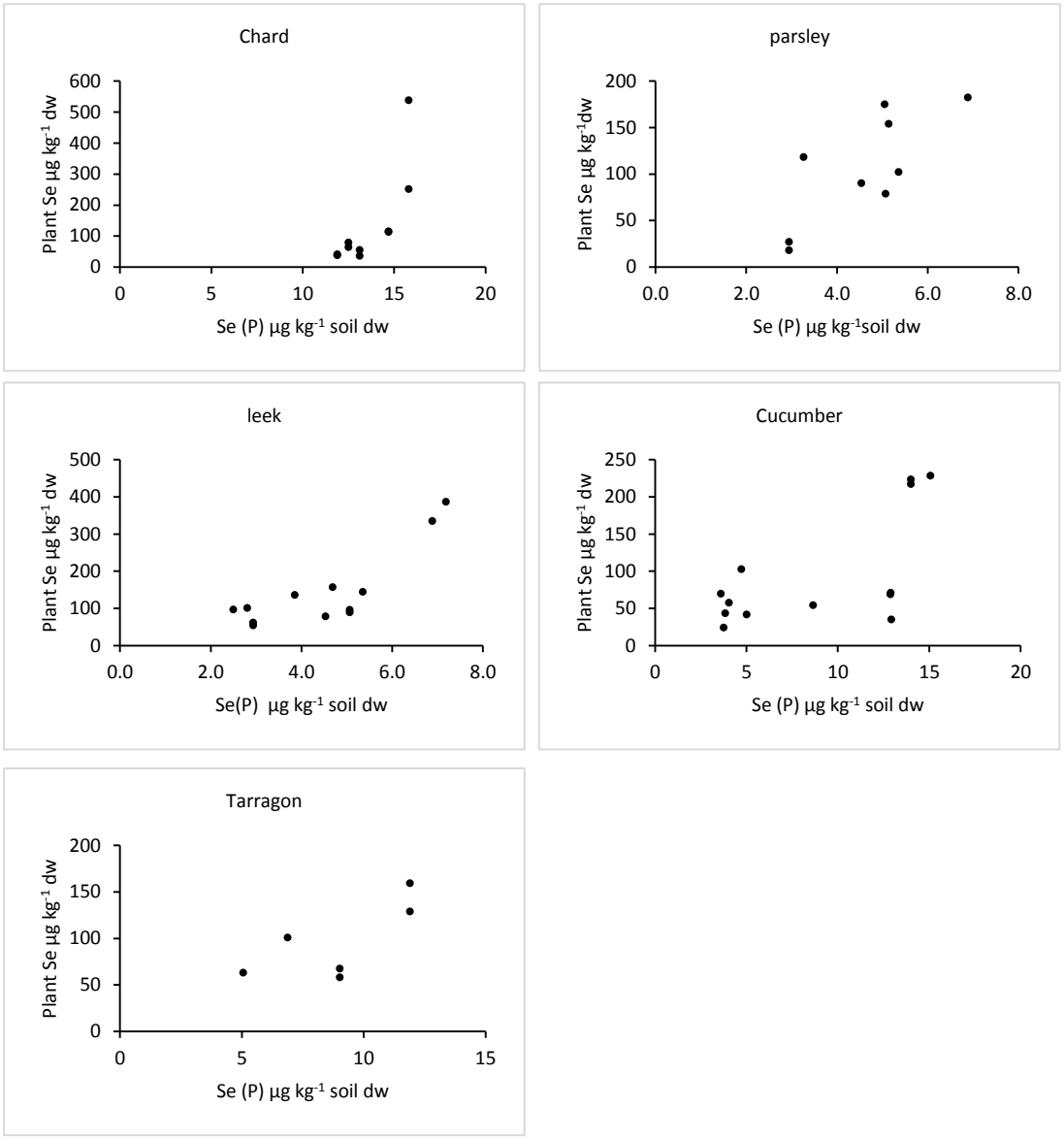


Figure 3-26. Plant selenium uptake from adsorbed Se (extracted by 0.016M KH_2PO_4) in leafy vegetables

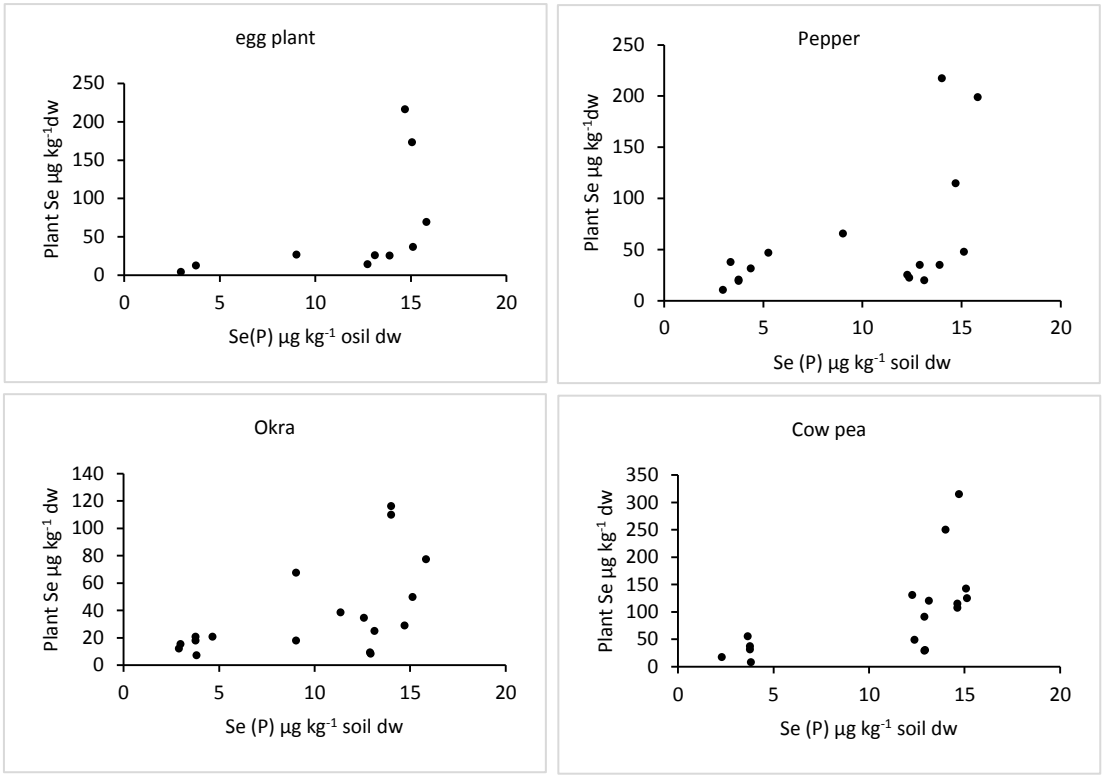


Figure 3-27. Plant selenium uptake from absorbed Se (extracted by 0.016M KH_2PO_4) in crop vegetables

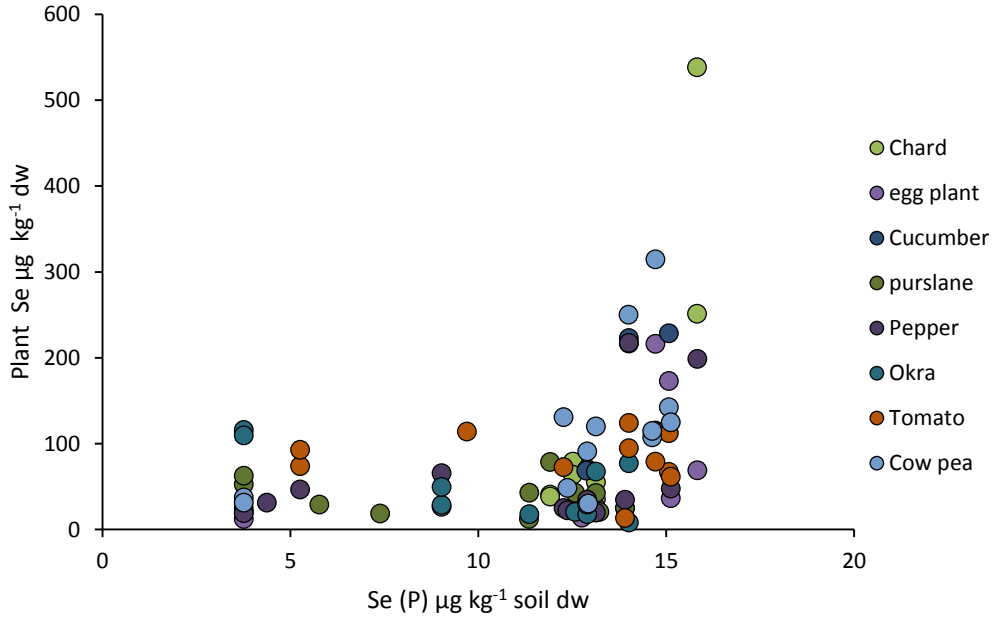


Figure 3-28. Plant selenium uptake from absorbed Se (extracted by 0.016M KH_2PO_4)

3.4.4.2 Soil-to-plant transfer factor

The simplest form of the soil-to-plant transfer factor (TF) is defined as total concentration in plant divided by total concentration in soil(Bitterli et al., 2010). However, values of TF can be calculated for total, extractable or soluble Se concentrations in soil depending on which fraction is regarded as the driver for plant uptake. Transfer factors based on total soil Se concentration are more likely to vary with soil properties while values of TF based on available Se fractions are likely to apply more widely (Bitterli et al., 2010). For all plant species of this study values of TF were calculated based on soluble Se_N ($Se\text{-}TF_N$), adsorbed ($Se\text{-}TF_{N+P}$), organic-bound ($Se\text{-}TF_{N+P+TMAH}$) and total fraction ($Se\text{-}TF_{HF}$) (Table 3.8).

Among leafy vegetables, $Se\text{-}TF_N$ was higher in celery, sp-onion, dill and leek ($Se\text{-}TF_N$ >100) showing a greater capacity to accumulate Se compared to other leafy vegetables ($Se\text{-}TF_N$ <70). Overall this factor fell in range of 31 to 164 when all leafy vegetables are considered. In the fruit vegetables group cow pea and tomato found to have the highest TF (>100) while the rest of the fruit vegetables had less values (<56). This factor for tubers was in the range of 34 to 68 and it was 44 for wheat.

Selenium-TF_{N+P} was distributed more evenly compared to $Se\text{-}TF_N$ and fell in range from 4.5 to 32 for purslane and celery respectively in leafy vegetables, ranged from 3.1-11 for fruit vegetables in Okra and Cowpea and 7.7 to 39 for onion and garlic respectively. This factor was

8.1 in wheat. The TF based on organic bound Se and total soil Se was <1 except one vegetable.

It was observed the values of TF in both soluble and adsorbed fractions varied widely in one type vegetable grown in different areas or sites which could rely on soil properties that mentioned in previous section. However difference in average of TFs can imply different inherent affinity of vegetables for Se. On the other hand, different types of vegetables growing the same site had different TF which reflects the physiologic difference for uptaking the Se by these plants. It can be concluded that plant's Se content seems to be controlled by a variety of factors including soil properties and plant factors. Soil pH was found the more important factor in this calcareous soil affecting plant Se content.

Table 3.8. Selenium soil to plant transfer factors based on each fraction of selenium in the soil

Plant species	n	$Se\text{-}TF_N$				$Se\text{-}TF_{N+P}$				$Se\text{-}TF_{N+P+TMAH}$				$Se\text{-}TF_{HF}$			
		min	max	Med	Mean	min	max	Med	Mean	min	max	Med	Mean	min	max	Med	Mean
Leafy Vegetables																	
Celery	20	20.2	545	85.4	164	6.8	130.8	23.1	32.1	0.51	7.07	1.38	1.86	0.14	2.42	0.48	0.75
Dill	17	43.2	413	73.0	110	10.5	70.0	23.8	27.6	0.45	3.53	1.25	1.52	0.24	1.84	0.66	0.71
Radish Leaves	6	26.4	144	59.2	67.8	4.7	31.8	16.3	18.0	0.41	2.15	1.03	1.10	0.21	0.85	0.49	0.51
Sp-Onion	13	28.3	323	76.6	116	5.7	81.8	21.7	28.0	0.25	4.42	0.95	1.32	0.13	1.52	0.52	0.62
Leek	16	25.0	339	64.2	107	4.4	41.4	16.2	19.3	0.53	2.22	1.00	1.09	0.15	1.25	0.40	0.50
Spinach	1	34.6	35	34.6	34.6	13.4	13.4	13.4	13.4	0.66	0.66	0.66	0.66	0.35	0.35	0.35	0.35
Chard	20	10.5	225	53.5	71.3	3.1	32.9	14.3	15.1	0.17	1.76	0.71	0.83	0.07	0.75	0.36	0.38
Parsley	10	13.9	174	48.5	67.2	3.6	24.3	12.1	14.3	0.44	2.43	0.85	1.03	0.14	0.85	0.42	0.45
Cress	4	20.3	75	57.0	52.4	8.8	27.4	16.1	17.1	0.35	2.26	1.16	1.23	0.21	0.79	0.47	0.48
Taragon	4	49.2	112	89.0	84.9	6.9	12.5	10.9	10.3	0.43	1.30	0.60	0.73	0.17	0.38	0.25	0.26
Thyme	2	66.0	72	68.9	68.9	13.3	14.5	13.9	13.9	0.57	0.63	0.60	0.60	0.30	0.33	0.31	0.31
Basil	4	16.0	39	30.2	28.8	6.7	16.1	8.5	10.0	0.32	1.12	0.42	0.57	0.17	0.43	0.24	0.27
Mallow	1	49.4	49	49.4	49.4	10.0	10.0	10.0	10.0	0.43	0.43	0.43	0.43	0.22	0.22	0.22	0.22
Pennyroyal	1	46.7	47	46.7	46.7	9.4	9.4	9.4	9.4	0.41	0.41	0.41	0.41	0.21	0.21	0.21	0.21
Mint	2	30.2	62	46.3	46.3	11.3	11.5	11.4	11.4	0.35	0.41	0.38	0.38	0.12	0.19	0.15	0.15
Purslune	14	8.0	67	26.2	31.5	1.0	13.5	3.2	4.5	0.14	0.65	0.22	0.30	0.05	0.30	0.09	0.12
Crop vegetables																	
cow pea	9	10.6	239	94.8	103	1.9	27.4	8.7	11.4	0.21	1.78	0.92	0.87	0.07	0.63	0.39	0.36
tomato	8	8.9	327	83.9	105	0.9	24.3	7.4	9.0	0.10	2.14	0.68	0.71	0.03	0.59	0.21	0.24
Cucumber	11	6.5	145	17.8	32.9	2.2	14.3	5.7	7.0	0.15	1.22	0.54	0.55	0.05	0.57	0.28	0.25
water melon	4	3.6	67	32.2	33.6	1.3	8.9	5.3	5.2	0.05	1.37	0.75	0.73	0.02	0.56	0.31	0.30
Pepper	13	8.8	264	23.5	56.2	1.4	14.0	3.5	5.3	0.13	1.38	0.28	0.41	0.04	0.56	0.10	0.17
Egg plant	10	3.5	135	15.8	37.6	1.0	10.6	2.0	3.1	0.08	1.12	0.21	0.31	0.02	0.47	0.07	0.12
Fava. Bean	6	13.7	30	23.1	22.0	4.1	15.0	6.1	7.5	0.18	0.63	0.28	0.34	0.09	0.42	0.16	0.21
Courgette	6	9.2	91	36.8	41.2	2.1	20.6	5.7	8.0	0.16	1.48	0.54	0.59	0.08	0.30	0.19	0.19
melon	2	3.7	46	24.8	24.8	1.3	6.0	3.7	3.7	0.06	0.82	0.44	0.44	0.03	0.35	0.19	0.19
okra	15	2.9	102	15.2	31.4	0.5	7.5	2.7	3.1	0.06	0.63	0.22	0.27	0.02	0.30	0.09	0.10
Tubers																	
Garlic	1	68.1	68	68.1	68.1	39.0	39.0	39.0	39.0	1.63	1.63	1.63	1.63	1.10	1.10	1.10	1.10
Radish	5	21.3	83	50.1	48.9	8.0	18.3	10.4	12.2	0.34	0.98	0.45	0.60	0.18	0.44	0.27	0.30
Onion	5	11.0	65	27.9	34.6	2.2	16.0	6.6	7.7	0.10	0.68	0.46	0.42	0.05	0.45	0.25	0.23
Cereals																	
Wheat	13	9.8	79	48.8	44.4	3.9	14.3	8.6	8.1	0.16	1.24	0.49	0.60	0.05	0.38	0.16	0.20

3.4.5 Soil iodine

3.4.5.1 Total soil iodine

Total soil iodine ranged from 1200 to 9310 µg kg^{-1}, mean 4140 µg kg^{-1} (n=97). Halabja and Sulaimani areas had higher mean soil iodine concentrations (5270 µg kg^{-1}, n=35) and (3980 µg kg^{-1}, n=41) respectively than the Sharazoor and Kalar areas (2950 µg kg^{-1}, n=8) and (2350 µg kg^{-1}, n= 13) respectively (Fig 3.29). Fuge and Johnson (1986) reported average values of 2400, 5000-200000, 2700, 2300 and 800 µg kg^{-1} for igneous rocks, recent sediments, carbonates and shales respectively. The British Geological Survey soil iodine survey (Johnson, 2003) found a wide range for totals soil concentration of the UK (500-36900 µg kg^{-1}) and mean of 8800 µg kg^{-1} for 80 sites where sampled during 1973 and 1999. This database also gave an average value of 2200, 14200, 3800 for German, Japanese and Russian soils respectively. In addition, Shacklette et al. (1984) calculated the whole U.S. soils iodine concentration in range of 500-

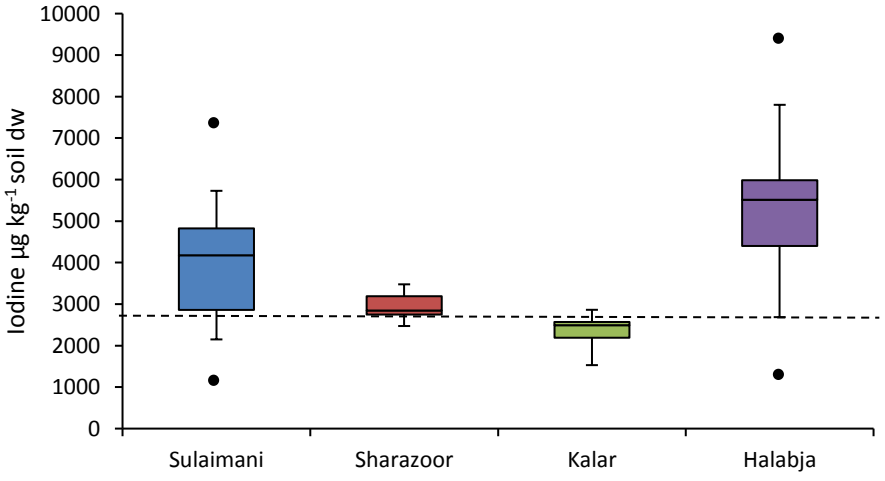


Figure 3-29. Total soil iodine content in the four main areas Sulaimani; n=41, Sharazoor; n=8, Kalar; n=13, Halabja; n= 35.

9600 µg kg^{-1}. Based on the value of 2700 µg kg^{-1} for sedimentary and carbonate soils, two of the studied areas were more than this value and two areas were in line with the value or in some locations found lower (Fig. 3.29). Zia et al. (2015) reported total iodine in 86 location of Pakistan as a low iodine soils ranged from 190 to 9590 µg kg^{-1} (mean = 660 µg kg^{-1}). Johnson (2003) investigated total iodine of 2151 soil and concluded the value of 5100 µg kg^{-1} as an average of total soil iodine. The same author also suggested that as the data were skewed the geometric mean of 3000 µg kg^{-1} was a better estimation of total soil iodine. Overall, the range of soil iodine concentrations measured in soils of the Kurdistan region were comparable with the mean of those reported for other soils.

3.4.5.2 Fractionation and extractability of soil iodine

Soluble soil iodine (I_N) was in the range of 25.8 to 173 µg kg^{-1} with a mean concentration of 51.0 µg kg^{-1}. The soils of the Sulaimani area had a higher mean I_N concentration (65.5 µg kg^{-1}) compared to the other areas (33 - 42 µg kg^{-1}) (Fig 3.30a). The I_N was typically 1.59% of total soil iodine content (I_{Tot}) when all areas considered; ranged from 0.7% to 12.0%. Calcareous soils from Pakistan (Zia et al., 2015) and Malawi (Watts et al., 2015b) had similar water soluble iodine concentrations; 2.36% and 1.38% of soil I_{Tot} respectively. Soluble iodine (extractable with KCl) comprised less than 10% of the total iodine of 183 UK top soils; approximately 80% contained <10% water extractable iodine (Hu et al., 2009).

Mean concentration of adsorbed soil iodine (I_P) was 332 µg kg^{-1} (ranged 107 to 566 µg kg^{-1}). The mean of I_P for four main areas ranged from

287 to 335 µg kg^{-1} (Fig 3.30 b). The ratio of I_P as a proportion of I_{Tot} was 10.7% (ranged 2.0- 26.2%) Table 3.9. Mean I_P concentration was 6 times greater than the mean of I_N (Table 3.9).

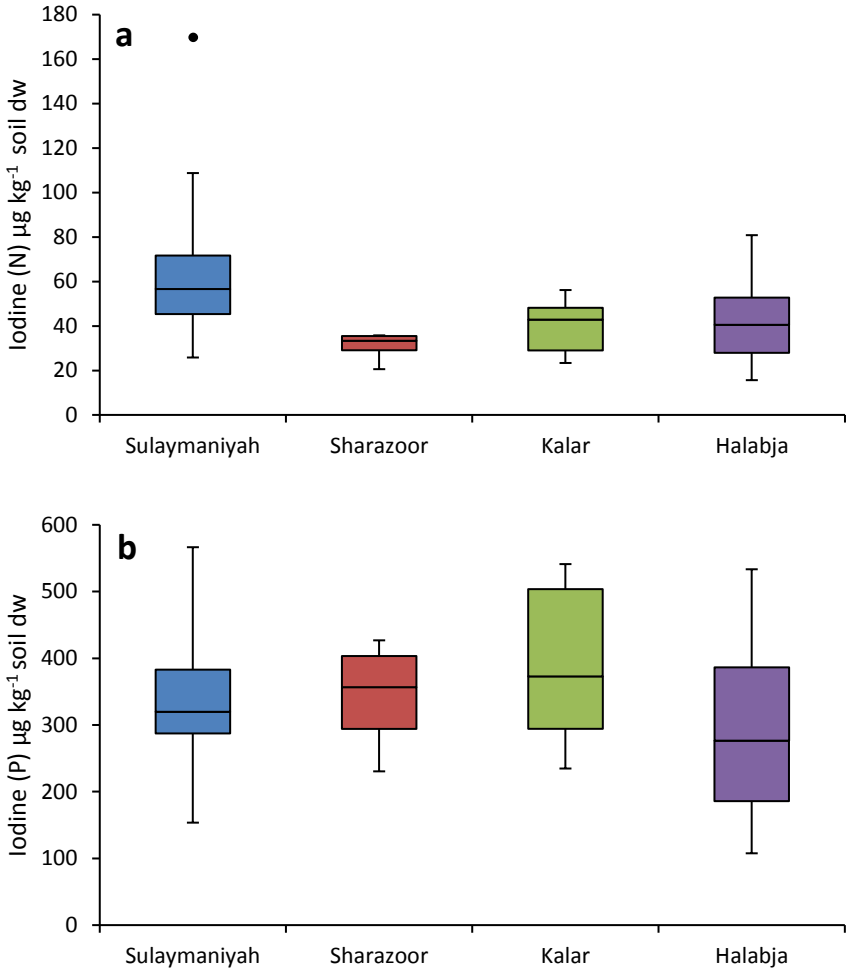


Figure 3-30. Soil iodine content in fractions a) soluble I extracted by 0.01M KNO_3 b) Adsorbed I extracted by 0.016M KH_2PO_4

Table 3.9. Iodine extractability as percentage of total soil iodine in fractions

Area	Sub area	n	I -N extractable			I -P extractable		
			Min	Max	Mean	Min	Max	Mean
					%			
Sulaimani	*Tanjaro*	15	0.772	4.48	1.76	8.65	14.8	10.9
	Baziyan	2	1.89	5.64	3.77	10.6	18.2	14.4
	Zhalla	2	4.39	5.28	4.84	15.3	26.2	20.8
	Dukan	2	1.87	3.84	2.85	8.36	15.7	12.01
	West area	3	1.18	12.0	1.84	11.6	4.54	11.8
	Industrial	2	1.79	2.22	2.00	7.44	7.48	7.46
	Kana Sura	9	0.923	2.43	1.44	4.40	8.33	7.04
	Kani Goma	6	1.35	2.03	1.66	6.02	8.50	7.28
Halabja	*Kani Kolka*	13	0.309	1.093	0.176	2.56	9.36	2.03
	Anab	8	0.323	1.22	0.730	3.60	7.42	5.77
	Kani Spi	5	0.533	1.39	0.868	2.01	4.98	3.21
	Bakrawa	4	2.04	3.48	2.67	9.95	12.3	11.4
	Zamaqi	2	1.33	2.19	0.430	13.7	14.8	0.542
	Ka Grdal	1			1.39			8.45
	Bia Wella	1			0.85			13.6
	Khormal	1			0.92			6.69
Sharazoor	*Sharazoor*	7	0.862	1.40	1.14	10.8	17.8	13.7
	New Halabja	1			1.53			10.4
Kalar	*Kalar*	13	1.07	2.96	2.11	11.9	32.7	20.6

3.4.5.3 Soil iodine speciation

The majority of I_N was organic (75.5, 69.4, 65.9 and 58.5%, mean =70%) for Sulaimani, Kalar, Halabja and Sharazoor respectively. The proportion of inorganic iodine of I_N was (41.5, 34.1, 30.6 and 24.5%, mean=30%,) for Sharazoor, Halabja, Kalar and Sulaimani respectively (Table 3.10). Among this 30%, the majority of inorganic iodine found in form of iodide (93.5, 91.1, 90.6, 78.9% mean 83.3%,) for Sulaimni, Halabja, Sharazoor and Kalar respectively. The iodine of I_P fraction found more organic compared to soluble fraction (>90%) the vast majority of inorganic I_P was iodide (Table 3.10).

Table 3.10. Iodine speciation in soil solution and absorbed fraction and proportion of iodate and iodide in each fraction a) Sulaimani b) Sharazoor c) Kalar and d) Halabja.

a)

Area	Sub area	Code	I (N) µg kg^{-1}	IO_3^- (N) µg kg^{-1}	I^- (N) µg kg^{-1}	In-org-I[a] %	Org-I[b] %	IO_3^- (N)[c] %	I^- (N)[d] %	I (P) µg kg^{-1}	IO_3^- (p) µg kg^{-1}	I^- (p) µg kg^{-1}	In-org %	Org-I %	IO_3^- (p) %	I^- (p) %
Sulaimani	*Tanjaro*	STA 12	55.2	4.43	3.58	14.5	85.5	55.4	44.6	293		27.9	9.51	90.5	0.00	100
		STA 13	109	3.43	26.8	27.8	72.2	11.3	88.7	335		26.8	7.98	92.0	0.00	100
		STA 14	57.9	0.665	21.7	38.7	61.3	2.97	97.0	297	0.783	30.0	10.4	89.6	2.54	97.5
		STA 15	41.6	0.375	1.43	4.34	95.7	20.8	79.2	280	0.129	24.6	8.84	91.2	0.524	99.5
	Baziyan	SBY 1	47.2	4.62	13.5	38.5	61.5	25.4	74.6	264		15.7	5.94	94.1	0.00	100
		SBY 2	173	0.578	164	95.3	4.71	0.35	99.6	559		161	28.8	71.2	0.00	100
	Zhalla	SZH 1G	108	0.477	21.7	20.6	79.4	2.15	97.9	313	0.550	29.1	9.46	90.5	1.85	98.1
		SZH 2	94.3	12.7	23.9	38.8	61.2	34.6	65.4	562	47.1	24.1	12.7	87.3	66.1	33.9
	Dukan	DUK 1	38.5	1.36	1.014	6.16	93.8	57.2	42.8	157	0.208	8.77	5.71	94.3	2.31	97.7
		DUK 2G	48.4	0.136	3.35	7.19	92.8	3.91	96.1	217		7.44	3.43	96.6	0.00	100
	Kana Sura	SKS 4	42.7	0.188	5.69	13.8	86.2	3.20	96.8	168		12.9	7.68	92.3	0.00	100
		SKS 5	56.6	0.255	13.1	23.6	76.4	1.91	98.1	328	0.196	12.3	3.81	96.2	1.57	98.4
		SKS 6	66.8	0.265	24.4	36.9	63.1	1.08	98.9	287	1.052	20.2	7.40	92.6	4.94	95.1
		SKS 7	70.8	0.177	1.69	2.63	97.4	9.50	90.5	306	0.127	22.4	7.36	92.6	0.56	99.4
		SKS 8	70.0	0.264	7.23	10.7	89.3	3.52	96.5	309	0.305	17.0	5.59	94.4	1.77	98.2
		SKS 9	60.0	0.278	0.0304	0.51	99.5	90.1	9.86	322	0.471	0.472	0.29	99.7	49.9	50.1
	Kani Goma	SKG 1	71.7	0.307	13.5	19.3	80.7	2.22	97.8	309	0.512	12.2	4.10	95.9	4.04	96.0
		SKG 2	101	0.197	24.9	24.7	75.3	0.784	99.2	424	0.271	18.8	4.49	95.5	1.42	98.6
		SKG 3	76.8	0.256	13.8	18.4	81.6	1.82	98.2	344	0.486	11.4	3.44	96.6	4.10	95.9
		SKG 4	89.3	0.343	31.2	35.4	64.6	1.09	98.9	422	0.533	15.6	3.82	96.2	3.31	96.7
		SKG 5	91.1	0.884	33.3	37.5	62.5	2.59	97.4	389	2.59	5.39	2.05	97.9	32.4	67.6
		SKG 6	63.0	0.015	14.4	22.9	77.1	0.107	99.9	281	2.70	5.25	2.83	97.2	33.9	66.1

b)

Area	Sub area	Code	I (N) µg kg^{-1}	IO_3^- (N) µg kg^{-1}	I^- (N) µg kg^{-1}	In-org-I %	Org-I %	IO_3^- (N) %	I^- (N) %	I (P) µg kg^{-1}	IO_3^- (p) µg kg^{-1}	I^- (p) µg kg^{-1}	In-org %	Org-I %	IO_3^- (p) %	I^- (p) %
Sharazoor	*Sharazoor*	SHA 1	21.6	0.99	7.97	41.5	58.5	11.1	88.9	268	1.44	10.6	4.49	95.5	11.9	88.1
		SHA 2	20.6	0.00	9.63	46.8	53.2	0.00	100	290	3.73	11.2	5.17	94.8	24.9	75.1
		SHA 3	29.2	1.98	17.3	66.0	34.0	10.3	89.7	414	5.28	8.021	3.21	96.8	39.7	60.3
		SHA 4	32.8	1.14	1.12	6.90	93.1	50.5	49.5	400	1.38	37.6	9.75	90.2	3.54	96.5
		SHA 5	35.8	1.28	23.2	68.5	31.5	5.24	94.8	427	5.81	14.7	4.81	95.2	28.3	71.7
		SHA 6	35.4	1.80	15.9	50.1	49.9	10.1	89.9	352	4.28	13.5	5.05	94.9	24.1	75.9
		SHA 7	35.7	1.52	9.16	29.9	70.1	14.2	85.8	362	3.031	21.1	6.67	93.3	12.6	87.4
	New Halabja	SHA 8	33.9	0.701	6.66	21.7	78.3	9.53	90.5	230	0.369	17.0	7.55	92.5	2.12	97.9

c)

Area	Sub area	Code	I (N) µg kg^{-1}	IO_3^- (N) µg kg^{-1}	I^- (N) µg kg^{-1}	In-org-I %	Org-I %	IO_3^- (N) %	I^- (N) %	I (P) µg kg^{-1}	IO_3^- (p) µg kg^{-1}	I^- (p) µg kg^{-1}	In-org %	Org-I %	IO_3^- (p) %	I^- (p) %
		KAL 1	23.4	0.959	0.666	6.96	93.0	59.0	41.0	315	0.691	3.57	1.35	98.6	16.2	83.8
		KAL 2	42.9	2.78	4.043	15.9	84.1	40.7	59.3	524	9.74	7.49	3.29	96.7	56.5	43.5
		KAL 3 W	27.5	0.976	12.3	48.3	51.7	7.37	92.6	504	3.64	2.90	1.30	98.7	55.6	44.4
		KAL 3	36.4	1.70	17.9	53.9	46.1	8.65	91.4	541	10.9	3.47	2.65	97.4	75.8	24.2
		KAL4a	45.8	1.29	5.19	14.1	85.9	19.9	80.1	345	1.51	4.89	1.85	98.1	23.6	76.4
		KAL4b	56.2	1.35	4.72	10.8	89.2	22.3	77.7	294	0.403	13.2	4.62	95.4	2.97	97.0
Kalar	***Kalar***	KAL4c	48.2	2.77	1.39	8.63	91.4	66.7	33.3	403	2.32	11.7	3.47	96.5	16.6	83.4
		KAL 5G	55.4	0.464	4.43	8.83	91.2	9.48	90.5	266	0.376	6.94	2.75	97.2	5.14	94.9
		Kal 6	44.0	2.30	7.90	23.2	76.8	22.5	77.5	373	3.58	1.58	1.39	98.6	69.4	30.6
		KAL 6W	53.5	2.13	9.33	21.4	78.6	18.6	81.4	518	3.58	5.04	1.67	98.3	41.5	58.5
		KAL 7	29.3	8.23	14.7	78.2	21.8	36.0	64.0	450	22.1	7.47	6.58	93.4	74.7	25.3
		KAL 8	29.1	1.19	12.7	47.8	52.2	8.55	91.4	269	1.16	5.91	2.63	97.4	16.4	83.6
		KAL 9	27.7	3.01	13.5	59.7	40.3	18.2	81.8	235	8.74	6.18	6.36	93.6	58.6	41.4

d)

Area	Sub area	Code	I (N) µg kg^{-1}	IO_3^- (N) µg kg^{-1}	I^- (N) µg kg^{-1}	In-org-I %	Org-I %	IO_3^- (N) %	I^- (N) %	I (P) µg kg^{-1}	IO_3^- (p) µg kg^{-1}	I^- (p) µg kg^{-1}	In-org %	Org-I %	IO_3^- (p) %	I^- (p) %
		HBK 1	63.5	2.20	11.9	22.3	77.7	15.5	84.5	243	1.072	25.6	11.0	89.0	4.02	96.0
	Bakrawa	HBK 2	80.8	0.00	29.4	36.4	63.6	0.00	100	285	2.79	23.8	9.33	90.7	10.5	89.5
		HBK 3	66.1	2.47	25.5	42.3	57.7	8.82	91.2	314	2.67	17.0	6.27	93.7	13.5	86.5
		HBK 4	52.7	2.07	18.5	39.1	60.9	10.0	90.0	295	1.13	18.6	6.71	93.3	5.71	94.3
Halabja	***Zamaqi***	HZM 1	74.4	1.21	14.1	20.5	79.5	7.91	92.1	465	0.00	3.73	0.80	99.2	0.00	100
		HZM 2	45.9	1.17	13.1	31.0	69.0	8.25	91.7	509	1.018	21.1	4.34	95.7	4.60	95.4
	Ka Grdal	HKG	41.9	0.876	11.7	30.0	70.0	6.96	93.0	255	0.00	16.3	6.38	93.6	0.00	100
	Bia Wella	HBW1	30.6	1.24	20.4	70.9	29.1	5.72	94.3	492	3.14	22.2	5.14	94.9	12.4	87.6
	Khormal	HKH	18.5	0.445	2.16	14.1	85.9	17.1	82.9	134	0.207	13.5	10.2	89.8	1.51	98.5

a) Inorganic iodine as a percentage of I_N b) Organic iodine as a percentage of I_N C) Iodate as a percentage of inorganic iodine d) Iodide as a percentage of inorganic iodine

3.4.5.4 Relationship between soil iodine fractions and soil properties

No clear relationship between I_{Tot} and %OC was observed possibly due to the relatively narrow range of %OC (< 5%) (Fig. 3-31). In other studies a positive correlation between total iodine and organic matter has been reported e.g. (Watts et al., 2015a). Zia et al. (2015) also reported a weak positive trend when 10% of outlier points were removed. Shetaya et al. (2012) reported that TMAH extraction recovered 75-80% rate of ^{129}I (in organic rich soils whereas this value was greater (85-90%) in soils with lower organic matter content suggesting more retention of iodine and less extractable in higher organic soils.

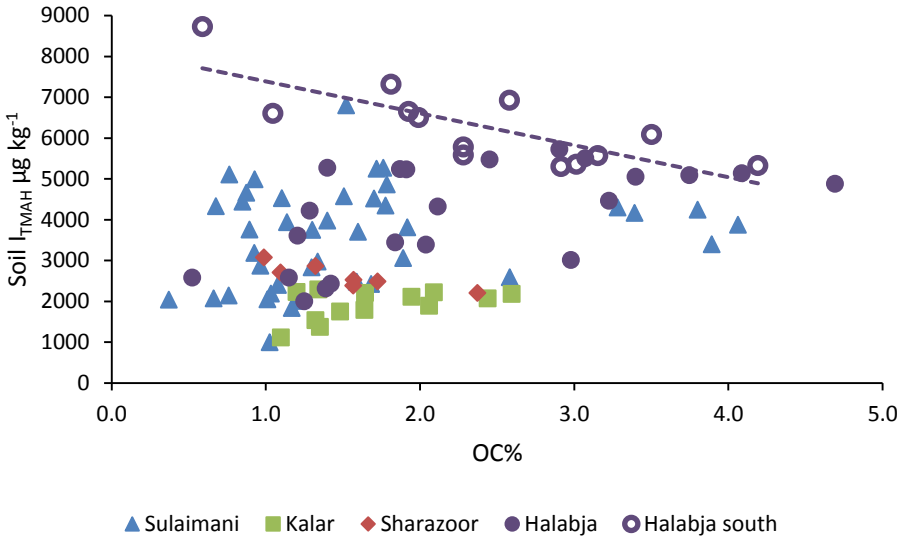


Figure 3-31. Relationship of soil organic carbon with soil iodine in 10% TMAH extract consider to areas or regional effects-Halabja south is (Anab+Kani Spi subareas) where are located next to each other

Figure 3-32a shows that a negative relationship between OC% and the I_P fraction exists in two sub areas of Halabja (Anab and Kanikolka) and in Sharazoor suggesting that, soils with a slightly higher %OC fix or retain iodine with strongly bound and cause less availability in those soils. The majority of I_P fraction was organic with a small amount of iodide. Iodide can bind strongly to aluminium and iron oxides and is a possible reasons for lower extractability of iodine in high organic soils. This relationship is not only for

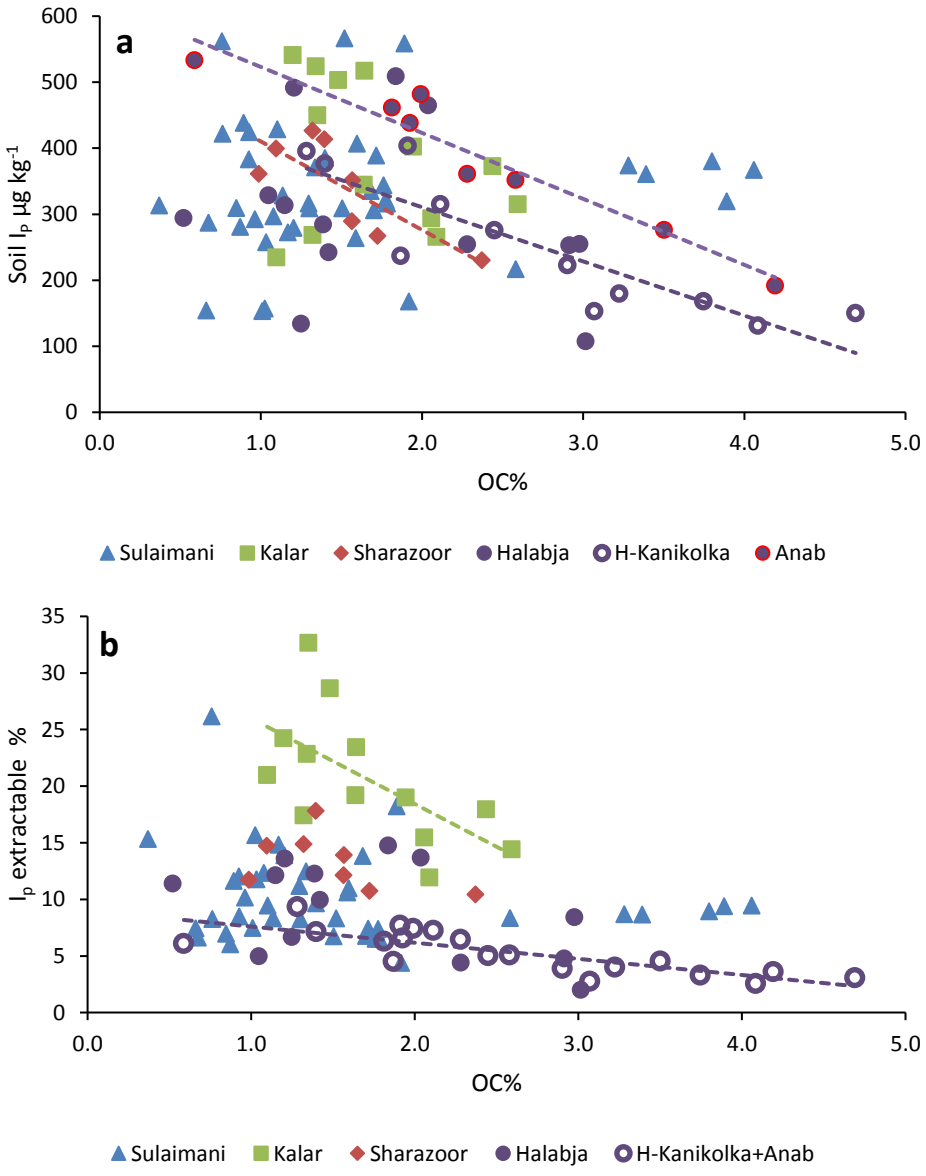


Figure 3-32. Relationship of soil organic carbon with (a) soil iodine in 0.016M KH_2PO_4 extract and (b) iodine extractable in this fraction as a proportion of total iodine according o areas or regional effects

the adsorbed fraction, it is true even for precentage of I_P extractable as a proportion of I_{Tot} in one of the areas and two subareas but no relationship when all areas considered (Fig. 3.32b).

No correlation was observed between OC% and I_N except in the Kani Kolka sub area of Halabja. The same result found for I_N extractable as proportion of I_{Tot}.

No correlation was observed between soil pH and total soil iodine content perhaps as a result of the narrow range of these soils pH (Fig. 3.33). Other studies have reported a negative correlation between soil pH and iodine content. (Watts et al., 2015b, Zia et al., 2015).

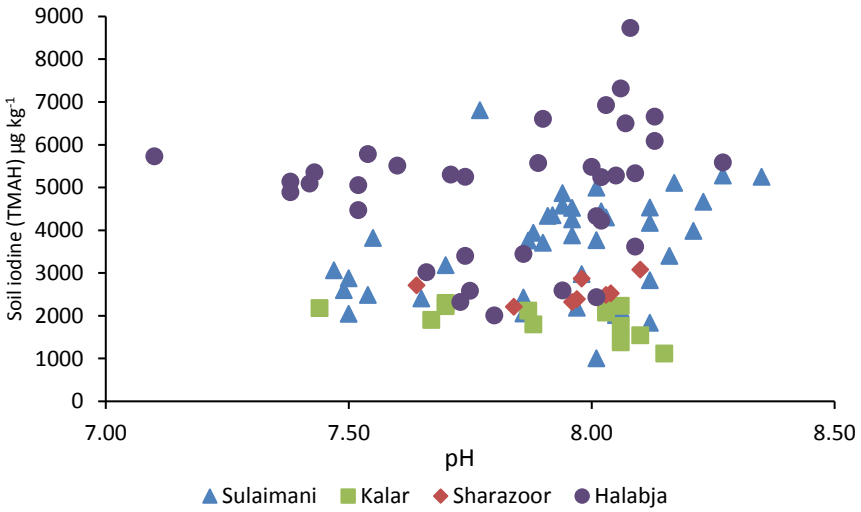


Figure 3-33. Relationship between soil pH and soil I in 10% TMAH extract

No correlation was found between soil I_P and I_N fractions with soil pH in areas and subareas. Soil soluble iodine seems to be not affected by variation of pH in that narrow range (7-8) .

Except Halabja area, in other three areas soil I_{Tot} was not affected by soil $\%CaCO_3$ however the subareas of Halabja correlated with soil $\%CaCO_3$. The reason for that could be different geology or parent material of these soils for example soils with higher $\%CaCO_3$ in those areas corresponded with parent material containing high iodine content.

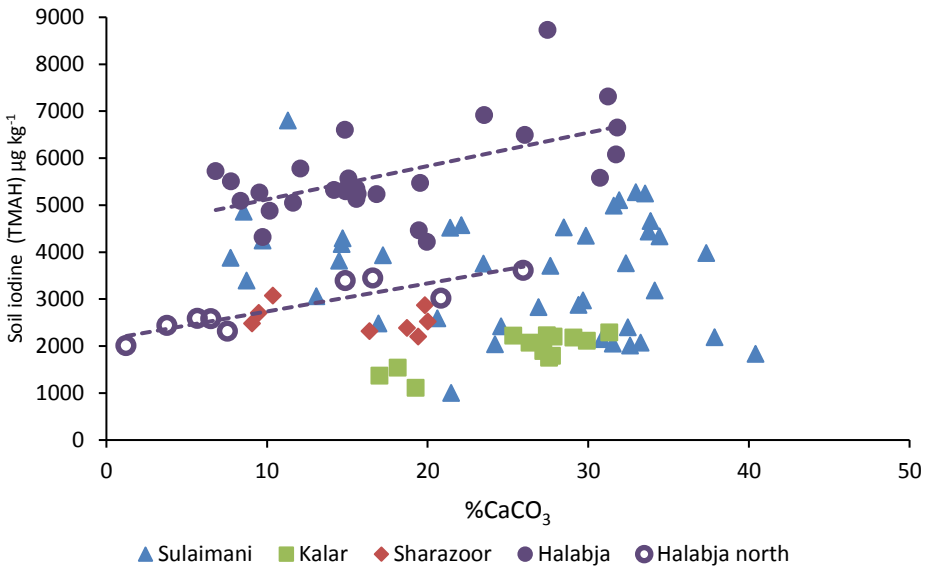


Figure 3-34. Relationship of soil $CaCo_3$ % (estimated based on soil in-organic carbon content) and total soil iodine extract 10% TMAH (r =0.56). Halabja north is subareas of (Bakrawa+Zamaqi+Kagrdal+Biawella) where are located next to each other.

3.4.5 Plant iodine

3.4.6.1 Plant iodine content

The range of iodine content in each plant is shown in Table 3.11 and Figure 3.35. In general the iodine content of the plants decreases in the order of leafy vegetables > fruit vegetables> tubers> grains.

Iodine concentrations were greatest in leafy vegetables (439 µg kg^{-1} n= 135). Within the leafy vegetable group, mallow (151 µg kg^{-1}, n=1) and leek (622 µg kg^{-1}, n=16) represented the extremes. Cowpea with an iodine content of 667 µg kg^{-1} (n=9) and watermelon with average of 215 µg kg^{-1} (n=4) represent the variation in iodine content in the fruit vegetables with the mean of 368 µg kg^{-1} (n=84). Tubers including garlic, onion and radish had lower iodine contents compared to other vegetables (140 µg kg^{-1}, n=11). Wheat had the smallest iodine content with a mean of 12.5 µg kg^{-1} (n=13) (Table 3.11).

The mean iodine content of plant species was comparable with other studies in the literature (Table 3.12).

Grain iodine content was comparable with data from Pakistan and less than reported by other workers for wheat from other countries (Table 3.12).

Table 3.11. Plants iodine content including leafy and crop vegetables, tubers and grains based on dry (DW) and fresh (FW) weight

Plant species	n	Iodine μg kg^{-1} (DW)			Iodine μg kg^{-1} (FW)		
		Mean	Median	Range	Mean	Median	Range
Leafy Vegetables							
Celery	20	554	505	196-1410	157	94.9	34.07-458
Dill	17	482	418	174-941	482	76.4	21.2-231
Radish Leaves	6	521	454	120-1090	101	64.7	8.17-342
Sp-Onion	13	275	233	138-434	34	28.4	11.7-102
Leek	16	622	438	189-2150	141	48.8	16.5-575
Spinach	1	462			52.7		
Chard	20	535	410	137-1490	112	85.5	15.3-388
Parsley	10	461	433	216-909	184	117	43.9-515
Cress	4	560	460	109-1210	77.2	57.6	20.5-173
Tarragon	4	528	524	204-860	77.2	229	19.1-490
Thyme	2	231	231	221-239	31.8	31.8	27.2-36.4
Basil	4	403	414	230-552	63.0	62.8	54.9-71.5
Mallow	1	151			36.3		
Pennyroyal	1	276			55.3		
Mint	2	491	491	322-659	54.0	158	65.7-249
Purslane	14	471	419	81.6-1070	83.3	74.3	6.49-226
Fruit vegetables							
Cow pea	9	667	639	275-951	270	253	119-503
Tomato	8	513	445	191-1010	65.5	58.2	21.9-132
Cucumber	11	231	187	95.3-603	25.0	9.6	4.36-97.8
Water melon	4	215	177	114-389	17.8	15.7	10.2-29.5
Pepper	13	394	307	116-1050	72.9	53.8	4.36-164
Egg plant	10	306	232	101-739	52.2	42.9	13.8-113
Fava. Bean	6	301	388	75.4-425	84.2	81.9	11.9-165
Courgette	6	286	184	124-572	24.4	16.3	10.4-59.6
Melon	2	507	507	127-886	37.9	37.9	8.72-67
Okra	15	255	247	134-431	86.3	82.8	48.3-158
Tubers							
Garlic	1	50.4			18.2		
Radish	5	224	126	101-570	31.0	11.4	8.17-113
Onion	5	145	47.6	37.3-439	26.7	6.64	5.11-77.05
Cereals							
Wheat	13	12.5	9.83	1.99-46.02	12.5	9.83	1.99-46.02

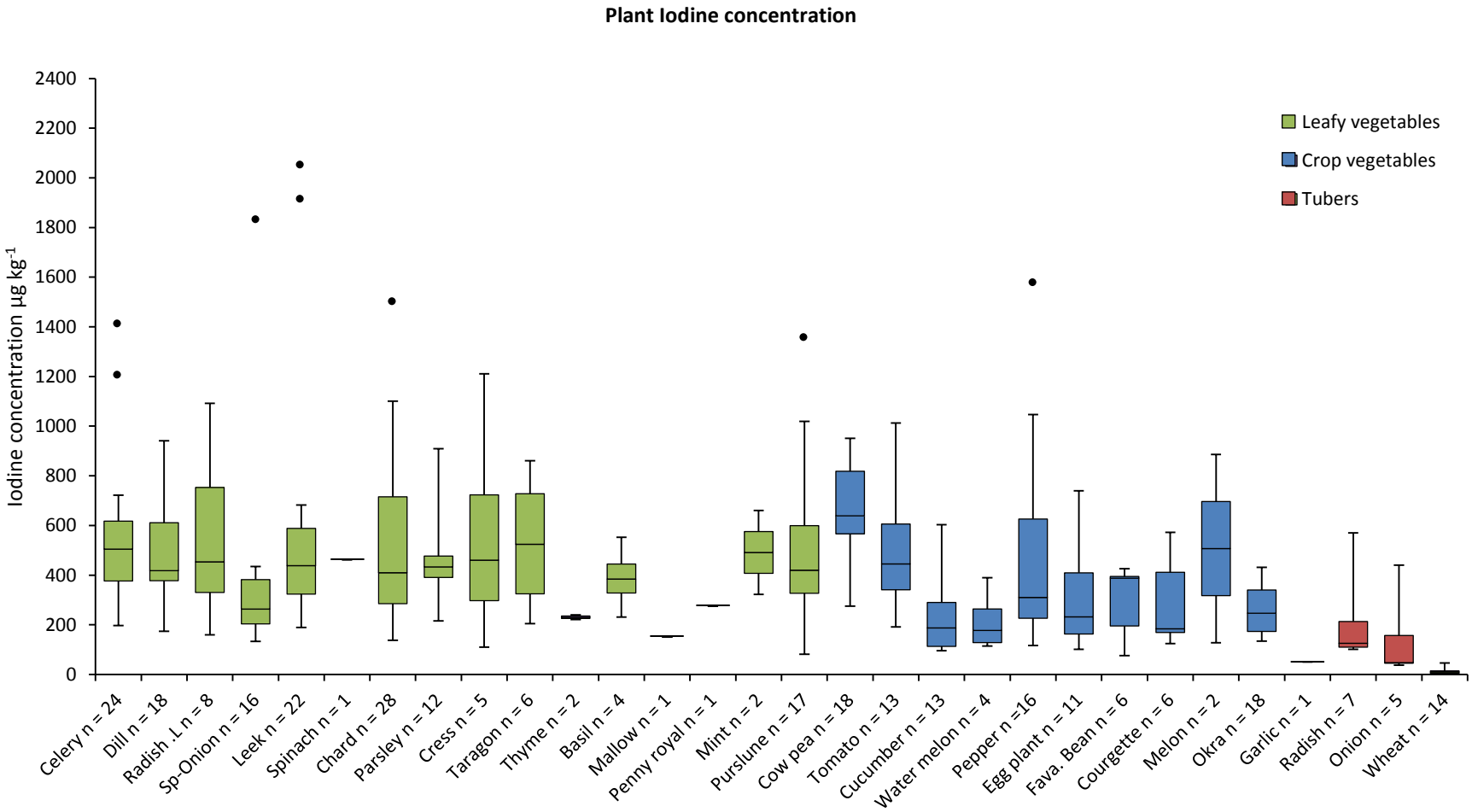


Figure 3-35. Total iodine concentration of plants including leafy vegetables, fruit vegetables, tubers and grains

3.12. Comparison of plant iodine content of this study with other studies as an a) individual plant species and b) averages as a food groups

a)

	Plants	n	This study I µg kg^{-1} (dw)	Other authors I µg kg^{-1} (dw)
Leafy vegetables	Celery	20	554	
	Dill	17	482	3.9[b], 388[b]
	Radish	6	550	
	Sp-Onion	13	267	2[b], 192[b]
	Leek	16	622	
	Spinach	1	462	1636[c], (19.5-690)[d],
	Chard	20	535	
	Parsly	10	461	3.2[b], 180[b],
	Cress	4	560	
	Taragon	4	528	
	Thyme	2	231	
	Basil	4	388	
	Mallow	1	151	
	Pennyroyal	1	276	
	Mint	2	491	87[e],
	Purslune	14	471	
Fruit vegetables	Cow pea	9	667	163[f], 390[f],
	Tomato	8	513	(1.4, 31)[b], 196[c], 82[g]
	Cucumber	11	231	(0.2, 43)[b], 400[c], 336[g],
	water	4	215	
	Pepper	13	394	(13.5, 157)[b], 356[e],
	Egg plant	10	306	
	Fava. Bean	6	301	201[f], 520[f],
	Courgette	6	286	
	Melon	2	507	
	Okra	15	255	
Tubers	Garlic	1	50.4	(66, 120)[f],
	Radish	5	224	
	Onion	5	145	(46-479)[h], 204[c],
Cereals	Wheat	13	12.5	10[i], 35[j], 74.4[a]

`

b)

Plant group	n	This study		Other authors	
		I µg kg^{-1} (dw)	I µg kg^{-1} (fw)	I µg kg^{-1} (dw)	I µg kg^{-1} (fw)
Leafy vegetables	135	439	109	315[a], 236[j],	
Fruit vegetables	84	368	73.6	163[a]	
Vegetables	209	412	95.0	123[k], 20[l],	(20-280)[l],
Tubers	11	140	25.3	233[a]	
Cereals	13	12.5	12.5	96.5[a], 107[a]	

a) (Fordyce, 2003), b)(Anke et al., 1995), c) (Chilean Iodine Educational Burean, 1952), d) (Remington et al., 1929), e)(Mahesh et al., 1992) , f)(Longvah and Deosthale, 1998) , g) (Anke et al., 1993), h) (Beeson and Matrone, 1976), i) (Zia et al., 2015), j) (Haldimann et al., 2005), k) (Hou et al., 1997), l) (Dahl and Meltzer, 2009).

3.4.6.2 Soil to plant transfer factor of iodine

A soil to plant transfer factor (TF) was calculated by dividing the total iodine content of the plant by of the soil iodine in the corresponding soil (TF_{Tot}). Transfer values were also calculated using I_N (TF_N) I_{N+P} (TF_{N+P}). The mean TF_{Tot} for leafy vegetables was 0.16 (range 0.07-0.39), fruit vegetables 0.09 (range 0.05-0.18,), tubers 0.06 (range 0.04-0.07) and for wheat was 0.01. The TF_{N+P} of leafy vegetables fell in range of (0.99-4.15, mean = 1.75), fruit vegetables (0.70-2.26, mean = 1.26), tubers (0.29-0.82, mean = 0.48) and wheat = 0.03. The TF_N was higher and for leafy vegetables was (6.20-36.6, mean = 13.6), fruit vegetables (3.41-16.5, mean = 8.90), tubers (1.31-7.23, mean = 4.77) and 0.42 for wheat. The order of $TF_N > TF_{N+P} > TF_{Tot}$ simply reflects the availability of iodine in those fractions for plant uptake. With different plant groups (TF_{Tot}, TF_{N+P}, and TF_N) were in the order of

leafy vegetables > fruit vegetables > tubers > grain.

3.4.6.3 Plant iodine content and relation to soil properties

A relationship between soil I_{Tot} and plant iodine content was observed in a few plant species e.g. chard, okra and cucumber. For the majority of plants the relationship was very weak or absent (Fig. 3.36).

In general no relationship was observed between Soil I_N and plant iodine concentration. Tarragon and cress appeared to have a linear positive relationship with soil I_N but were limited in both cases to only four samples (Fig. 3-38). Considering soil soluble iodine is the more available fraction for plant uptake but the proportion of this fraction of iodine was only 1% of total iodine compared to absorbed iodine which was 10% of soil total iodine.

Table 3.13. Soil-to-plant transfer factor according to vegetable groups and soil iodine fractions

Plant species	n	$I\text{-}TF_N$				$I\text{-}TF_{N+P}$				$I\text{-}TF_{Tot}$			
		Min	Max	Median	Mean	Min	Max	Median	Mean	Min	Max	Median	Mean
Leafy Vegetables													
Celery	20	3.92	29.70	10.99	12.96	0.72	5.02	1.45	1.66	0.08	0.30	0.15	0.17
Dill	17	5.07	34.88	9.42	12.57	0.80	2.59	1.41	1.53	0.09	0.41	0.16	0.19
Radish Leaves	6	4.62	169.86	18.93	36.65	1.19	19.04	1.62	4.15	0.08	1.57	0.20	0.39
Sp-Onion	13	2.34	45.51	7.32	10.04	0.61	1.87	0.93	1.07	0.06	0.33	0.10	0.13
Leek	16	2.02	53.15	10.50	14.43	0.66	6.75	1.39	1.72	0.09	0.41	0.13	0.17
Spinach	1	6.20	6.20	6.20	6.20	0.99	0.99	0.99	0.99	0.14	0.14	0.14	0.14
Chard	20	2.10	33.91	8.51	10.96	0.50	5.43	1.36	1.66	0.07	0.72	0.13	0.17
Parsley	10	6.35	20.03	10.15	11.02	0.94	3.24	1.01	1.47	0.07	0.23	0.16	0.16
Cress	4	5.92	13.29	10.47	10.03	0.82	3.11	1.06	1.51	0.05	0.24	0.22	0.18
Taragon	4	11.05	18.64	14.41	14.63	0.74	2.18	1.61	1.53	0.10	0.20	0.12	0.13
Thyme	2	11.97	12.96	12.46	12.46	1.65	1.78	1.72	1.72	0.11	0.12	0.11	0.11
Basil	4	8.92	23.67	12.41	14.35	1.18	1.82	1.74	1.62	0.11	0.25	0.21	0.20
Mallow	1	8.14	8.14	8.14	8.14	1.12	1.12	1.12	1.12	0.07	0.07	0.07	0.07
Pennyroyal	1	14.90	14.90	14.90	14.90	2.05	2.05	2.05	2.05	0.14	0.14	0.14	0.14
Mint	2	2.97	31.06	17.01	17.01	0.96	3.93	2.44	2.44	0.13	0.13	0.13	0.13
Purslane	14	1.60	31.05	9.84	11.34	0.49	4.09	1.13	1.75	0.04	0.29	0.10	0.12
Fruit vegetables													
Cow pea	9	9.40	30.17	14.63	16.58	1.16	3.28	2.70	2.26	0.05	0.15	0.13	0.11
Tomato	8	4.73	38.11	9.13	13.42	0.47	3.08	1.44	1.51	0.04	0.15	0.07	0.08
Cucumber	11	0.61	9.65	2.77	3.74	0.19	1.66	0.52	0.73	0.03	0.09	0.05	0.06
Water melon	4	2.04	5.51	3.05	3.41	0.31	1.27	0.68	0.74	0.04	0.09	0.06	0.06
Pepper	13	1.92	38.58	7.55	10.81	0.29	8.23	1.04	1.62	0.03	0.30	0.08	0.10
Egg plant	10	1.12	21.53	6.63	8.20	0.26	2.25	0.76	0.87	0.02	0.11	0.06	0.06
Fava. Bean	6	1.80	21.13	10.26	11.23	0.30	2.91	2.02	1.77	0.03	0.39	0.19	0.18
Courgette	6	1.77	24.21	5.99	8.18	0.30	1.48	0.84	0.84	0.03	0.12	0.07	0.07
Melon	2	2.82	12.65	7.73	7.73	0.34	2.87	1.61	1.61	0.04	0.19	0.12	0.12
Okra	15	1.63	15.89	4.29	5.63	0.37	1.74	0.65	0.70	0.03	0.08	0.05	0.05
Tubers													
Garlic	1	1.31	1.31	1.31	1.31	0.32	0.32	0.32	0.32	0.05	0.05	0.05	0.05
Radish	5	1.98	9.13	5.94	5.79	0.52	1.35	0.76	0.82	0.05	0.11	0.05	0.07
Onion	5	0.97	28.14	2.46	7.23	0.17	0.41	0.24	0.29	0.02	0.09	0.04	0.04
Cereals													
Wheat	13	0.07	1.58	0.35	0.42	0.01	0.11	0.02	0.03	0.00	0.02	0.00	0.01

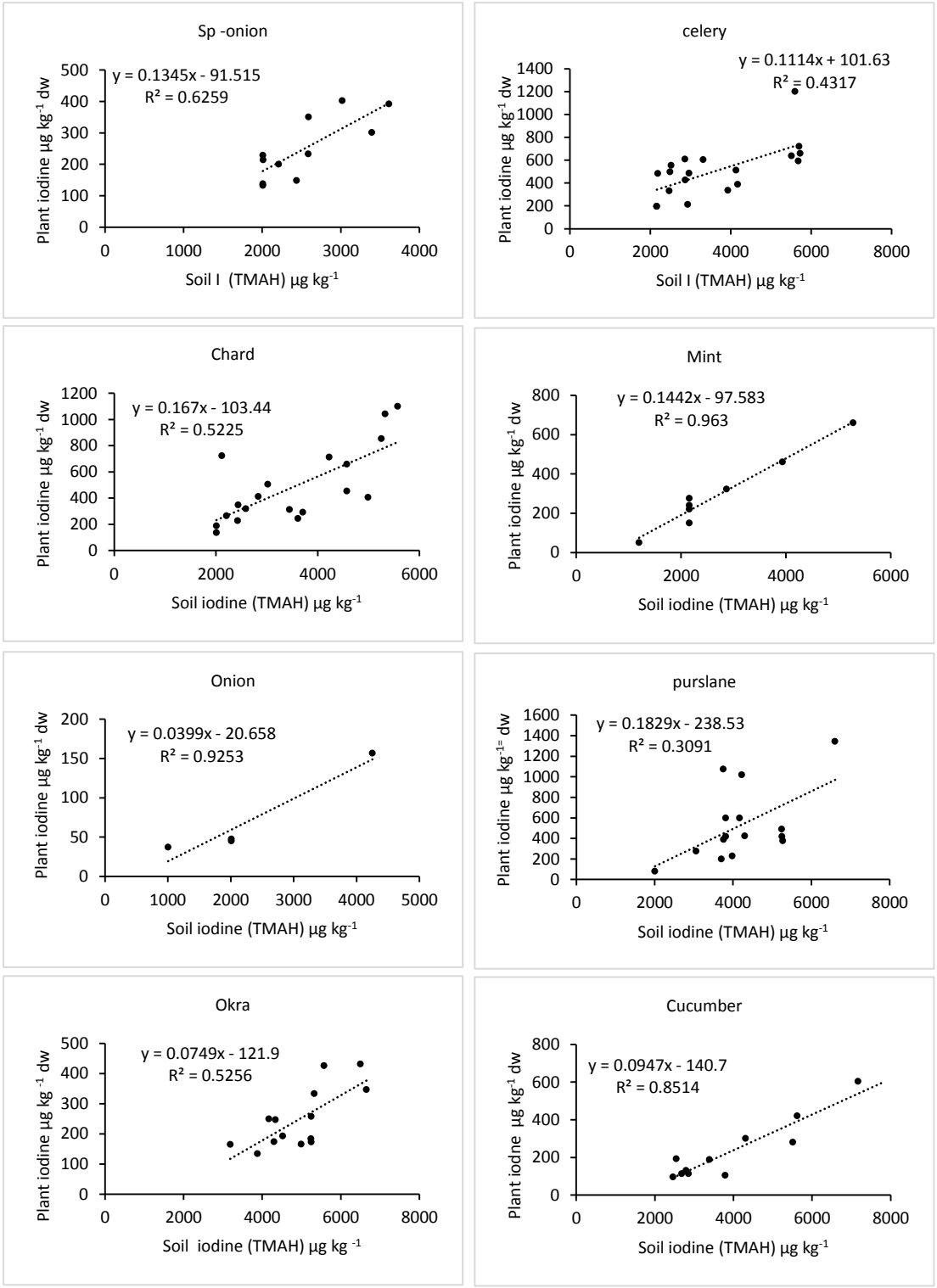
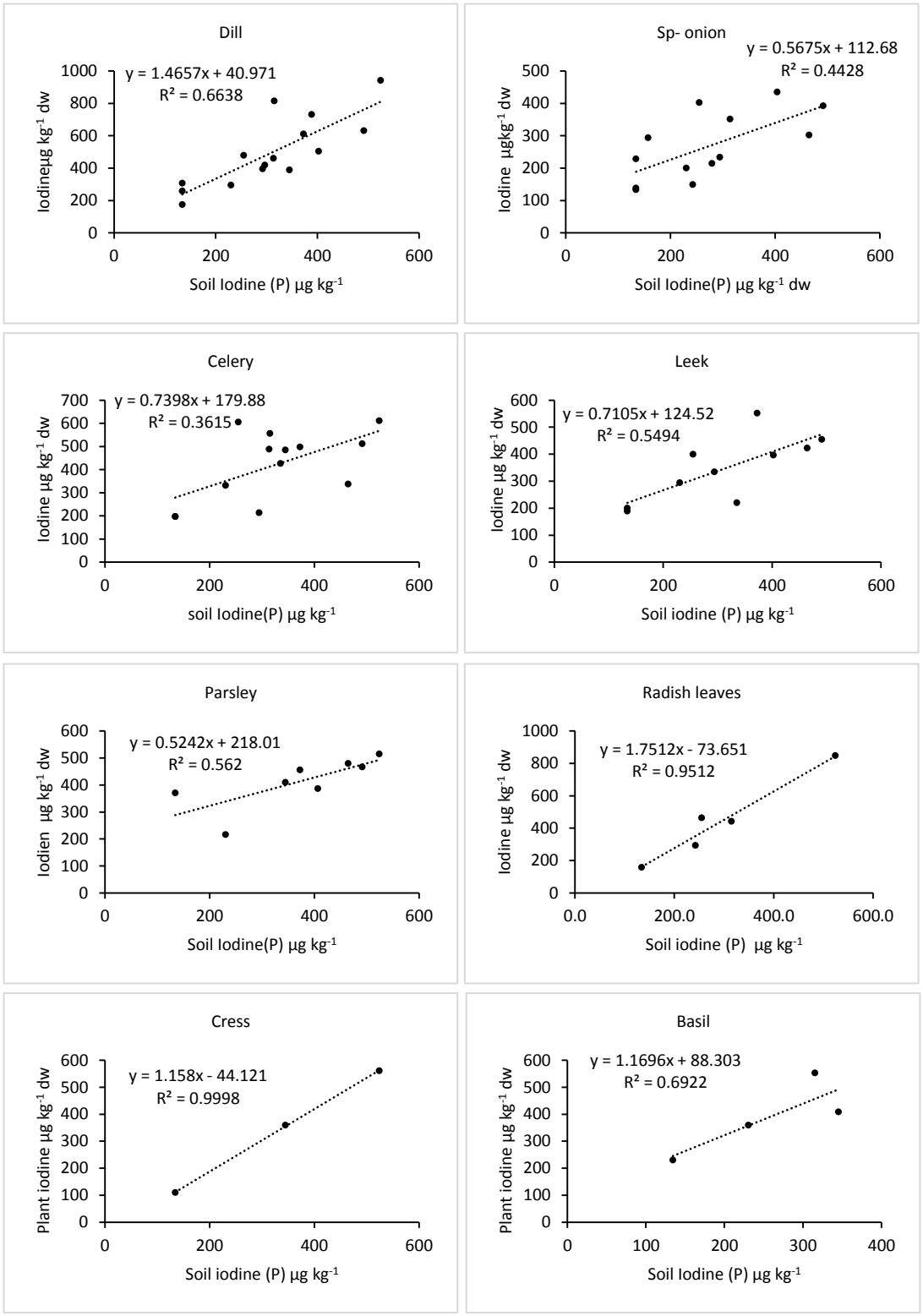


Figure 3-36. Plant iodine uptake and relationship with total soil iodine (extracted by 10% TMAH) in leafy vegetables

A)


Dill
y = 1.4657x + 40.971
R² = 0.6638
Iodineµg kg-1 dw
Soil Iodine (P) µg kg-1
Sp- onion
y = 0.5675x + 112.68
R² = 0.4428
Iodine µgkg-1 dw
Soil Iodine(P) µg kg-1 dw
Celery
y = 0.7398x + 179.88
R² = 0.3615
Iodine µg kg-1 dw
soil Iodine(P) µg kg-1
Leek
y = 0.7105x + 124.52
R² = 0.5494
Iodine µg kg-1 dw
Soil iodine (P) µg kg-1
Parsley
y = 0.5242x + 218.01
R² = 0.562
Iodien µg kg-1 dw
Soil Iodine(P) µg kg-1
Radish leaves
y = 1.7512x - 73.651
R² = 0.9512
Iodine µg kg-1 dw
Soil iodine (P) µg kg-1
Cress
y = 1.158x - 44.121
R² = 0.9998
Plant iodine µg kg-1 dw
Soil iodine (P) µg kg-1
Basil
y = 1.1696x + 88.303
R² = 0.6922
Plant iodine µg kg-1 dw
Soil Iodine (P) µg kg-1

B)

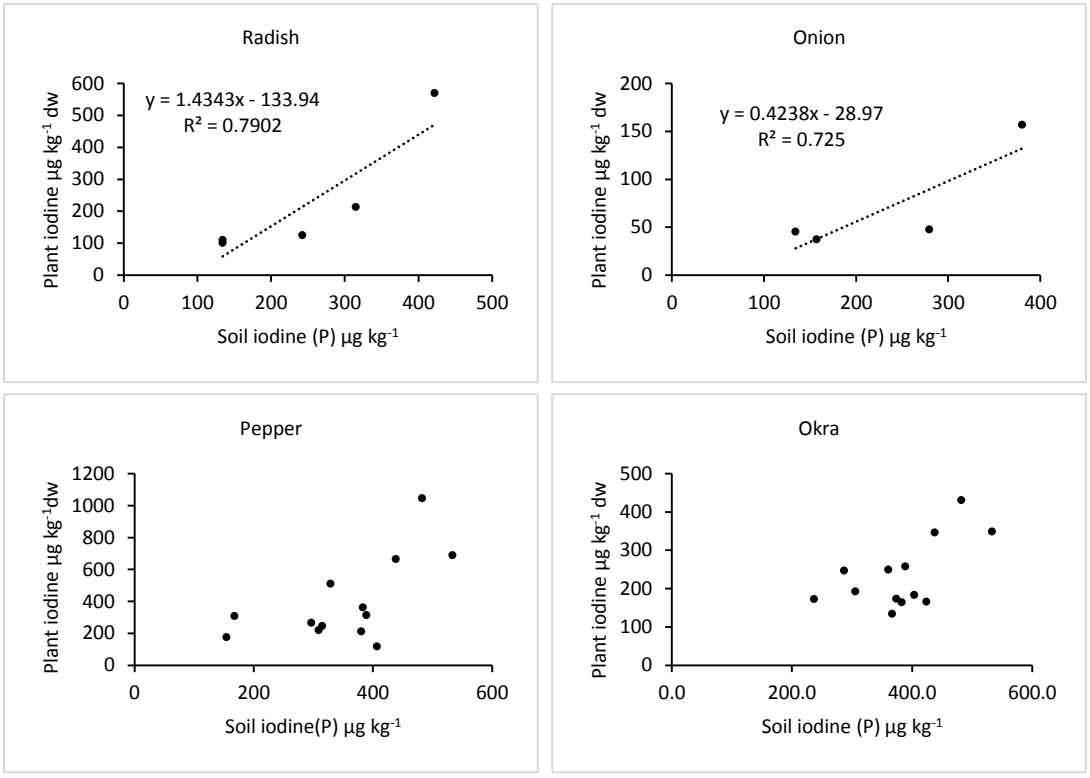


Figure 3-37. Plant iodine uptake in fruit and leafy vegetables (A and B) as a function of soil adsorbed iodine (extracted by 0.016M KH_2PO_4) I_P.

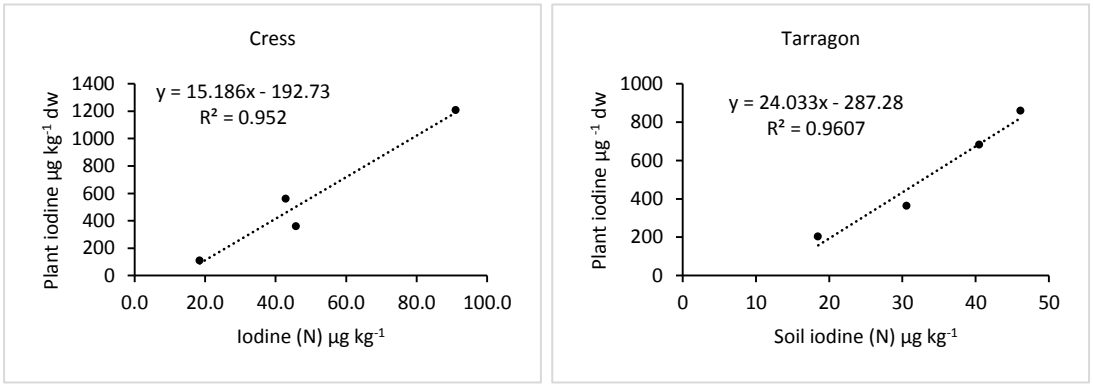


Figure 3-38. Plant iodine uptake as a function of soil soluble iodine (extracted by 0.01M KNO_3) (I_N)

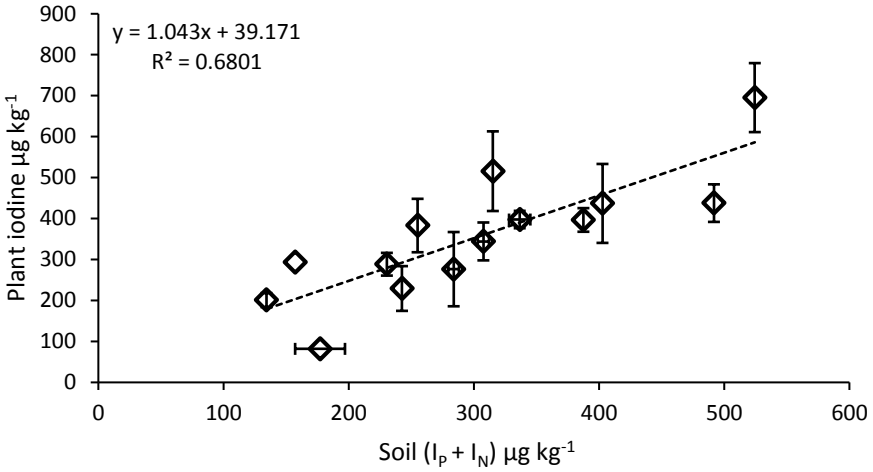


Figure 3-39. Relationship of soil adsorbed + soluble iodine of each site with average of iodine content of plants grown in those sites

In addition to role of soil adsorbed iodine in plant uptake of iodine, figure 3.37 plots soil adsorbed iodine and plant iodine content in each plant species whilst figure 3.39 plots soil (I_P +I_N) iodine for each site and average of iodine content of all plants grown in corresponded site. Since the I_N was considerable lower than I_P it seems the majority of available iodine in soil was I_P. It implies that considering any difference in uptake mechanism or inherent affinity of plant species for iodine, the adsorbed fraction overall is influential factor for plant iodine uptake and found as a bioavailable fraction of soil iodine in these soils. This relationship was examined for soil solution and total iodine individually but no correlation was observed.

3.4.6 Irrigation water

3.4.7.1 Irrigation water characteristics

Mean DOC in irrigation water was 6.8 mg L^{-1}. The highest DOC was recorded at Zamaqi location 25.1 mg L^{-1} for a spring water that traverses through

irrigation channels and it is possible to have a soil contamination and other organic material compared to other sources. The second and third highest DOC values were recorded in the Tanjaro and Dukan sub areas, and were a wastewater and river respectively and expected to have a high level of DOC as the river may contain waste water of some villages. The average DOC excluding these three samples was 4.2 mg L^{-1}.

Dissolved inorganic carbon (DIC) in irrigation water was relatively constant and ranged from 34.3 mg L^{-1} (n=4) in Kalar to 56.2 mg L^{-1} (n=6) in Sulaimani . The average of DIC across all irrigation water samples was 47.6 mg L^{-1}.

Total dissolved solid (TDS) ranged from 85.8 to 305 mg L^{-1}. This range is <500 mg L^{-1} and means that the water can be classified as a low salinity hazard irrigation water (Chapman, 1996).

Water alkalinity was estimated based on Ca and all were classified as hard waters. Water containing >200 mg L^{-1} $CaCO_3$ has classified as an alkaline water and severe hazard level of alkalinity and in range of 100-150 classified as a significant hazard levels (Chapman, 1996) which most of water samples were in this range except Zhalla area which had a low $CaCO_3$ content. Alkalinity indicates the ability of the water to increase the pH of the soil or growing media.

Sodium adsorption ratio (SAR) in all areas was <3 which is classified as a non hazard level except. Zhalla area which had considerably high SAR value (23.3) and determined as a severe hazard level. This is due to having a high Na concentration in that water sample.

Table 3.14. Irrigation water characteristics

Areas	Location	Source	OC	IC	Cl^-	NO_3^-	SO_4^{2-}	Na	Mg	K	Ca	S	B	$CaCO_3$	TDS	SAR
									mg L^{-1}							
Sulaimani	Tan1	waste	18.1	74.7	73.1	0.65	25.0	63.01	18.5	9.38	93.9	35.2	0.0368	235	222	1.55
	Duk1	Well	14.6	74.4	56.7	2.57	8.82	78.88	40.8	3.19	39.4	13.1	2.703	98	179	2.10
	Duk2	River	n.d*	42.1	7.15	0.77	7.81	7.44	14.98	1.43	49.7	11.4	0.0719	124	85.8	0.237
	Baz1	Well	7.93	46.6	20.9	10.1	1.52	6.98	25.13	0.63	68.5	2.48	0.0158	171	105	0.183
	Baz2	Well	7.67	46.6	7.24	3.49	2.05	3.94	6.29	0.813	68.6	6.19	0.0273	171	86.1	0.122
	Zhalla	Well	1.32	52.7	48.9	n.d	11.0	142	0.239	10.3	2.41	16.0	0.952	6.030	175	23.3
Sharazoor	New-Hal	Well	4.68	62.4	20.6	3.85	2.67	8.87	25.4	2.80	83.1	4.14	0.0309	208	126	0.218
Kalar	Kal1	Well	4.50	32.3	14.0	7.51	102	25.9	35.2	1.15	121	118	0.0998	303	305	0.532
	Kal2	Well	3.58	36.9	25.3	6.74	37.9	15.7	13.5	0.89	56.7	17.6	0.0508	142	106	0.486
	Kal4ab	Well	3.72	34.8	11.8	4.45	13.6	16.4	14.7	0.88	64.8	20.05	0.0581	162	118	0.477
	Kal4c	Well	3.069	33.0	10.3	3.43	12.3	20.5	21.9	1.10	91.4	48.7	0.0556	228	186	0.499
Halabja	Zam	Spring	25.1	60.6	48.4	0.00	17.2	53.6	9.37	8.01	78.5	25.9	0.0192	196	177	1.52
	Zam	Well	4.37	46.3	7.87	3.43	4.85	3.77	14.3	0.514	78.5	7.30	0.0148	196	105	0.103
	Bak1	Well	3.96	45.1	4.16	2.69	2.66	5.62	13.3	0.522	64.2	3.87	0.0227	160	88.2	0.167
	Bak2	Well	3.94	46.4	8.24	3.90	5.40	4.28	10.94	1.15	80.0	7.72	0.0125	200	105	0.119
	Bak3	Well	3.85	45.0	5.09	3.03	2.58	4.29	13.9	0.405	71.7	4.08	0.0159	179	95.2	0.121
	Bia	Spring	3.98	43.3	1.73	1.16	1.68	2.03	6.48	0.659	78.4	2.96	0.0215	196	91.4	0.059
	Khor	River	2.88	38.3	5.47	0.923	4.01	6.56	20.2	0.539	60.6	3.40	0.0228	151	92.0	0.186
	Kag	Well	4.33	41.8	6.15	3.12	2.06	3.74	9.67	2.34	70.6	3.29	0.0141	177	90.4	0.110

n.d: Not determined

3.4.7.2 Selenium and iodine content in the irrigation water

Selenium concentration in all water sources was <1μg L^{-1} (mean = 0.495 μg L^{-1}, n=19) and ranged from 0.05 to 0.859 μg L^{-1}. Kalar and Halabja areas had higher water selenium concentration compared to Sulaimani and Sharazoor (Fig 3.40b and Table 3.15). No any correlation was observed between water selenium content and each one of soil selenium soluble, adsorbed and plant selenium content. It can implies that water selenium, due to very low concentration, is not a factor which controls plant selenium uptake in the studied area.

Iodine concentration in the water samples varied from 2.49-36.21 μg L^{-1} (mean= 11.9 μg L^{-1}, n=19). The average value of iodine in river water has been reported as 5 μg L^{-1} (Fuge and Johnson, 1986). Mehra et al. (2014) reported the mean of water iodine content originated from limestone areas to be 4.8 μg L^{-1}. Waters in the Sulaimani and Kalar areas had the highest water iodine concentration (16.6 and 15.9 μg L^{-1} respectively), Halabja had the lowest 4.83 μg L^{-1} (n=8) (Table 3.15, Fig 3-40a).

Table 3.15. Selenium and iodine water content in main areas

Area	n	Selenium concentration μg L^{-1}			Iodine concentration μg L^{-1}		
		Min	Max	Mean	Min	Max	Mean
Sulaimani	7	0.050	0.631	0.372	4.782	36.185	16.569
Sharazoor	1	0.373	0.373	0.373	11.636	11.636	11.636
Kalar	4	0.453	0.866	0.635	15.610	20.407	15.889
Halabja	8	0.181	0.859	0.548	2.467	6.850	4.835

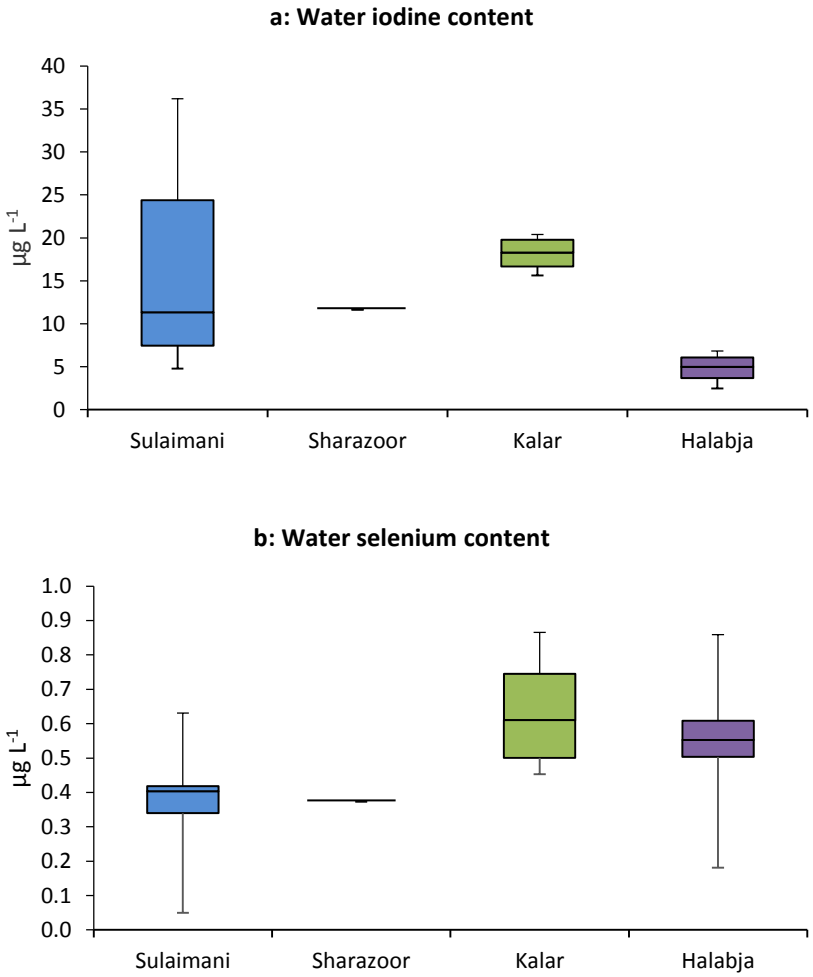


Figure 3-40. The amount of selenium and iodine concentration in irrigation water of four main areas.

Figure 3-41 shows that a strong correlation exists between soil $CaCO_3$ % (estimated from soil IC%) and underground water iodine content in the local water supplies. This may reflect the geology of the reservoir where water originated from. Speciation of the waters indicated that the iodine was mainly present as inorganic species. At most sites the dominant species was iodate e.g. in Kalar and its subareas 100% of the iodine was iodate. In Halabja three subareas had 100% iodate while one subarea had 100%

iodide. Only one site in Sulaimani had a mixture of three species; 50, 40, and 10% iodide, iodate and organic iodine respectively.

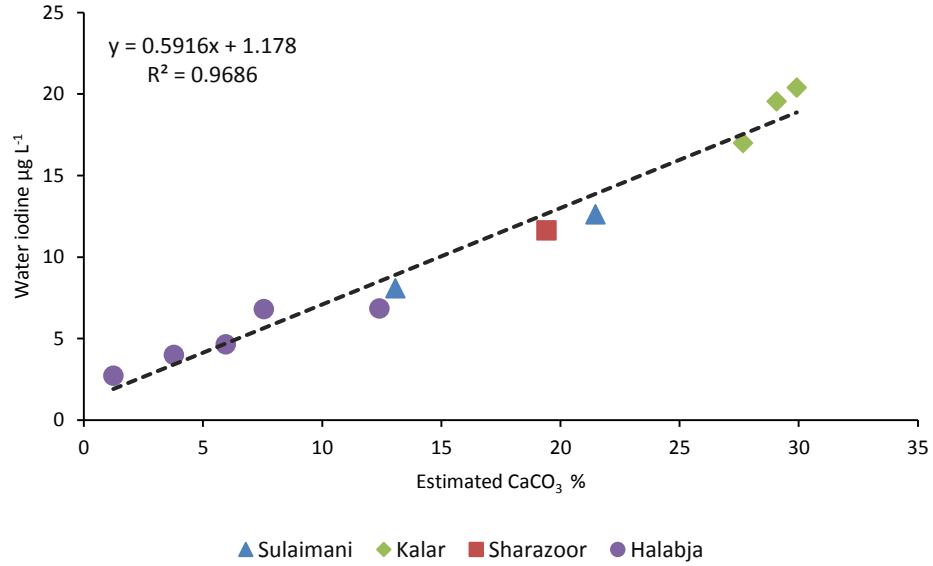


Figure 3-41. Relationship of soil calcium carbonate (estimated from soil in-organic carbon) amount (%) and iodine content of corresponding irrigation water (underground water source)

3.4.7.3 Water iodine content and relationship with soil and plant iodine status

It was not observed a relationship between water iodine concentration and soil I_N or I_P when all the locations considered. It is acceptable since iodine can be adsorbed by soil component, such as organic material, relatively quick (Shetaya et al., 2012) and soil samples had variable organic material content.

A correlation was observed between water iodine content and extractability of I_p+I_N in proportion of soil I_{Tot}; % (I_P+I_N/I_{Tot}) across all the areas (Fig. 3.42). This is could be attributed to replenishing iodine from irrigation water continuously and soils which received water with high iodine concentration

may contain more iodine available or more iodine which is not fixed yet and easier to extract.

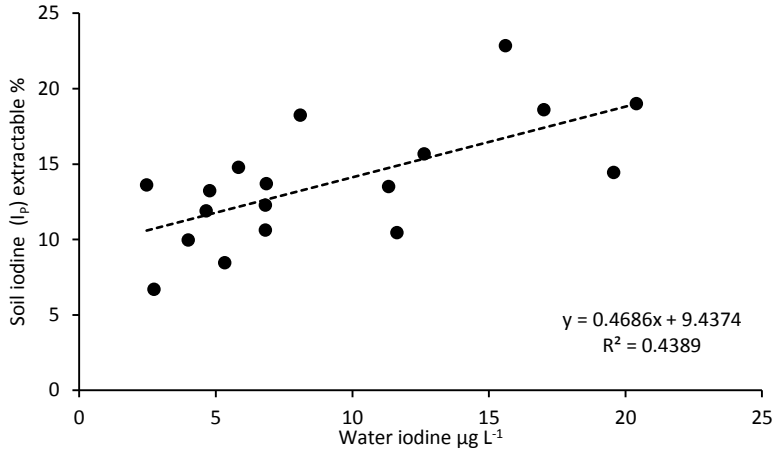


Figure 3-42. Relationship of water iodine content and extractability of iodine in proportion of total soil iodine %(I_P+I_N/I_{Tot})

A relationship was observed between water iodine content and average of iodine content in all plant species grown in the area irrigated with that water for two areas Halabja and Sulaimani (Figure 3.43 a & b). This suggests that irrigation water iodine content seems to be the factor controlling plant iodine content. One of the reasons for this could be that the majority of iodine was in inorganic forms that are more bioavailable for plant uptake (Ashworth, 2009).

The role of iodine content of irrigation water in replenishing soil iodine pools was examined. The estimated total amount of iodine supplied by irrigation water to a hectare of soil over a 6 months growing season was calculated as 537 g ha^{-1} $season^{-1}$. This calculation carried out based upon the total water consumption of a plant growing in 1 kg of Iraqi soil in a pot trial (6 L kg^{-1} $season^{-1}$) at temperatures similar to those anticipated in Kurdistan.

This amount was higher than soil soluble iodine I_N for 1 ha of soil (127 g ha^{-1}) and less than amount of adsorbed iodine I_P for 1 ha soil (830g ha^{-1}) while the total amount of iodine in 1 ha soil estimated as (10 kg ha^{-1}). Since, the majority of iodine in both soluble and adsorbed fraction (I_N+I_P = 957 g ha^{-1}) was found as organic compared to the amount of inorganic iodine derived from irrigation water (537 g ha^{-1}) and plant preferentially uptake inorganic forms of iodine and these are mainly supplied by the irrigation water. Consequently, water iodine seems have a considerable role in plant's iodine content.

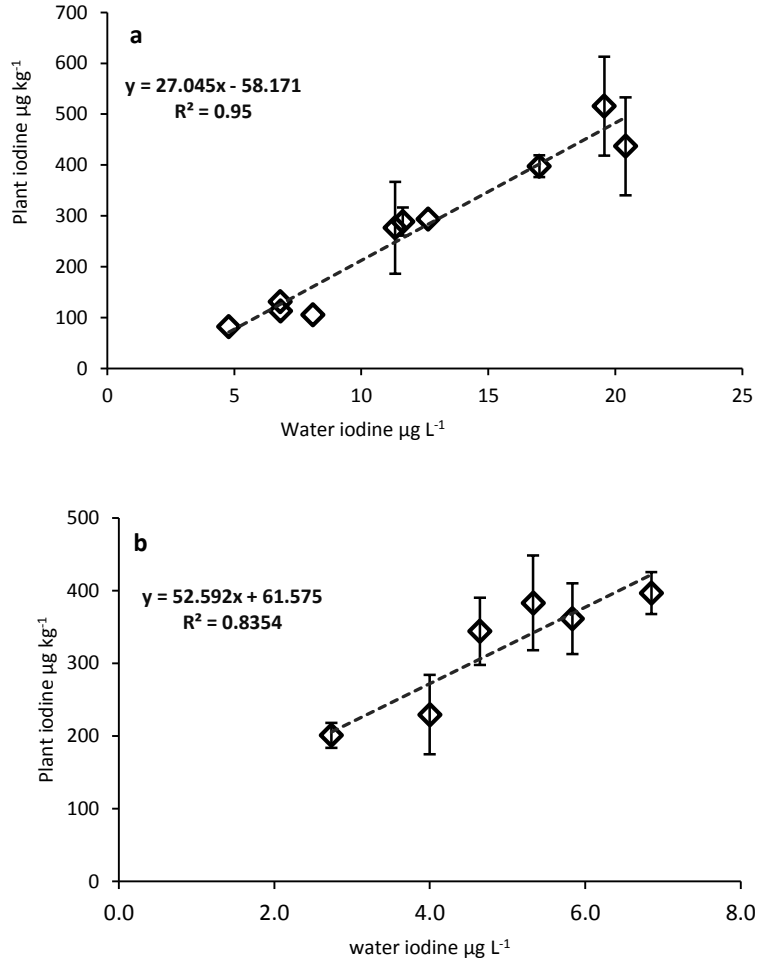


Figure 3-43. Relationship of irrigation water iodine concentration with average of iodine concentration in the corresponding plants grown in that area a)Halabja b)Sulaimani

3.5 Conclusion

The mean concentration of Se_{Tot} in the areas studied was 309 μg kg^{-1} which is lower than the global mean for soils of 400 μg kg^{-1}. The mean concentration of the Se_N fraction was 1.90 μg kg^{-1}, just 0.69% of Se_{Tot}. The mean concentration of Se_P was greater, at 5.65 μg kg^{-1} - 1.88% of Se_{Tot}. Thus, combining the soluble and adsorbed fractions (Se_{N+P}), only 2.57% of Se_{Tot} could be regarded as immediately available Se for plant uptake. Furthermore, speciation of Se_N and Se_P showed that 51% of Se_N was present as inorganic anions with more selenate (Se^{VI}) than selenite (Se^{IV}) and 78% of Se_P was present as inorganic Se with the vast majority being Se^{IV} (96%). Thus, assessing the 'bioavailable' pool of Se in these soils by simple chemical extraction is complicated by differences in speciation in soluble and adsorbed forms - both in terms of inorganic Se valence and in the proportions of inorganic and organic forms in both fractions. It is unlikely that the plant uptake rates of selenite, selenate and soluble organic Se are the same and so it seems unlikely that simple extractions without speciation will provide a consistent prediction of Se bioavailability.

Soil %OC was positively correlated with Se_{TMAH} suggesting retention of Se by soil organic matter. This correlation was not observed for Se_p or Se_N when all the areas were considered together. The proportion of Se that *resisted* extraction by 10% TMAH ($\%Se_{TMAH}$) varied in the range 20.3-89.0% (mean value = 41%) suggesting a substantial residual form of Se in these soils. A negative correlation was found between soil pH and Se_{TMAH} in some sub-areas but not when all the areas were considered, probably due to the very narrow range of pH variation in some areas.

This residual Se may be associated with $CaCO_3$, perhaps as selenite incorporated into the calcite structure. Further investigation would be required to resolve this.

The plant Se concentration varied widely across all vegetables, from 4.34 to 678 µg kg^{-1} and was comparable, or generally higher, compared to data reported from other countries. The mean concentration of Se in vegetables as a group was 113, 112, 69 and 49 µg $kg^{-1}{}_{dw}$ for leafy vegetables, tubers, fruit vegetables and wheat respectively. Plants grown in soils with pH>8 generally had higher Se concentrations compared to those grown in soils with lower pH values which mostly applied for fruit vegetables than leafy vegetables which could be attributed to plant transfer mechanisms, growing season time or internal Se mobilization. Overall, however, there was not a strong correlation between any of the soil Se fractions, $Se_{Tot,}$ $Se_{TMAH,}$ Se_P and Se_N, and plant Se content when all plants were considered together. However there was a correlation between both Se_N and Se_P with plant Se in the cases of a few vegetables – notwithstanding the variation in speciation within the fractions Se_N and Se_P seen between soils.

The mean concentration of soil iodine (I_{Tot}) was 4140 µg kg^{-1} across all studied areas, which is comparable with the global average, but some of the areas were close to concentrations reported for iodine deficient areas, from other studies. The average concentration of soluble I_N was 51.0 µg kg^{-1} which is 1.23% of the average $I_{Tot.}$ There was a greater concentration of adsorbed iodine (I_p) with a mean value of 332 µg kg^{-1}, comprising more than 8% of I_{Tot} and suggesting almost 10% of soil iodine (I_N + I_P) is available for plant uptake in these soils. However, iodine speciation revealed that only 33% of I_N was inorganic, mainly iodide, whilst only 6% of I_P was

present in an inorganic form – again with the majority present as iodide. There was no clear relationship between I_{Tot} and %OC when all areas were considered, possibly due to the relatively narrow range of %OC in the studied soils; exceptionally in one of subareas a negative correlation was observed. A negative correlation was also found between %OC and I_P within some sub-areas, perhaps indicating that soils with a higher %OC were better able to fix or retain iodine in a strongly bound form and thereby reduce iodine availability. No correlation was observed between pH and soil iodine for any of the fractions.

Iodine concentrations were greatest in leafy vegetables (439 μg kg^{-1}_{dw}); concentrations in fruit vegetables, tubers and wheat were 368, 140 and 12 μg kg^{-1}_{dw} respectively. The mean concentrations of iodine in the crops were comparable, or in some cases higher, than other reported studies except in grains which were comparatively very low. A relationship between soil I_{Tot} and plant iodine content was observed in a few plant species - e.g. chard, okra and cucumber. For the majority of plants, however, the relationship was very weak or absent. A better correlation was found between I_P and plant iodine content but very poor correlation was observed for I_N. In addition 'available iodine' (soil I_{N+P}) was correlated (r = 0.66) with average plant iodine content grown at the corresponding site.

Selenium concentrations in irrigation water were very low, with a mean value of 0.495 μg L^{-1} (n = 20); the average iodine concentration in water was 11.9 μg L^{-1}. There was no correlation between Se in irrigation water and plant Se content but plant iodine content was significantly correlated with iodine in water (r=0.97 and 0.91 for Halabja and Sulaimani areas respectively). A strong correlation observed between soil $CaCO_3$ content

(%) and underground water iodine content could reflect the geology of the reservoir from where water originated and indicate the presence of iodine rich sedimentary parent material. There was also a strong positive correlation between water iodine content and the average iodine content for all plant species grown in the area and irrigated with that water supply. There is therefore evidence that iodine in irrigation water plays an important role in determining plant iodine content in the area under study.

4 FEASIBILITY OF SELENIUM BIOFORTIFICATION USING ^{77}SE AS A TRACER

4.1 Introduction

As a component of selenoproteins, selenium (Se) is considered an essential element for human health. One of the main ways that Se enters the body is via the consumption of fresh vegetables, whose concentration of this element depends on soil Se content and bioavailability (Funes-Collado et al., 2013). Agricultural biofortification with Se-enriched fertilisers is a successful strategy for increasing dietary Se intake by humans and animals that has been implemented in some countries (Broadley et al., 2010, Smoleń et al., 2014). Selenium is typically applied as sodium selenate (Na_2SeO_4), sodium selenite (Na_2SeO_3) or as a trace component in commercial fertilizers (e.g. 'Selcote Ultra', 'Top Stock') (Mathers et al., 2017). It is consistently reported that uptake of Se by crops is a remarkably linear function of application rate (Chilimba et al., 2012). A wide range of selenium application rates have been tested in biofortification research, but rates of approximately 10 g ha^{-1} have been accepted as a realistic level to achieve safe and useful dietary enrichment with Se (Chilimba et al., 2012, Gupta et al., 1993). It is also generally accepted that plant uptake of selenate is substantially greater than that of selenite because of (i) the greater availability of selenate in the pH range of agricultural soils and (ii) greater ease of translocation within plants compared to selenite (Ros et al., 2016).

Selenium uptake by plants is still the subject of extensive study and the efficiency of different fertilizer formulations and residual effects remain areas of particular interest (Keskinen et al., 2011). Quantification of fertilizer Se in biomass, and residues in soil, strictly requires some means of discrimination between the Se applied as a fertilizer (Se_f) and native Se from soil (Se_s). Because the amount of Se applied (10 g ha^{-1}) is negligible compared to Se_s (c. 600 g ha^{-1} in this study) assuming a 2500 t ha^{-1} mass of top soil (bulk density =1.25 g cm^{-3}; soil depth = 20 cm; Se concentration = 240 μg kg^{-1}). This issue can be addressed by using an enriched (> 99%) stable isotope of Se such as ^{77}Se (7.63% natural abundance). Most Se biofortification studies have been carried out on staple grain crops such as wheat and maize; comparatively little is known about Se biofortification of green vegetables. There have been some studies in hydroponic systems or other culture media but not in arable soils.

The primary aims of this study were:

a) To examine the feasibility of biofortification using a single application of Se (10 g ha^{-1}) to calcareous soils and commonly grown vegetables (local genotypes) of Kurdistan;
b) To determine the fate of applied Se and source apportionment of plant Se between fertilizer and soil Se using enriched ^{77}Se as a tracer.

4.2 Material and methods

4.2.1 Soil sampling and preparation

The soil used in this experiment was sampled from agricultural land normally used for growing vegetables. Soil was taken from shallow soils (0-15 cm) of the Tanjaro area of Sulaimani, Kurdistan. The soils were homogenised, air dried, <2 mm sieved in the Technical Institute of Halabja before shipping to Nottingham University, UK. A portion of 50 g was finely ground using an agate ball mill (Retsch, PM400); sub samples were acid digested for elemental analysis (see section 2.4).

4.2.2 Spiking soil with enriched ^{77}Se and potting

The soil was moistened (top c. 10% w/w) with deionised water using a food processor mixer and incubated in a cold room (4 ^{o}C) for three days before being spiked with Se. The amount of required ^{77}Se for each pot was calculated based on the mass of topsoil in one hectare. The hectare mass was estimated based on an assumed soil depth of 20 cm and bulk density of 1.25 g cm $^{-3}$. Assuming 2.5×10^{6} kg ha^{-1} topsoil mass and 10 g ha^{-1} of ^{77}Se application, a gravimetric application of 0.004 mg kg^{-1} is required which equals 2 μg pot^{-1} (500 g soil (dw) per pot). The same calculation was applied based on the area of one hectare compared to the area of one pot (11 cm pot diameter) and the estimated amount was 9.49 μg pot^{-1}; but applying a conversion factor to allow for topsoil depth in the pot the application would then be reduced to 2.3 μg pot^{-1}. The mass based calculation (2 μg pot^{-1}) was applied in this experiment. The primary stock solution of 2000 μg L^{-1} was prepared from a primary stock of 259 mg L^{-1} (Isoflex; USA). One ml of

stock solution (equivalent to 2 μg ^{77}Se) was diluted with 19 ml of Milli-Q water and gently mixed with 550 g soil (moist weight) for each pot using a food processor to ensure homogenisation while still maintaining an aerobic crumb aggregate structure.

4.2.3 Pot experiment design

The experiment consisted of a randomised block design and included 5 vegetable types with 12 pots for each type. Four of the 12 pots received no biofortification treatment while 8 were spiked with ^{77}Se and 4 of these also received $^{129}I^-$ while the remaining 4 pots received $^{129}IO_3^-$ solution (0.5 μg L^{-1}) as a daily irrigation treatment. The 60 pots were distributed in 4 blocks of 15 pots; each block included one replicate of all treatments, randomly positioned within the block. All pots were labelled prior to sowing the seeds.

4.2.4 Vegetable sowing

Five vegetables widely used in the Kurdish diet were selected for this experiment. The seeds were of Kurdish genotypes commonly planted in the local area. These vegetables included celery, chard, lettuce, radish and spring onion (Fig 4.1).

The seeds were sown just below the surface of the soil (1-2 cm depth) depending on seed size. The density of sowing was (0.5 g) per pot. To limit surface evaporation and prevent weed growth the soil surface in the pot was covered by a thin layer of perlite (1 cm). The pots were irrigated and covered by a plastic sheet to aid germination. After 3 days the plastic sheets were removed and irrigation was applied daily. One week after germination the vegetables were thinned to 10 celery, 10 spring onion, 2 chard, 2 lettuce

and 2 radish plants. Environmental conditions in the growth room were initially intended to mimic conditions during mid spring in Kurdistan: 15°C/10 h night and 25°C/14 h day, 50% humidity. After one month control settings were changed to 28°C (day) and 18°C (night) and 25% humidity (Fig 4.2 and 4.3). The photon flux was 650 µmol m^{-2} s^{-1} for



Figure 4-1. Vegetable species used in this experiment

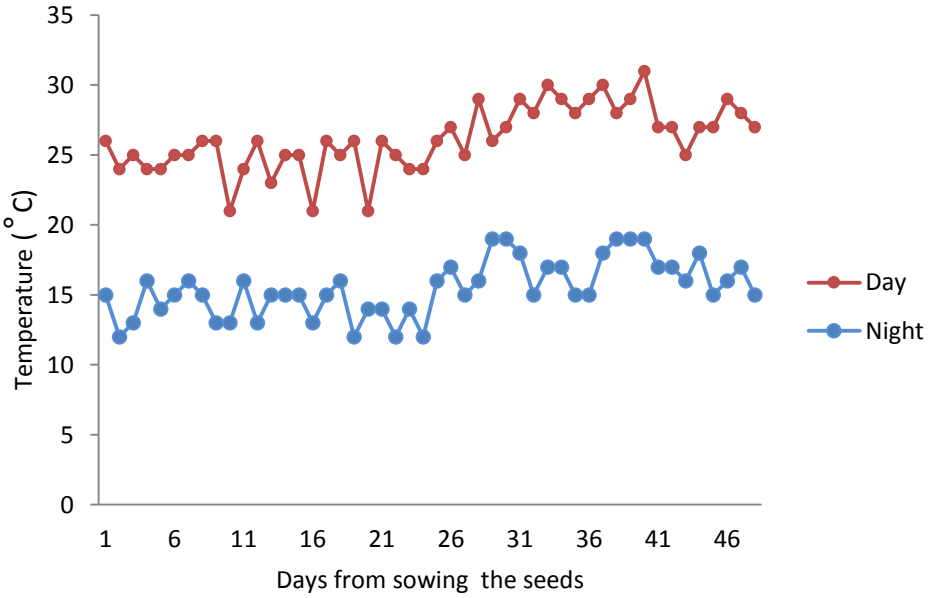


Figure 4-2. Daytime and night temperatures during the experiment

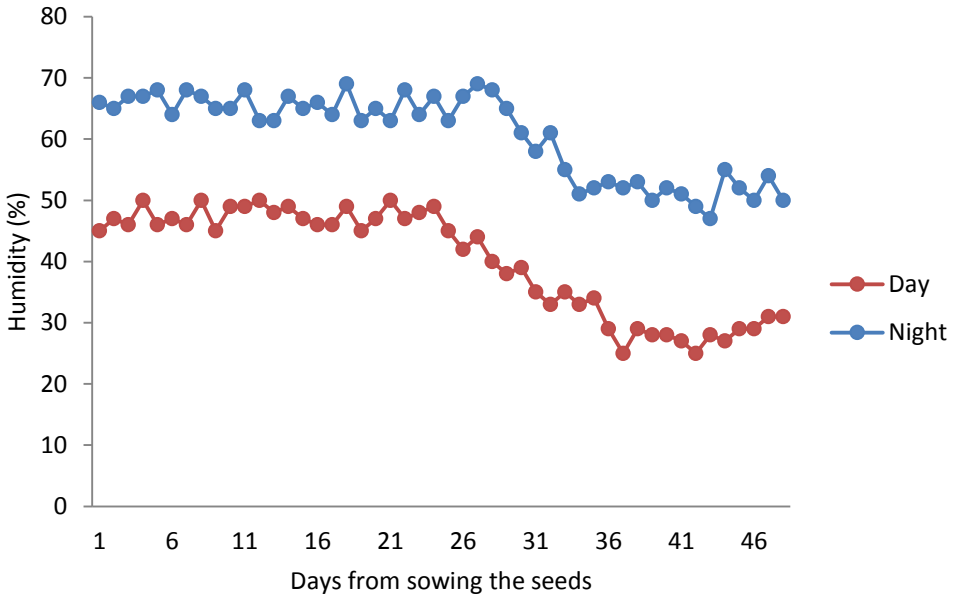


Figure 4-3. Daytime and night humidity during the experiment

the daytime period. Water was applied every day but the amount of water for each vegetable type differed during the growing period according to their individual water requirements: daily applications included 1.76, 2.21, 3.08, 3.09 and 3.16 L for spring onion, celery, chard, lettuce and radish respectively (growing season collectively). Fertilizer was applied twice, after 3 and 6 weeks of growth, to achieve normal growth conditions. Application rates were 50 kg ha^{-1} of nitrogen and phosphorus, as KNO_3 and $(NH_4)_2HPO_4$, applied in irrigation water.

4.2.5 Plant harvesting

Plants were harvested after 8 weeks of growth, when they reached the stage of having sufficient edible material. Shoots were harvested above the soil surface, using stainless steel scissors. Care was taken to avoid contamination from soil particles during harvesting. Plants were weighed

immediately after harvesting to determine the fresh weight and then dried in a paper bag. The roots were carefully separated from soil particles.

Soils including roots were sieved (<1 mm) and the roots removed in the sieve were washed with deionized water. This process was repeated 2-3 times until all the soil particles adhering on root surfaces were removed. Radish bulbs were washed by deionized water (Fig 4.4). Roots and bulbs were dried with absorbent paper and dried in a paper bag.



Figure 4-4. Separating plant ‘s root from shoot and cleaning

4.2.6 Plant and soil, preparation and analysis

Following harvesting and cleaning, plant tissues were dried at 40°C for 72 hr and weighed. Samples were milled and subsamples were (i) microwave acid digested and (ii) microwave TMAH extracted, in duplicate for all 60 pots pending selenium and iodine analysis (see sections 2.6). Samples from the sieved potted soil were air dried and ball-milled; a sequential extraction was then carried out to determine soluble, adsorbed and organic-bound fractions (See section 2.4, 2.5). Soil selenium speciation (Se^{+4} and Se^{+6}) was carried out (HPLC-ICPMS) on the soluble and adsorbed fractions (see section 2.7).

4.2.7 Calculation of Se source apportionment

Considering the application of ^{77}Se as a fertilizer and the multiple Se isotopes available in soil (including native ^{77}Se), both 'total' Se (measured by ICPMS at m/z = 78) and total ^{77}Se (m/z = 77) were determined by separate external calibration. The apportionment of plant ^{77}Se from the fertilizer ($^{77}Se_f$) and soil ($^{77}Se_s$) were estimated using the natural abundance of ^{77}Se and estimates of total Se from soil sources (Se_{Tot}) measured by calibration at m/z = 78.

$$^{77}Se_s = (Se_{Tot} / 78.96) \times 0.0763 \times 76.919 \qquad \text{Eq.4.1}$$

Which $^{77}Se_s$ is the amount of native ^{77}Se estimated from natural Se source, Se_{Tot} is the total Se from soil sources (all isotopes), 0.0763 is natural abundance of ^{77}Se in soil and other values are the atomic weight of the single isotope ^{77}Se (76.919) and the average of all isotopes of Se (78.96). The value of $^{77}Se_s$ subtracted from total ^{77}Se in the plant ($^{77}Se_{Tot}$) then

provides an estimate of the amount of ^{77}Se in the plant originating from the fertilizer ($^{77}Se_f$). The source apportionment of ^{77}Se in each fraction of soil was calculated using the same method as above.

The statistical analysis (one way and two way ANOVA) of this chapter have been done by Graph Pad Prism 7.02.

4.3 Results and discussion

Due to application of low concentration of ^{129}I and $^{129}IO_3^-$, the iodine concentrations in plants were below the limit of detection. Therefore this chapter presents only Se results. A second experiment looking at uptake of iodine is presented in the following chapter.

4.3.1 Soil properties

The mean soil pH for 12 replicates of soil samples before planting was 7.93. The organic carbon content was 2.38% and the total soil Se content was 240 μg kg^{-1}. The calcium carbonate content of the soil was 35%.

4.3.2 Selenium fractionation

4.3.2.1 Soil Se

The sequential extraction results showed that only 1% (2.37 μg kg^{-1}) of soil Se_{Tot} was present in soluble fraction (Se_N). The amount of adsorbed Se (Se_P) was 4.19% (10.07 μg kg^{-1}) and organic-bound fraction (Se_{TMAH}) was 43.2% (103 μg kg^{-1}) (Fig 4.5). The remaining soil Se (50.8%) may be regarded as a recalcitrant Se phase; <1% of soil Se was taken up by the vegetables

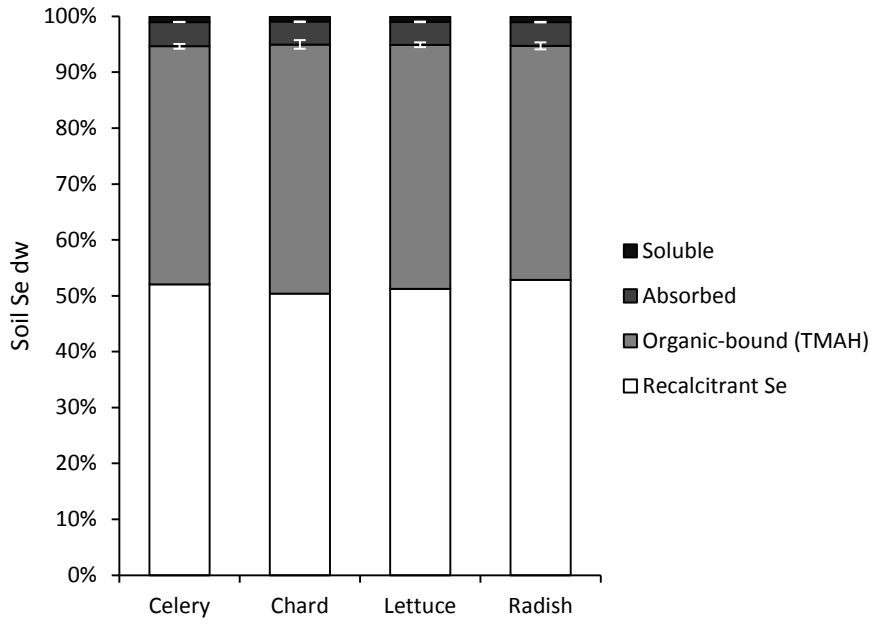


Figure 4-5. Fractionation of soil Se after harvesting

(see section 4.3.5). Similar results were found for other soil samples taken from that area (Tanjaro-SUL), presented as part of the soil survey of the region (see section 3.4.3.2).

The result of Se *speciation* in each fraction showed that 30% of Se_N was inorganic, divided between selenite (20%) and selenate (10%), and 70% was organic. By contrast, about 50% of the Se_p fraction (adsorbed) was inorganic (only selenite) and 50% was present as organically bound Se.

4.3.2.2. Fertilizer ^{77}Se

Sequential extraction was carried out after harvesting. Therefore, in soil fractionation calculations the amount of $^{77}Se_f$ taken up by the plants (see section 4.3.5) was accounted for by initial subtraction from the total applied $^{77}Se_f$ (Figure 4.6b). Results were similar to those for the soil Se results: the mean value for the $^{77}Se_N$ fraction was 1.55%, the $^{77}Se_P$ fraction 3.54% and

$^{77}Se_{TMAH}$ was 55% of $^{77}Se_f$ applied (allowing for plant uptake). The residual fraction of $^{77}Se_f$ (40%) suggests that recently added Se can be rapidly transferred to a recalcitrant form. Losses of ^{77}Se during plant growth were unlikely as there was negligible drainage from the pots during the course of the experiment (Figure 4.6a). The ^{77}Se speciation results showed that the ^{77}Se present in $^{77}Se_N$ was >70% Se^{+6} and the rest was Se^{+4} and in $^{77}Se_P$

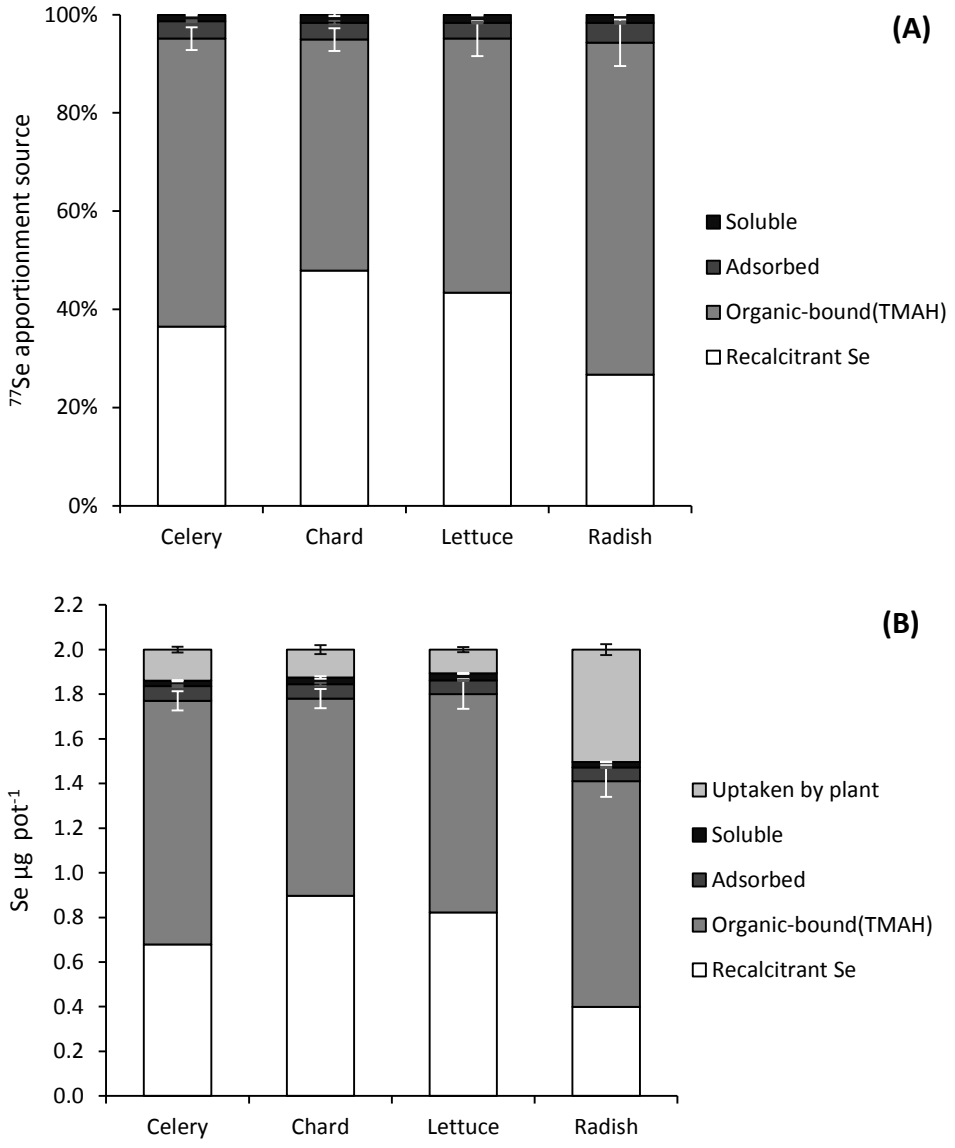


Figure 4-6. Fractionation of ^{77}Se (A) Proportional (%) distribution of Se fractions according to the ^{77}Se remaining after subtracting biomass uptake (B) Recovery of $^{77}Se_f$ according to the amount applied per pot.

100% was present as Se^{+6} – i.e. the entire fraction was inorganic suggesting a part of Se^{+6} applied from the fertilizer converted to Se^{+4} at the end of growing season in Se_{N+P} fraction.

These results imply that at the end of growing season (8 weeks) the availability of $^{77}Se_f$ has been substantially decreased in the soil. Only 5% of $^{77}Se_f$ was present in a form likely to be available for plant uptake ($^{77}Se_N$ and $^{77}Se_P$) and approximately 40% was recalcitrant (excluding plant uptake amount) equals 35% of applied $^{77}Se_f$ not even extractable by 10% TMAH. Furthermore, the recalcitrant ^{77}Se apparently varied slightly in the soil used to grow different vegetables. The reasons for this remain unclear, considering that from <1% to 25% of the Se_f was taken up by the vegetable plant species. Figure 4.6b presents this based on $\mu g^{77}Se$ per pot.

4.3.3 Plant uptake of native soil Se

There was no significant difference in plant Se_s concentration between treatments which had received either ^{129}I and $^{129}IO_3^-$ (α=0.05; p>0.05). The average concentrations of Se_s across all plants which had received ^{129}I and $^{129}IO_3^-$ are shown in Figure 4.7.

Smoleń et al. (2014) biofortified lettuce by Se and I simultaneously in hydroponic system using different treatments and reported that introduction of iodate and selenate in dose of 0.5 mg Se and 1.0mg I dm^{-3} had no negative interaction on uptake of these elements by roots.

Values of shoot Se_s of celery and spring onion (107 and 115 $\mu g\ kg^{-1}$ respectively) were significantly higher (P<0.01) than those of lettuce, radish and chard (70.6, 59.4 and 58.2 $\mu g\ kg^{-1}$ respectively) (Fig 4.7). There

was no difference between chard, lettuce and radish. There was no difference in shoot Se_s concentrations between controls and ^{77}Se treatments (α=0.05;

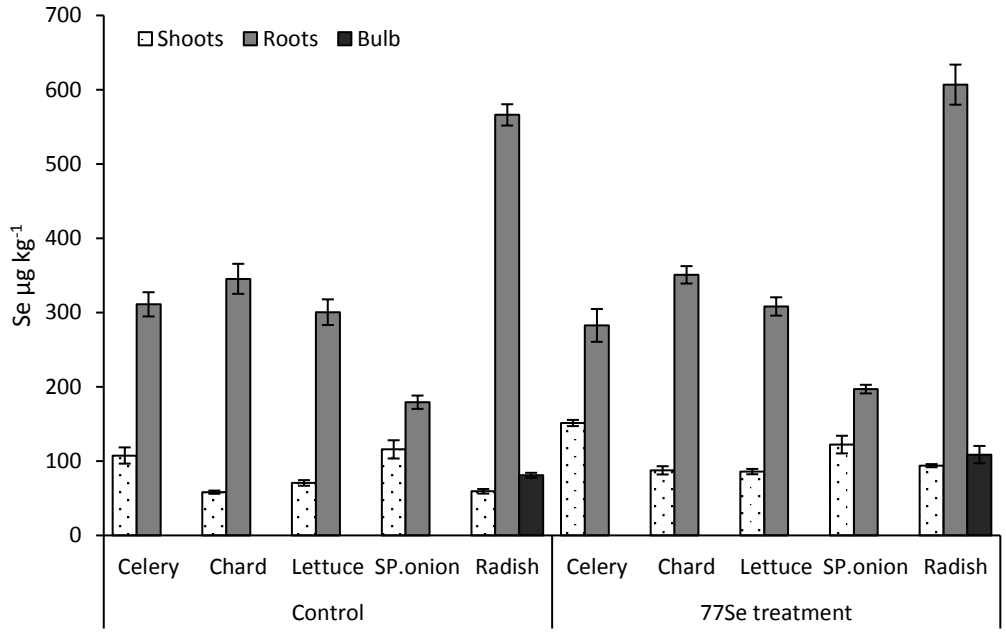


Figure 4-7. Concentration of plant tissue Se originating from the native soil Se source for control and ^{77}Se treatment.

$p>0.05$) with the exception of celery.

The root Se_s concentration ranged from 179 to 566 µg kg^{-1} in spring onion and radish respectively (Fig 4.7). In all vegetables, root Se_s was significantly higher than shoot Se_s (α=0.05; $p<0.001$) but there was no significant difference in root Se_s between control and ^{77}Se treatments. Variation in shoot and root Se_S concentration where all plants have been grown in the same soil must reflect inherent differences in affinity of the plants for Se (Zhu et al., 2009).

4.3.4 Plant uptake of Se from fertilizer ^{77}Se

The concentration of ^{77}Se from fertilizer origin ($^{77}Se_f$) in shoots varied between vegetable varieties (α=0.05; P<0.01). The highest concentration of $^{77}Se_f$ was for radish whilst lettuce had the lowest value (46.9 and 10.5 $\mu g\ kg^{-1}$ respectively). This pattern was true for shoot Se_s of the same plants (Figure 4.8).

Concentration of $^{77}Se_f$ in radish roots was significantly higher (147 $\mu g\ kg^{-1}$) (α=0.05; P<0.01) whilst the roots of other vegetables had lower concentration of $^{77}Se_f$ varying in the range 21.02 to 41.9 $\mu g\ kg^{-1}$ (Fig 4.8). Concentrations of $^{77}Se_f$ in roots were higher than in shoots for all vegetables except celery. The affinity of celery for Se uptake could be one of the reasons for this difference. In the crop survey undertaken previously (see section 3.4.4.1) celery had the highest Se concentration among 28 plant species.

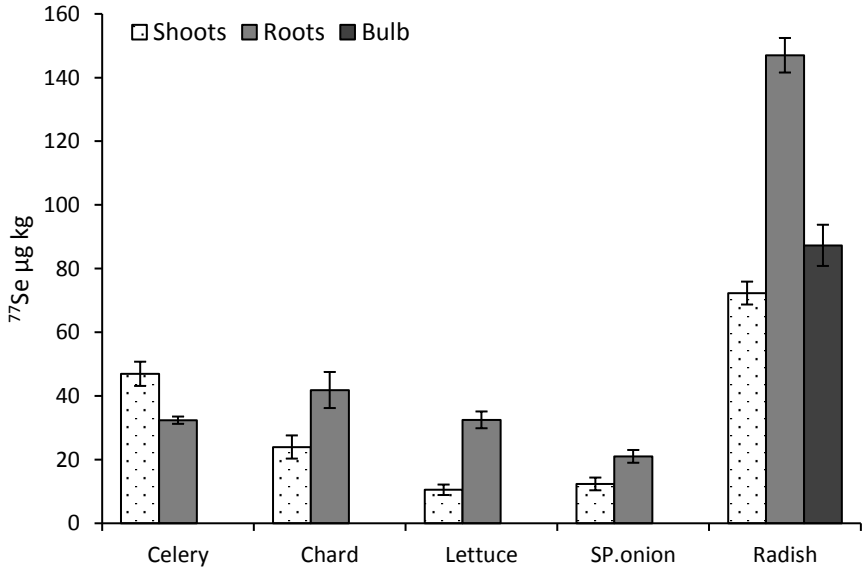


Figure 4-8. Concentration of Se from the fertilizer source ($^{77}Se_f$) in vegetable shoots, roots and bulbs.

Figure 4.9 shows the total Se concentration in the vegetable plants originating from soil and fertilizer sources. The source apportionment of Se shows that in all cases, for roots and shoots, concentrations of $^{77}Se_f$ were considerably lower than those originating from Se_s. This result is not in agreement with other Se biofortification studies reported earlier. One of the

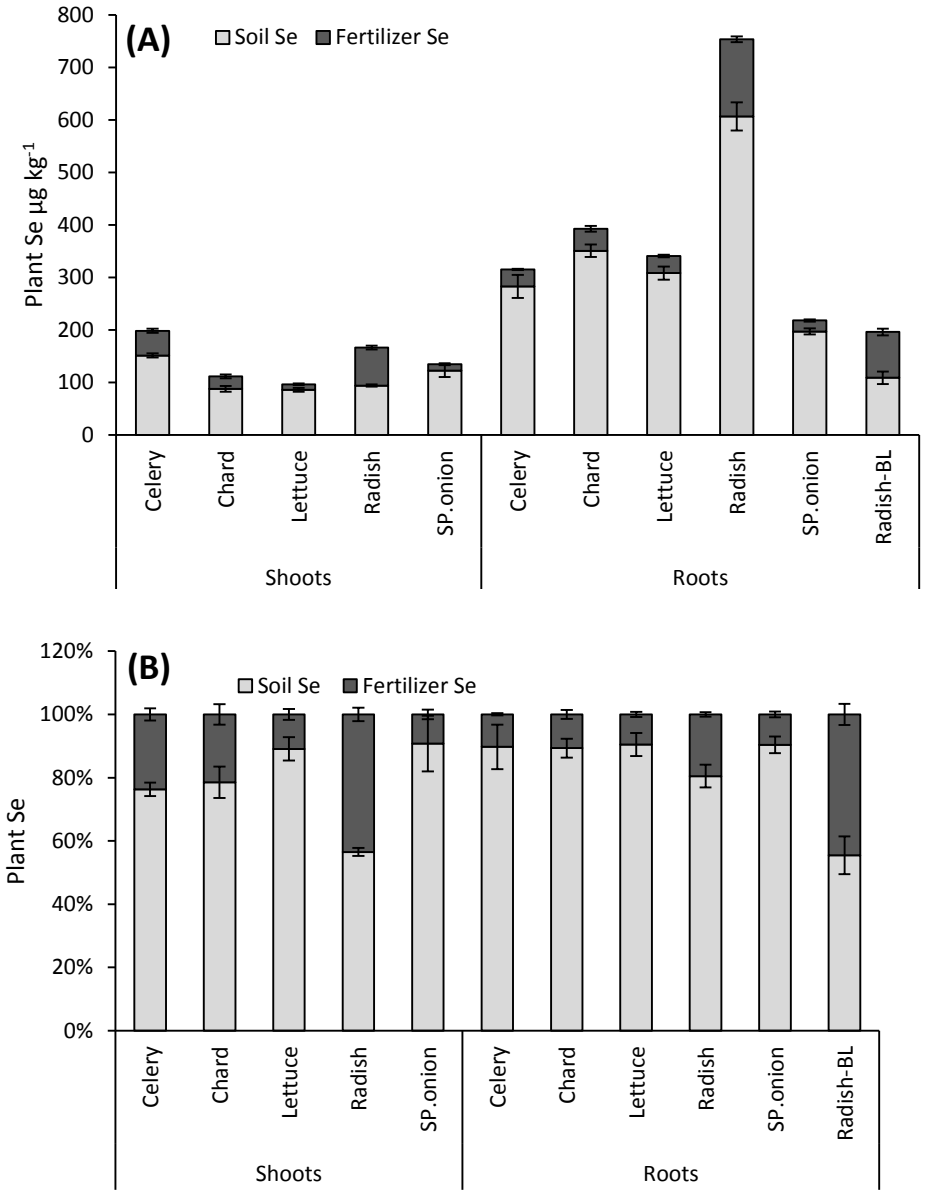


Figure 4-9. Total plant Se from native soil and fertilizer sources (A) Se concentration (µg kg^{-1}) (B) proportion of total (%)

possible reasons for this could be higher availability of native soil Se in these soils due to their high pH. Moreover, the large amount of calcium carbonate in these soils (35%) may be the reason for low availability of the added $^{77}Se_f$. After harvesting only 50% of $^{77}Se_f$ was extractable by TMAH suggesting that the $^{77}Se_f$ had readily assimilated into the native soil Se_s pool. This again would cause an increase in the ratio Se_s/Se_f in the plant tissue compared to previous studies. The ratio Se_s/Se_f also varied between varieties (Fig. 4.9 B). This may reflect the growth patterns of the different crops against a background of gradually decreasing Se_f availability in soil during the growing season and consistent availability of Se_s. Consequently, those plants which grow more rapidly or having rapidly extending root systems are more likely to take up more $^{77}Se_f$ in the beginning of the growing season when it is more available.

Mathers et al. (2017) reported that applying 10 g ha^{-1} of ^{77}Se (foliar spray 1.0 L m^{-2}) could boost the total wheat grain Se concentration to 4-13 times that of controls. This difference in result may be attributed to method of Se application and soil characteristic of studied soil in this experiment (35% $CaCO_3$).

Soil-to-plant transfer factor from Se_f, determined by dividing plant $^{77}Se_f$ by soil $^{77}Se_f$, was considerably higher than for soil Se source (Fig 4.10). So although more soil Se was taken up than fertilizer-derived Se, the latter was intrinsically more available than the native soil Se. The transfer factor for Se_f was 20 times larger than that for Se_s; total Se_s in the soil was 60 times larger than the amount of Se_f added to the soil (Fig. 4.10).

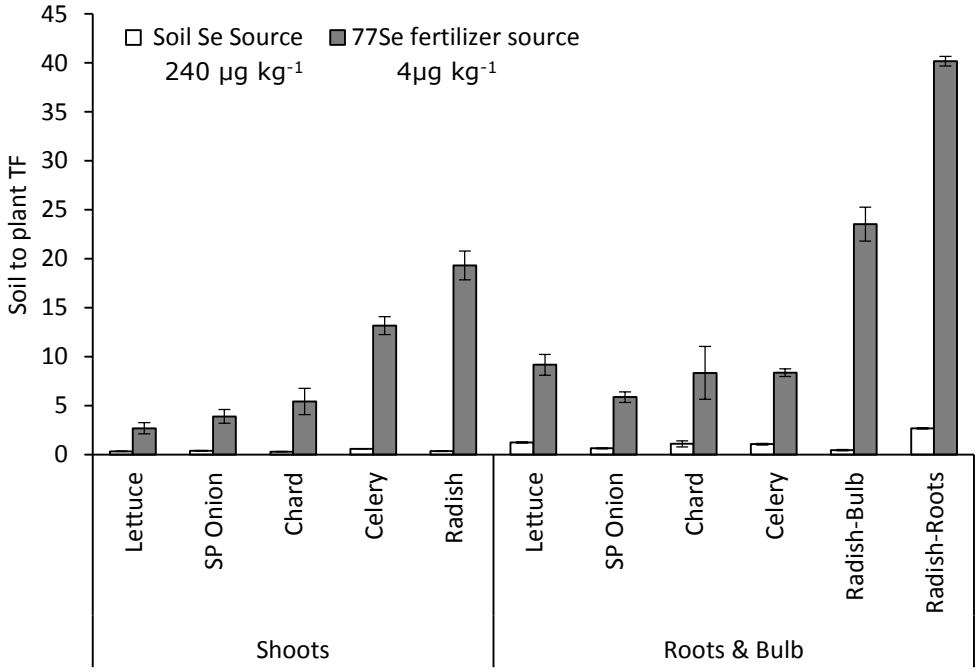


Figure 4-10. Comparison of soil to shoot and root transfer factor for Se from soil (Se_s) and fertilizer (Se_f) origin

4.3.5 Selenium recovery

The recovery of Se by plant biomass from soil Se was low and ranged from 0.064 to 0.80 % for spring onion and lettuce respectively. The recovery of Se_f in plant biomass ranged from 0.44 to 25.2 % for spring onion and radish respectively whilst other vegetables had recovery of 6-7% (Fig 4.11). Significant differences between recoveries ($P < 0.01$) in different vegetable varieties probably reflects differences in plant growth rate against progressive fixation of the Se_f in the soil. Recoveries in plant tissues are shown in Table 4.1 according to shoots and roots.

Table 4.1. Recovery of Se by plant biomass according to soil Se and fertilizer origin Se

Pants		Se taken up by plants compared to total Soil Se*		Se taken up by plants compared to ^{77Se} applied**	
	n	µg	%	µg	%
Shoots					
Celery	12	0.310	0.258	0.106	5.30
Chard	12	0.241	0.201	0.0747	3.74
Lettuce	12	0.373	0.311	0.0420	2.10
Radish	12	0.362	0.301	0.315	15.7
SP-onion	12	0.0512	0.0426	0.00468	0.234
Roots					
Celery	12	0.303	0.253	0.0332	1.66
Chard	12	0.489	0.407	0.0505	2.52
Lettuce	12	0.587	0.489	0.0648	3.24
Radish	12	0.274	0.228	0.0645	3.23
SP-onion	12	0.0255	0.0213	0.00419	0.209
Bulb					
Radish bulb	12	0.144	0.120	0.12	6.21

*240 µg kg^{-1} **4 µg kg^{-1}

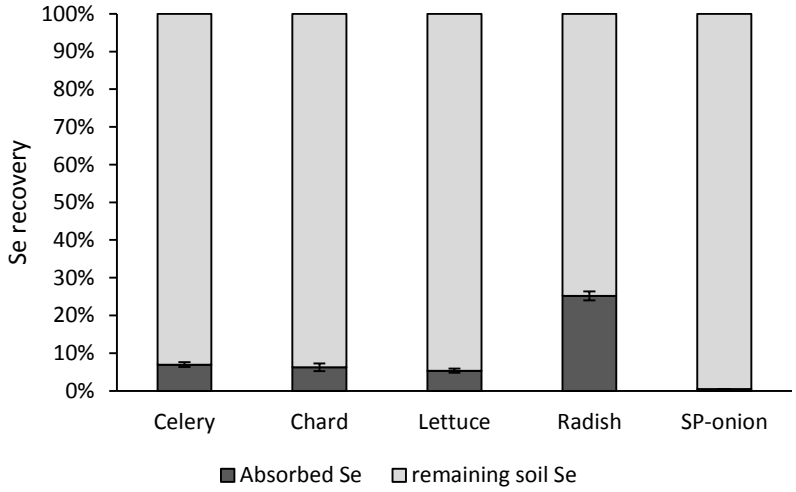


Figure 4-11. Proportional recovery of Se_f in plant biomass and in the soil

Mathers et al. (2017) after biofortification of wheat by 10 g ha^{-1} Se found a biomass recovery rate of $^{77}Se_f$ in the range of 25 to 44% at three different sites, 15-30% retention in top soil and almost 40% losses from the system during a wet spring season.

The current experiment highlights the importance of the soil characteristics in determining the outcome of Se biofortification and the importance of time of application and plant growth rate in the case of green vegetables.

Funes-Collado et al. (2013) biofortified lettuce and chard grown in a peat-based pot trial by adding sodium selenate and selenite (1:9) at levels of 2-8 and 9-17mg Se kg^{-1}. They reported total Se concentrations in leaves as: 34-113, 108-171 mg kg^{-1} corresponding to the first and second levels of Se applied above. Most studies on the biofortification of vegetables have been undertaken with considerably higher applications of Se than used in the experiment presented in this chapter. For example in the Funes-Collado et a.l study 1000 times more Se was applied than used in the Chapter 4 experiment (2-8 mg kg^{-1} and 4 μg kg^{-1} respectively). This tends to result in very high, but possibly unrealistic, Se concentrations in plants in some of the published literature.

Puccinelli et al. (2017) carried out Se fortification of basil plants in a hydroponic system using levels of (0, 4, 8, and 12 mg L^{-1} of growth solution). At the higher concentrations of 8 and 12 mg L^{-1} there was a decreasing trend in Se uptake 13 days after application. However the 4 mg L^{-1} Se treatment demonstrated an almost stable concentration during the growing season (15-25 mg $kg^{-1}{}_{DW}$). In agreement with the current experiment, at the end of the growing season the concentration of Se in

roots was higher than in leaves. Overall this implies that leafy vegetables can take up and tolerate a high concentration of Se when fortified by high Se concentrations in the growth media where there is no limiting factor in the growth medium as regards Se availability.

Ríos et al. (2008) biofortified lettuce with sodium selenate and selenite solutions in a concentration range of (5 to 120 μmol L^{-1}) in the pots (vermiculite growth media) and found the most suitable treatment for lettuce was 40 μmol L^{-1} (3.12 mg L^{-1}) which resulted in 33 mg kg^{-1} Se concentration in lettuce plants.

Businelli et al. (2015) reported an increase in lettuce Se concentration of up to 8.5 times higher than untreated controls by sowing seeds in peat containing 10-20 mg kg^{-1} Se prior to transplanting into the field.

The main difference comparing the current experiment with other Se biofortification projects is that the current Se biofortification trial was undertaken in a naturally calcareous arable soil, with a large amount of calcium carbonate, compared to hydroponic and other growth media. In addition, a considerably lower concentration of Se was applied (4 μg kg^{-1} compared to few mg kg^{-1} in mentioned studies above). Moreover, in the current experiment Se was applied before planting and, due to the soil characteristics, the availability decreased before some of the plants reached their full potential to take up Se. Therefore, for short growth-period green vegetables applying Se during the growing season and via foliar spray may improve the efficiency of biofortification in these type of soils.

Smoleń et al. (2014) after biofortification of lettuce by Se and I in hydroponic system noted a higher efficiency of Se and I biofortification in foliar spraying application compared to introduce via nutrient medium.

4.4 Conclusion

Application of 10 g ha^{-1} of Se increased the Se concentration of vegetables but, unexpectedly, in the soils studied the majority of Se uptake originated from the native soil Se (Se_S) and not from the isotopically labelled Se application ($^{77}Se_f$). Variation was found in shoot and root $^{77}Se_f$ of different vegetable types, even though all plants have been grown in the same soil. There were also differences in the ratio of $^{77}Se_f$:Se_S between the different vegetable types. This pattern may have resulted from gradually decreasing availability of $^{77}Se_f$ (added once at the start of the experiment) against a more constant level of Se_S availability during the growth period. Consequently, plants that grow more rapidly are likely to take up more $^{77}Se_f$ in the beginning of the growth season when it is more available. Therefore this apparent disparity between crop plants may be less if the fertilizer was applied in midseason when the plants were more uniform in their level of maturity or if the Se application was carried out via periodic foliar dressings. Alternatively, the variation in $^{77}Se_f$ recovered in plant biomass across all vegetable types (<1% to 25%) may simply have resulted from different intrinsic affinities of the plants for Se arising partly from differences in capacity (eg root binding site or transporter density) or different physiological uptake mechanisms.

Fractionation of soil $^{77}Se_f$ at the end of growing season showed that 55 and 40% of the remaining $^{77}Se_f$ had been transformed to the organically-bound and recalcitrant fractions of $^{77}Se_f$ respectively and therefore only 5% of $^{77}Se_f$ remained available for plant uptake (in the Se_N and Se_P fractions). However, in contrast to native soil Se, the available $^{77}Se_f$ (Se_N and Se_P) remaining in the soil was entirely inorganic Se and therefore, presumably, easily available for plant uptake. The mechanism whereby 40% of the $^{77}Se_f$ is rapidly transformed to a recalcitrant phase is still not clear but it may attributed to high amount of $CaCO_3$% in these calcareous soils. Timing and frequency of fertilizer application are perhaps the main factors requiring future research into biofortifictaion of vegetables in these types of soils with high carbonate calcium contents.

5 IODINE BIOAVAILABILITY IN SOIL AND THE FEASIBILITY OF BIOFORTIFICATION

5.1 Introduction

Producing iodine-enriched crops could be an efficient method of overcoming iodine deficiency in regions dependent on locally grown crops. Thus biofortification may be an alternative to established strategies such as supplementary treatments or the use of iodine-enriched additives such as iodized salts (Hong et al., 2008). The latter is widely used, and may be the most economical and practical method, but iodized salt has its own drawbacks (Bülow Pedersen et al., 2006). Inorganic iodine is volatile and iodised salt is subject to losses during cooking and storage, especially at high temperatures. Therefore, producing iodine-enriched vegetables has been investigated as an alternative approach. Iodine of food stuffs can be assimilated easily by humans; bioavailability is up to 99% (Longvah and Deosthale, 1998, Weng et al., 2008). It is also been reported in many studies that the iodine concentration of crop plants can be increased significantly by iodine concentrations in the growth medium but the response varies depending on species and plant cultural method (Zhu et al., 2003, Landini et al., 2011). Differences in iodine uptake by plants appear to depend on (i) the particular form or chemical species applied (ii) the physiochemical properties of soil that control interaction with iodine and (iii) the method of application. In addition, plant species differ in their preference for different form of iodine (Smoleń et al., 2016). Generally iodine is added via aggregated fertilizers, foliar application or irrigation water. It has been reported that iodine application via irrigation water or

fertigation is more efficient and economical than other methods. Ren et al. (2008) applied 40-80 μg L^{-1} of potassium iodate to soil in remote areas of China via irrigation water and reported an increase in soil total and soluble iodine and in animal and human urinary iodine for 4 years following the single application.

Moreover, the distribution, transformation and translocation of iodine from soil to plant are not mechanistically understood. It has been reported that iodine in soil undergoes strong sorption by organic matter in reactions that can occur from 2 h to a few days after iodine introducing to the soil (Hakimi, 1996; Kashparov et al., 2005, Shetaya et al., 2012). Iodine readily interacts with aromatic compounds within fulvic and humic acids, resulting in strong sorption by soil (Schlegel et al., 2006). The process of iodine adsorption from Al/Fe hydroxides has also been reported (Pless et al., 2007).

Very little is known about the residual availability of iodine after fortifying soil. The objectives of this study were to better understand the dynamics of iodine in a soil-plant system with different plant varieties and to determine the efficiency of different iodine species, their behaviour and recovery rate by crop plants using soil and seed from the Kurdistan region. Therefore, the data discussed in this chapter include the ^{127}I uptake from the trial undertaken in the previous chapter (Experiment I) and uptake of both ^{127}I and ^{129}I in the new trial described in this chapter (Experiment II).

The main objectives of this study were:

a) To evaluate iodine biofortification in calcareous soils and efficiency of different species of iodine as a fertilizer

b) Determining the fate of daily iodine applied during the growing season and source apportionment of plant iodine from soil I and ^{129}I as a tracer.

5.2 Material and methods

OVERVIEW

The majority of procedures undertaken in this study are described in the previous chapter (see section 4.2.1 to 4.2.6).This section will therefore only consider *differences* in the methods applied to the new pot experiment discussed in this chapter. This second biofortification pot experiment (II) was designed using similar approaches but with higher ^{129}I concentrations. The number of plant varieties was decreased to three green leafy vegetables including celery, chard and lettuce.

The statistical analysis (one way and two way ANOVA) was carried out using Graph Pad prism 7.02.

5.2.1 Spiking soil with ^{129}I

The ^{129}I was applied daily, throughout the trial, in irrigation water. The $^{129}IO_3^-$ treatment was prepared from a stock solution (total activity of 4.52 kBq and activity concentration in solution of 0.143 kBq ml^{-1}) and the $^{129}I^-$ treatment was prepared from (total activity of 3.25 kBq and activity concentration solution of 1.72 kBq ml^{-1}). The ^{129}iodine (^{129}I) (SRM 4949c)

was obtained from the American National Institute of Standards (NIST), Gaithersburg, Maryland, USA. The half-life of ^{129}I is 1.57×10^{7} years.

Irrigation with iodine was started after seed germination using a diluted irrigation solution consisting of 1 mL of stock solution in 1 L of DI water (mQ water in pot experiment II); this was applied according to the plant's water requirements and the volume applied to the pots recorded. The final concentration of $^{129}IO_3^-$ and $^{129}I^-$ in irrigation water for the experiment (I) was 0.5 µg L^{-1}. For the experiment (II) the exact concentration of irrigation water was confirmed by ICP-MS and the concentrations were 6.89 and 5.56 µg L^{-1} for $^{129}IO_3^-$ and $^{129}I^-$ respectively.

5.2.2 Soil iodine analysis

All soil preparation after harvesting and the subsequent analysis was as described in the methods described in sections (2.4, 2.5, and 2.6) for Se analysis. The only difference was that in the pot experiment (II) soil fractionation of iodine, was carried out on moist soils immediately after harvesting, when the soil was at field capacity; the measurements were then repeated after air-drying.

5.3 Results and discussion

5.3.1 Plant uptake of ^{127}I (Experiment I)

Celery, lettuce and spring onion had significantly (Two way ANOVA, $p < 0.0001$) higher iodine concentrations compared to chard and radish. As shown in Figure 5.1 the mean ^{127}I concentration of vegetables across all treatments fell in the range 113-696 µg kg^{-1}_{dw} for radish and celery

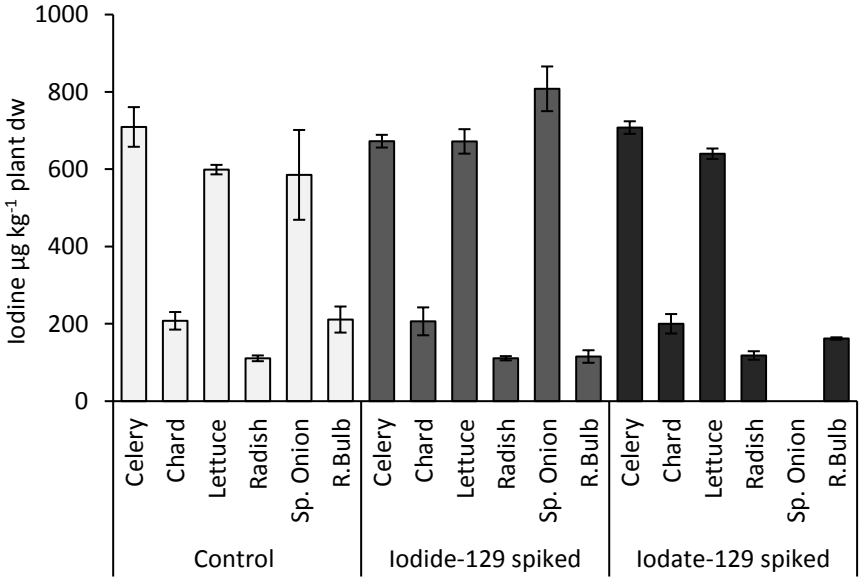


Figure 5-1. Concentration of ^{127}I in various plant species

respectively. No significant differences were observed ($\alpha = 0.05$, $P = 0.74$) for plant ^{127}I concentration in the control and two ^{129}I treatments. Thus, addition of $^{129}IO_3^-$ or $^{129}I^-$ spikes did not affect the uptake of ^{127}I. Due to the delayed germination of Sp.onion, there was insufficient biomass for analysis of ^{127}I in the $^{129}IO_3^-$-spiked Sp.onion.

Soil used for the pot trial had identical ^{127}I concentration in all treatments so, variation in ^{127}I plant concentration in different vegetables appears to be a result of plant physiological factors e.g differences in biomass causing dilution of native soil ^{127}I, stage of plant maturity affecting uptake rate or simply differences in the inherent affinity of plant for iodine uptake.

5.3.2 Fractionation and speciation of ^{127}I in soil (experiment I)

5.3.2.1 Soil soluble iodine ($^{127}I_N$)

Soil soluble iodine ($^{127}I_N$) was determined in the soils in which different vegetable varieties had been grown. No significant difference was observed for $^{127}I_N$ between control soils and soil subject to both ^{129}I spike treatments when similar vegetables had been grown (α=0.05, P=0.183). The value of $^{127}I_N$ varied in the range 23 - 97 µg kg^{-1}_{dw} depending on the vegetable type grown (Fig. 5.2). The concentration of $^{127}I_N$ in soils used to grow chard, lettuce and radish was considerably higher than $^{127}I_N$ in fallow soil (22 µg kg^{-1}_{dw}). This suggests that plant growth may have changed the availability of iodine in the rhizosphere (α=0.05, p<0.0001). Moreover, there were differences in the volume of roots between vegetables. The $^{127}I_N$

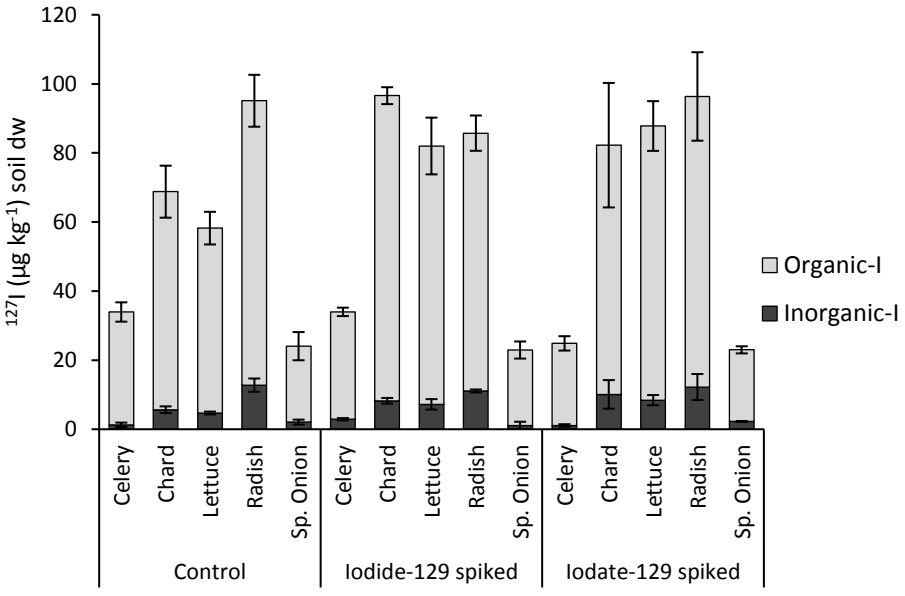


Figure 5-2. Total soluble ^{127}I concentrations ($^{127}I_N$) with apportionment between organic and inorganic I species

concentration was lower in the soils contained lower root densities e.g celery and Sp.onion. Organic acid exudates from roots may mineralize iodine bound to soil organic matter and transforms to organic soluble fraction. The soluble iodine $^{127}I_N$ as a fraction of total soil iodine ($^{127}I_{Tot}$) varied from 1 to 4% depending on the vegetable type grown; the mean value was 2.7% (n=60) for planted soil and 1.09% for fallow soil.

Speciation of $^{127}I_N$ showed that the majority of $^{127}I_N$ was organic (>90%); inorganic iodine fell in the range of 4 to 12 % of $^{127}I_N$ (1-15 μg kg^{-1}_{dw}) depending on which plants had been grown (Fig. 5.2). The mean concentration of inorganic iodine was 8% when all vegetables were considered whilst fallow soils had 2% inorganic iodine. The vast majority of inorganic iodine was in the form of iodide as <2% of inorganic iodine in $^{127}I_N$ was found in the form of iodate. Soil on which radish had been grown had the greatest amount of inorganic iodine in contrast to soil on which celery and Sp.onion, with their lower root density, had been grown. It should be noted that speciation of $^{127}I_N$ was carried out on air dry soil following harvesting and so may have been affected by the drying process. Nevertheless, the amount of $^{127}IO_3^-$(N), $^{127}I^-$(N) and $^{127}I_{Org}$(N) were significantly different in soils depending on which vegetables had been grown.

5.3.2.2 Soil adsorbed iodine ($^{127}I_P$)

As was found for $^{127}I_N$, the adsorbed iodine fraction ($^{127}I_P$) showed no significant difference between control soils and both ^{129}I treatments (α=0.05, p=0.807). Values of $^{127}I_P$ varied in range of 39 -70 µg $kg^{-1}{}_{dw}$ in soil which Sp.onion and chard had been grown respectively (Fig 5.3). This range was more limited compared to that of $^{127}I_N$ but differences were statistically significant (α=0.05, p<0.0001). It seems the factors which caused

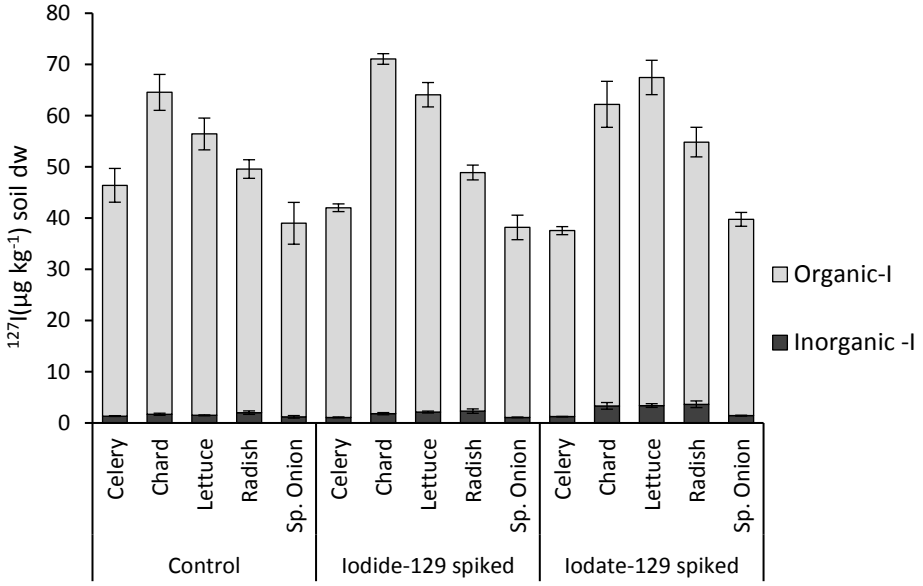


Figure 5-3. Total adsorbed ^{127}I concentrations ($^{127}I_P$) with apportionment between organic and inorganic I species.

differences in $^{127}I_N$ also affected $^{127}I_P$.

The $^{127}I_P$ as a fraction of $^{127}I_{Tot}$ ranged between 1.8-2.8% (Mean=2.3%; n=60). Thus, speculatively, the iodine 'available for plant uptake' ($^{127}I_N$+ $^{127}I_P$) was 6% of $^{127}I_{Tot}$ but strongly affected by the vegetable type previously grown. The great majority of $^{127}I_P$ was organically bound (96%). The inorganic $^{127}I_P$ varied between 2-6% with a mean value of 4%. The majority of inorganic iodine was again iodide which ranged from 1.15 µg $kg^{-1}{}_{dw}$ for

celery and Sp.onion jointly to 2.03 µg kg^{-1}_{dw} for chard. Concentrations of $^{127}I_P$ were lower than those of $^{127}I_N$.

5.3.3 Plant uptake of ^{127}I and ^{129}I (Experiment II)

Plant ^{127}I concentration was in the range of 204-462 µg kg^{-1}_{dw} for lettuce and celery with statistically significant differences between plants (α=0.05, P<0.0001) (Table 5.1). The ^{127}I concentrations in roots were significantly higher than in shoots (α=0.05, P<0.002). It seems unlikely that this was due to soil contamination of roots as the roots were washed and variation between replicates was very small (standard error was negligible). Weng et al. (2008) found higher concentrations of iodine in roots compared to the shoots of Chinese cabbage when the plants were biofortified by both organic and inorganic fertilizers. Similarly, Weng et al. (2013a) reported higher concentrations of iodine in the roots of leafy vegetables compared to leaves when they applied algal organic iodized fertilizers to spinach, cabbage, coriander.

The analysis of ^{129}I showed that vegetables irrigated by $^{129}I^-$ had considerably lower iodine concentration (6.2 - 12 µg kg^{-1}_{dw}) than those irrigated with $^{129}IO_3^-$ (53.3 - 479 µg kg^{-1}_{dw}) (Fig 5.4). Chard had the greatest ^{129}I concentration which may attributed to the innate affinity of this plant for iodine.

Table 5.1.Concentration of ^{127}I according to diiferent plants and treatments (n=4)

		^{127}I (µg kg^{-1}) plant dw		
		Control	$^{129}IO_3^-$ spiked	$^{129}I^-$ spiked
Shoot	Celery	330	462	412
	Chard	216	218	336
	Lettuce	227	204	252
Root	Celery	931	794	721
	Chard	856	1180	1300
	Lettuce	936	963	1170

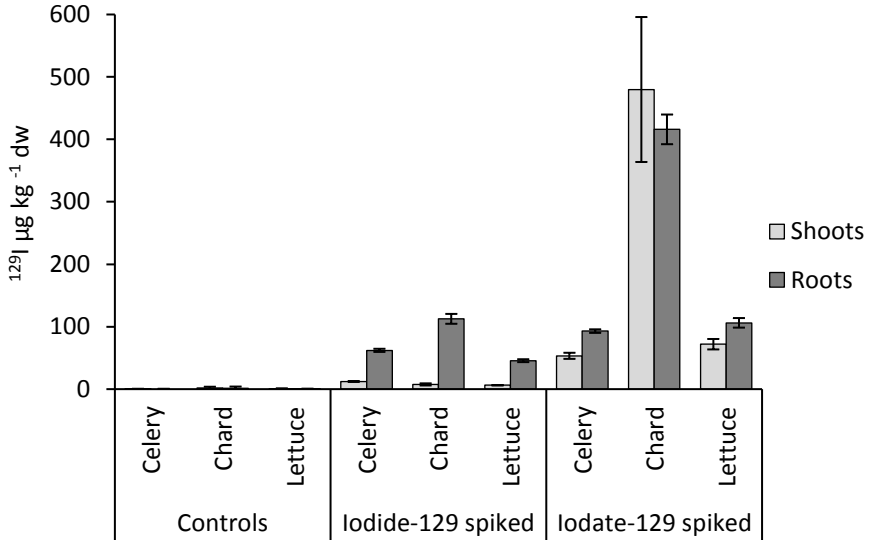


Figure 5-4. Concentration of ^{129}I in shoots and roots according to iodate and iodide treatments (n=4)

Unlike the shoots, ^{129}I concentration in the roots of $^{129}I^-$ treated vegetables was comparable to those treated by $^{129}IO_3^-$ with the exception of chard that had the greatest amount of ^{129}I in roots (Fig 5.4). Thus although iodide was taken up by roots there seemed to be limited subsequent transfer to the shoots.

Compared with the ^{127}I concentration of plants (native soil iodine), celery and lettuce had greater ^{127}I concentration than ^{129}I whilst for chard the reverse was true (Fig 5.5a). The ratio of ^{127}I:^{129}I must be a balance between the greater availability of ^{129}I which was added in irrigation water as an inorganic compound and the greater overall pool of soil ^{127}I in the soil. The differences between the plant types may be ascribed to intrinsic differences in physiology and affinity for iodine or may arise from differences in timing of uptake as the ^{129}I gradually becomes fixed within the soil. Figures 5.5 and 5.6 present the absolute and proportional distributions of ^{129}I and ^{127}I in shoots and roots as a function of iodine species added. For vegetable shoots treated with $^{129}I^-$ (iodide) only 3% of the iodine of the three test

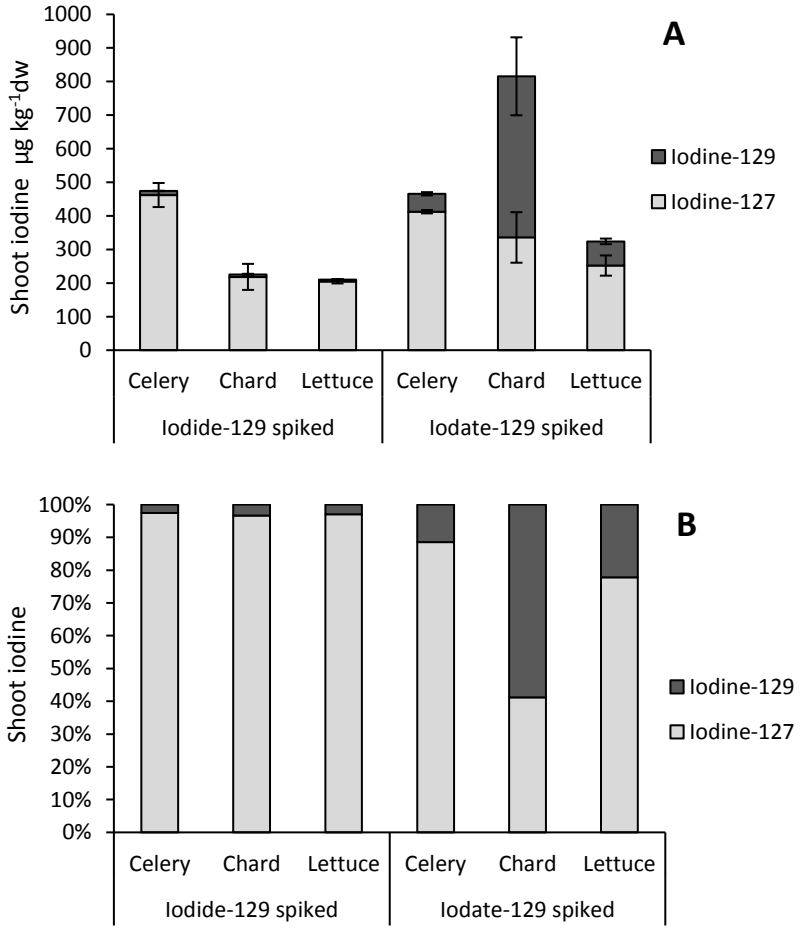


Figure 5-5. Distribution of ^{129}I and ^{127}I concentration in shoots for three plant types and contrasting iodine species (a) total iodine concentration (b) proportion of total concentration (n=4)

plants accumulated originated from the ^{129}I treatment. By contrast, for $^{129}IO_3^-$ (iodate) 11, 22 and 58% of iodine in the shoots of celery, lettuce and chard were from the ^{129}I addition respectively. For the roots of plants treated with $^{129}I^-$ (iodide), the ratio of $^{129}I/I_{Tot}$ was slightly greater (4 – 8%) than for shoots (3%). This ratio was not significantly different for celery and lettuce for $^{129}IO_3^-$ treatment compared to $^{129}I^-$ but for chard 24% of root iodine originated from added $^{129}IO_3^-$.

A possible reason for low ratio of $^{129}I/^{127}I$ overall can be attributed to high calcium carbonate content of this soil. Mehra et al. (2014) investigated the bioavailability of iodine in two type of UK soils with different calcium content

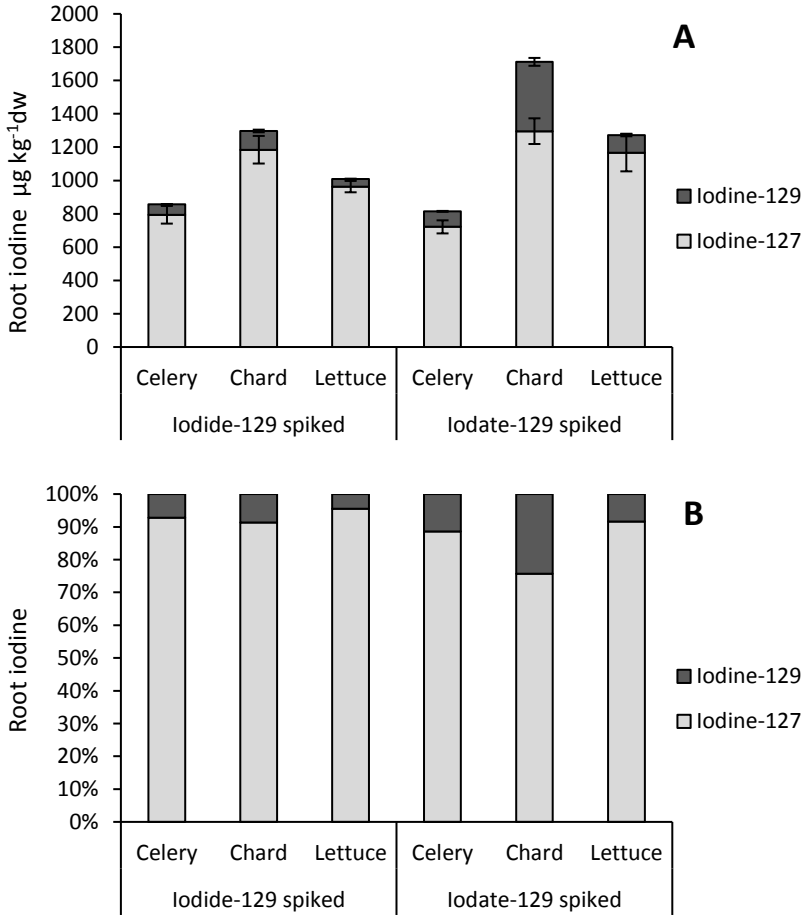


Figure 5-6. Distribution of ^{129}I and ^{127}I concentration in roots for three plant types and contrasting iodine species (a) total iodine concentration (b) proportion of total concentration

and reported that higher soil pH and calcium content of the limestone (higher Ca) area soils restrict iodine availability and mobility in that areas and also they reported a lower soil to plant transfer factor in the limestone area with high Ca content.

Smoleń et al. (2016) reported that a pot trial investigating fertigation of spinach with iodate and iodide showed a transfer factor several-fold higher for iodate than for iodide fertigation. In a similar study Weng et al. (2008) confirmed that iodine uptake by Chinese cabbage was more effective when iodine was in the form of IO_3^- rather than I^-.

It appears that biofortification with iodide is not suitable in this soil and that the plant's iodide uptake is restricted by plant or soil factors. Soil factors may include fixation of iodide by soil components such as organic matter. As a result, availability of iodide would be decreased during the growth period. Alternatively, the low bioavailability of iodide may reflect the plant's low intrinsic affinity for that species of iodine. Furthermore, regardless of iodine species, there may be physiological limitations to the translocation of iodine from root to shoot as seen in the chard plants.

5.3.4 Fractionation of ^{127}I in wet and air-dried soil (Experiment II)

Concentrations of soluble iodine, $^{127}I_N$, determined after plant harvest on moist potted soil ranged between 6.51 and 12.6 µg kg^{-1}_{dw}. However, this range was significantly ($p<0.01$, multiple t test) lower than $^{127}I_N$ concentrations in the same soil when it was air-dried prior to measurement

(86.8 - 273 µg kg $^{-1}_{dw}$); 20-25 times greater than values for wet soils. Mineralization of iodine resulting from drying the soil could be one reason for such a disparity. A higher value of $^{127}I_N$ in control soils planted with lettuce was observed compared to the other two treatments to which ^{129}I was added but for the soils on which celery and chard were grown this trend was not observed (Fig 5.7a).

Similarly, the soil $^{127}I_P$ fraction measured in wet soils ranged from 41 to 58 µg kg $^{-1}_{dw}$ which was significantly lower compared to air-dried soils which

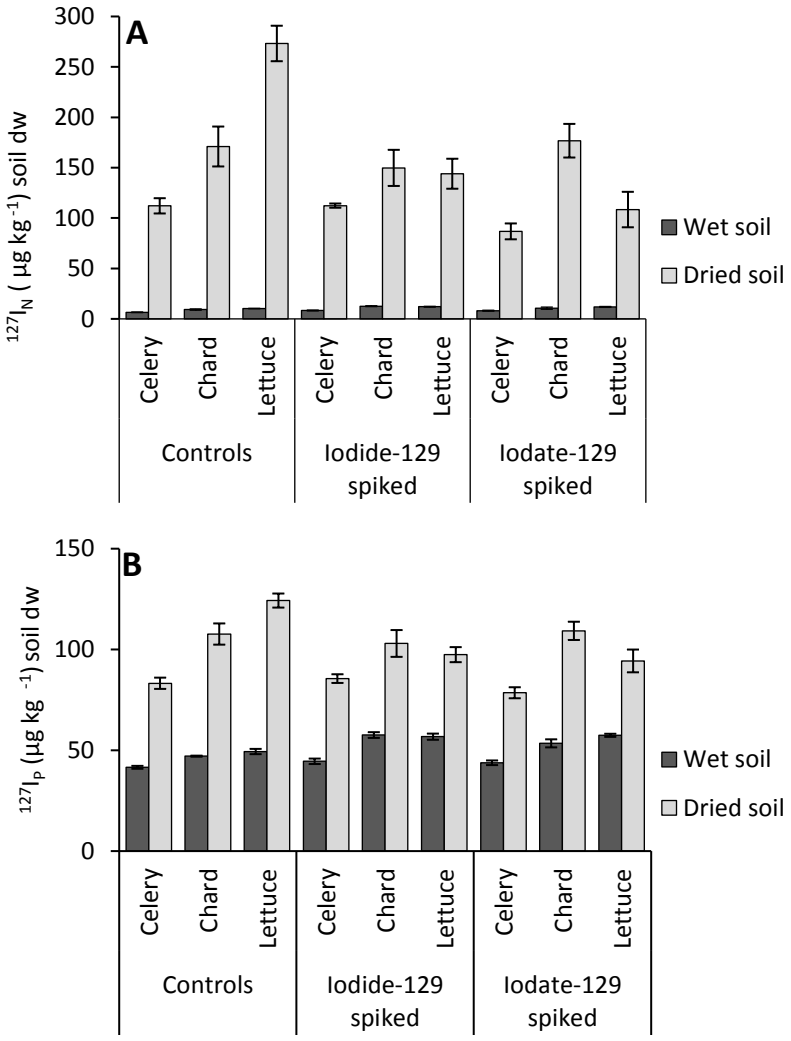


Figure 5-7. Soil fractionation of ^{127}I measured on moist and air-dried soil: (a) Soluble $^{127}I_N$ extracted with 0.01M KNO_3 (b) Adsorbed $^{127}I_P$ extracted with 0.016M KH_2PO_4

ranged 78-124 μg kg^{-1}_{dw} (Fig 5.7b). The ratio of $^{127}I_N$:$^{127}I_P$ was also affected as the change in $^{127}I_P$ as a result of drying was only 2 fold.

The total amount of 'available' iodine ($^{127}I_N$ + $^{127}I_P$) as a fraction of $^{127}I_{Tot}$ was 2.6% in the wet soils but 8.2% when measured on air-dried soils. Translating these findings into the field it may be implied that the availability of soil iodine is likely to change seasonally and possibly between irrigation events in farms - particularly in the study area with infrequent irrigation and the soil susceptible to drying out due to the climate.

5.3.5 Soil ^{127}I speciation measured on potted soil after harvest

The speciation of $^{127}I_N$ on air-dried soil showed that 50-68% (mean=60%) of the iodine present in this fraction was inorganic and 32- 50% (mean= 40%) was organic. This may confirm the mineralization of organic iodine during the air-drying soil preparation process. The majority of inorganic iodine was iodide (3% $^{127}IO_3^-$ and 57% $^{127}I^-$ as a fraction of $^{127}I_N$) (Fig 5.8a). The iodate and iodide concentrations were a fairly constant ratio of $^{127}I_N$ and were also significantly correlated.

By contrast, for $^{127}I_P$ only 10% was found as inorganic iodine (3% $^{127}IO_3^-$ and 7% $^{127}I^-$) and 90% was present as organic iodine (Fig. 5.8b). Soil inorganic iodate and iodide in both $^{127}I_N$ and $^{127}I_P$ soil fractions were statistically different ($P < 0.05$) in soil on which different vegetables had been grown (discussed in experiment I).

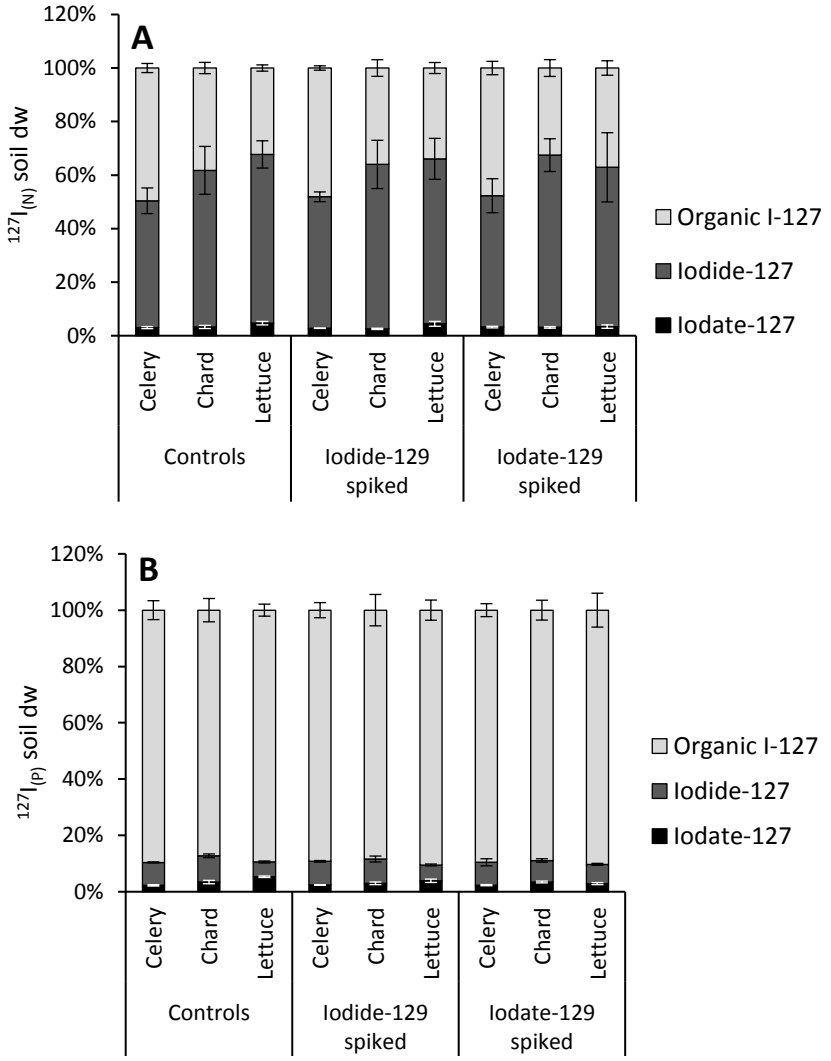


Figure 5-8. Speciation of soil ^{127}I as organic and inorganic iodine: (a) Soluble iodine fraction $^{127}I_N$ (b) Adsorbed iodine fraction $^{127}I_P$

5.3.6 Soil ^{129}I fractionation of wet and dry soils

The $^{129}I_N$ concentration measured in wet soils ranged from 0.275 to 2.38 µg kg $^{-1}_{dw}$ across all treatments whilst this range was 1.18-3.14 µg kg $^{-1}_{dw}$ in air-dried soils. Soils irrigated with $^{129}I^-$ had a significantly lower ^{129}I concentration in the soluble fraction compared to those treated with $^{129}IO_3^-$ (Fig 5.9a). This difference could explain why plant ^{129}I concentrations were

lower when grown in $^{129}I^-$ treated soils (Fig 5.9 and 5.4). Iodide may be immobilized within humus faster than iodate. Dai et al. (2006) obtained a similar result when they applied 0.5, 1 and 2 mg kg^{-1} iodate and iodide to potted soils sown with spinach and reported a higher soil solution iodine concentration for iodate treatments. Soil $^{129}I_N$ concentrations also varied depending on which vegetable type had been grown, suggesting that plant factors also influenced ^{129}I availability in soluble fractions.

As was found for ^{127}I, soil $^{129}I_N$ concentrations were increased following air drying in both the iodate and iodide treatments. Soils irrigated with $^{129}I^-$ were more affected by the drying process, producing a greater difference between iodine concentrations in the wet and air-dried soils compared to soils irrigated $^{129}IO_3^-$.

The effect of air drying suggests that $^{129}I^-$ absorbed by organic matter during the growing season can be re-mineralized as a result of drying and re-wetting. The mean proportion of $^{129}I_N$ as a fraction of soil $^{129}I_{Tot}$ was 1.5% and 6% in wet soils and 5 and 9 % in dried soils for soils irrigated by $^{129}I^-$ and $^{129}IO_3^-$ respectively.

Soil $^{129}I_p$ concentration ranged between 1.68-4.49 µg kg^{-1}_{dw} in wet soils but was only slightly different (1.87-4.29 µg kg^{-1}_{dw}) in air-dried soils. The mean proportion of $^{129}I_p$ as a fraction of $^{129}I_{Tot}$ was 8% and 12% for soils treated by $^{129}I^-$ and $^{129}IO_3^-$ respectively in both wet and air-dried soils. Values of $^{129}I_p$ for iodide treatments

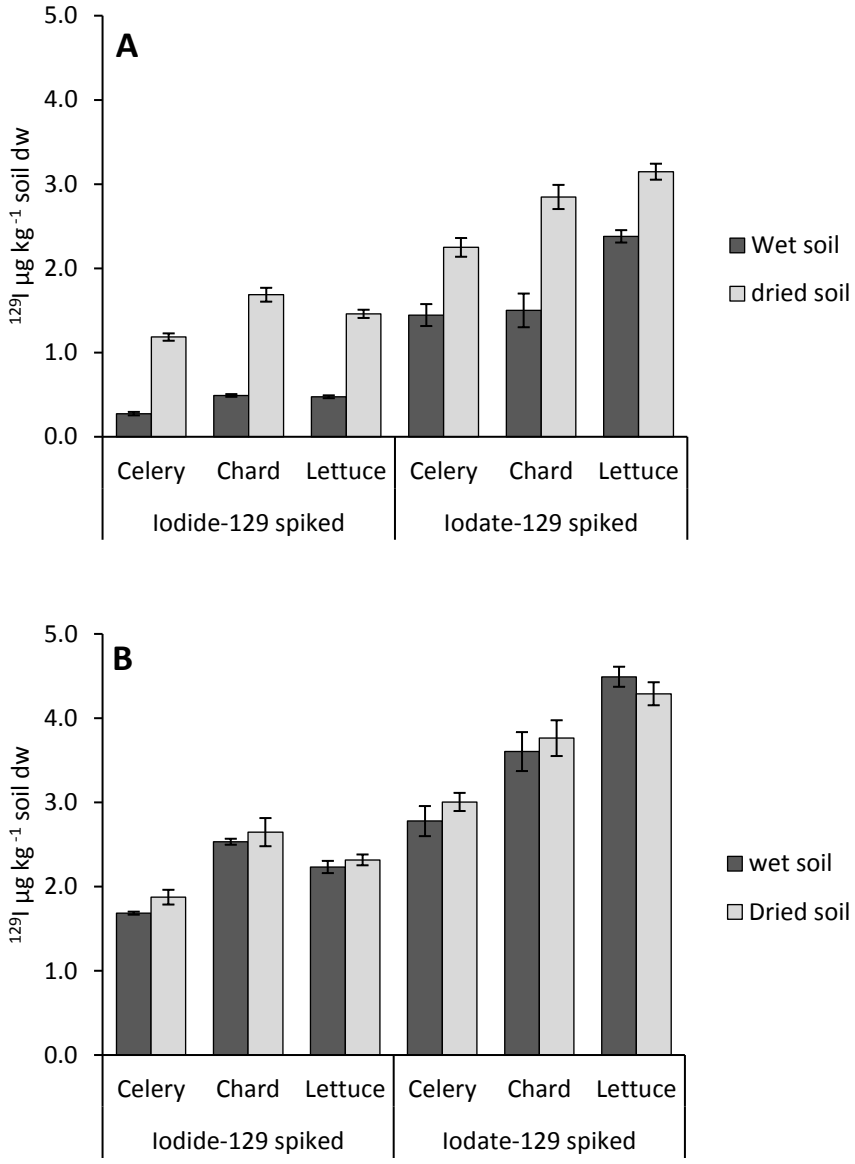


Figure 5-9 Soil fractionation of ^{129}I measured on moist and air-dried soil: (a) Soluble $^{129}I_N$ extracted with 0.01M KNO_3 (b) Adsorbed $^{129}I_P$ extracted with 0.016M KH_2PO_4

were greater than values of $^{129}I_N$ but they were still lower than those irrigated by $^{129}IO_3^-$ (Fig 5.9 A&B). The differences between iodate and iodide treatments were smaller in $^{129}I_P$ compared to $^{129}I_N$ however they were statistically significant ($\alpha=0.05$, $p<0.01$). The air drying process had no significant effect on $^{129}I_P$ concentration in both iodate and iodide treatments ($\alpha=0.05$, $p=0.63$).

The combined concentrations of ($^{129}I_N$ +$^{129}I_P$), representing 'available' iodine for plant uptake were 12 and 19% (iodide and iodate) measured in wet soils whilst these values for air dried soils were 13 and 21% (iodide and iodate) which shows a much higher availability of ^{129}I spiked compared to soil native ^{127}I.

The mean concentration of ^{129}I in TMAH extractions representing total organic iodine ($^{129}I_{TMAH}$) was 23.8 and 24.5 μg kg^{-1}_{dw} for wet and air-dried soil respectively. There was no significant difference between soils receiving iodide or iodate and also no significant difference between dried and wet soils (α=0.05, p>0.064). However the total iodine concentration ($^{129}I_N$+$^{129}I_P$+$^{129}I_{TMAH}$) was 27.8 and 29.6 μg kg^{-1}_{dw} in wet and dry soils. More details will be discussed in the recovery section (section 5.3.8).

5.3.7 Soil ^{129}I speciation in $^{129}I_N$ and $^{129}I_P$ fractions

Speciation of ^{129}I showed that the mean proportion of organic ^{129}I in the $^{129}I_N$ fraction accounted for 52% of $^{129}I_N$ in soils irrigated with $^{129}I^-$ but only 18% for soils irrigated by $^{129}IO_3^-$ (Fig 5.10a). Thus almost half of the remaining soluble inorganic iodide of this fraction has been converted to a soluble organic form. By contrast, the majority of $^{129}I_N$ in the iodate-irrigated treatment was inorganic in the end of growing season. Thus not only does a greater proportion of iodide become immobilized into organic-bound forms in the solid phase (than iodate), but even in the soluble fraction the majority of the ^{129}I-iodide originally added is in an organic form which may be unavailable to plants. Moreover, the whole 48% of inorganic iodine in the $^{129}I^-$ irrigated system was iodide whilst 82% of inorganic iodine

in the $^{129}IO_3^-$ irrigated system was distributed 60% and 22% as iodate and iodide respectively (Fig.5.10a). This implies that there was no $^{129}I^-$ oxidation

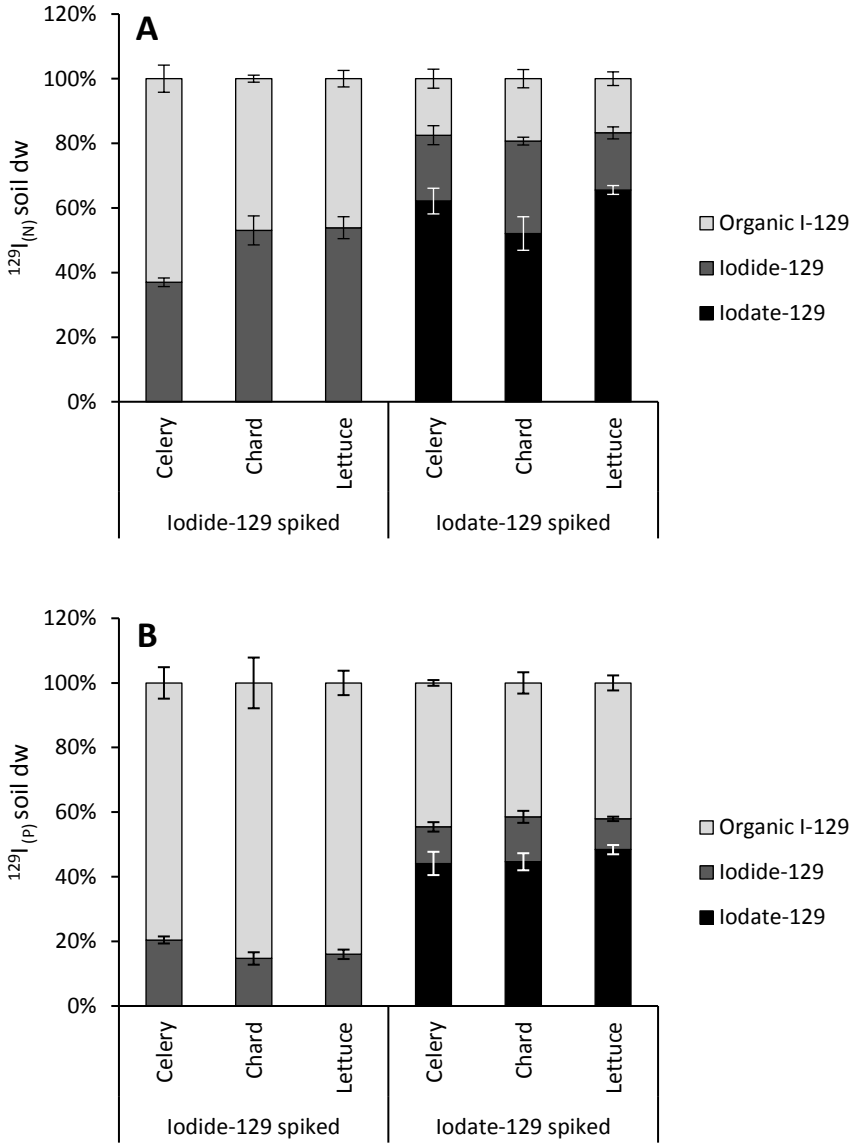


Figure 5-10. Speciation of ^{129}I in both iodate and iodide irrigated system according to organic and inorganic iodine at the end of growing season (a) soluble $^{129}I_N$ fraction (b) Adsorbed ^{129}I fraction

to $^{129}IO_3^-$ in the $^{129}I^-$ irrigated system whereas 22% of $^{129}IO_3^-$ was transferred to $^{129}I^-$ in the $^{129}IO_3^-$ irrigated system. This is consistent with the finding that inorganic iodine was greater in the iodate irrigated system.

Shetaya et al. (2012) incubated a range of soils with $^{129}IO_3^-$ and $^{129}I^-$ and, after sequential extraction, they found that iodide sorption was faster than iodate. Also they reported greater $^{129}IO_3^-$ adsorption in low pH and in soils with high iron-oxide contents (20-36% adsorption in 1 h) whilst the slowest time-dependent $^{129}IO_3^-$ loss was found in soils with high pH and low organic carbon (detectable after 3975 h) – those characteristics are similar to the soils used in this study. In keeping with the result of this study they also found that in arable soils with low organic carbon and high pH values after 800 h >60% of $^{129}I_N$ remained as $^{129}IO_3^-$, By contrast in grassland soils with higher organic matter contents all $^{129}IO_3^-$ was converted to soluble organic forms in 800 h. It should be acknowledged that ^{129}I was added incrementally in this study, in daily applications of very small amounts but the overall pattern of fractionation and speciation appears still comparable with the results of the Shetaya study. In addition, Shetaya et al, did not observe any conversion of $^{129}I^-$ to $^{129}IO_3^-$ and reported the rate of conversion of $^{129}I^-$ to soluble organic iodine was greater than that of iodate which is comparable with result of this study.

Speciation of $^{129}I_P$ (the adsorbed iodine fraction) in soils irrigated by $^{129}I^-$ indicated that 83% of adsorbed iodine remaining in the soil after the growing season was organic; by contrast the proportion was 43% in soils irrigated by $^{129}IO_3^-$. Thus there was a greater proportion of organic iodine in $^{129}I_p$ compared to the speciation of $^{129}I_N$. The proportion of inorganic iodine (17%) left in $^{129}I^-$ irrigated systems was all iodide whilst the 57% of inorganic ^{129}I remaining in soils irrigated with $^{129}IO_3^-$ was distributed as 11% iodide and 46% iodate.

The proportion of organic $^{129}I_P$ for both iodate and iodide treatments was greater than in $^{129}I_N$. This may suggest that the soluble fraction is composed of more recently added iodine which remains in an inorganic form or, alternatively, if equilibrium exists between adsorbed and soluble fractions, then it may simply mean that the kd value for organic iodine is higher than those of its inorganic counterparts (I^- and IO_3^-). In the study of Shetaya et al. (2012) they found the majority of $^{129}I_P$ (90%) was in inorganic from in arable soils but over time the proportion of inorganic iodine decreased to 3% in iodate-spiked soils and (1.6 -6.6%) in iodide-spiked soils. Those proportions are lower than in this study which probably reflects the incremental addition approach used in which inorganic iodine was effectively replenished via irrigation water in the current study. This is reasonable evidence that iodine bio-fortification via irrigation water may be more efficient in sustaining iodine supply to crops than applying a single iodine dressing.

5.3.8 Soil-to-plant transfer factor (TF)

The soil-to-plant transfer factors for ^{129}I in the $^{129}I^-$ treatment were (0.23, 0.24 and 0.47) for chard, lettuce and celery respectively - slightly higher than those of ^{127}I at 0.07 for chard and lettuce and 0.16 for celery. In contrast the TF for ^{129}I in the $^{129}IO_3^-$ treatment was considerably higher than that of ^{127}I from native sources of iodine: 1.75, 2.36 and 15.5 for ^{129}I uptake by celery, lettuce and chard respectively, compared with 0.14, 0.09 and 0.11 for ^{127}I uptake by the same plants. Therefore the ^{129}TF of chard was more than 100 times greater than ^{127}TF whilst this ratio was 26 and 12 for

lettuce and celery (Fig 5.11). This was expected due to greater availability of ^{129}I added incrementally in an inorganic form even though the concentration of soil ^{127}I was approximately 100 times greater than ^{129}I when measured at the end of the experiment. Values of ^{129}TF for soils irrigated with $^{129}I^-$ were lower than those of $^{129}IO_3^-$. The root transfer factors were greater than the shoot TF values for all vegetables and both treatments, except for chard (α=0.05, p>0.0001). The variable TF for different plant types can imply different inherent affinities of those plants for iodine uptake suggesting either (i) different physiological uptake mechanisms or (ii) simply differences in uptake capacity caused by, for example, different densities of root adsorption sites or transporters.

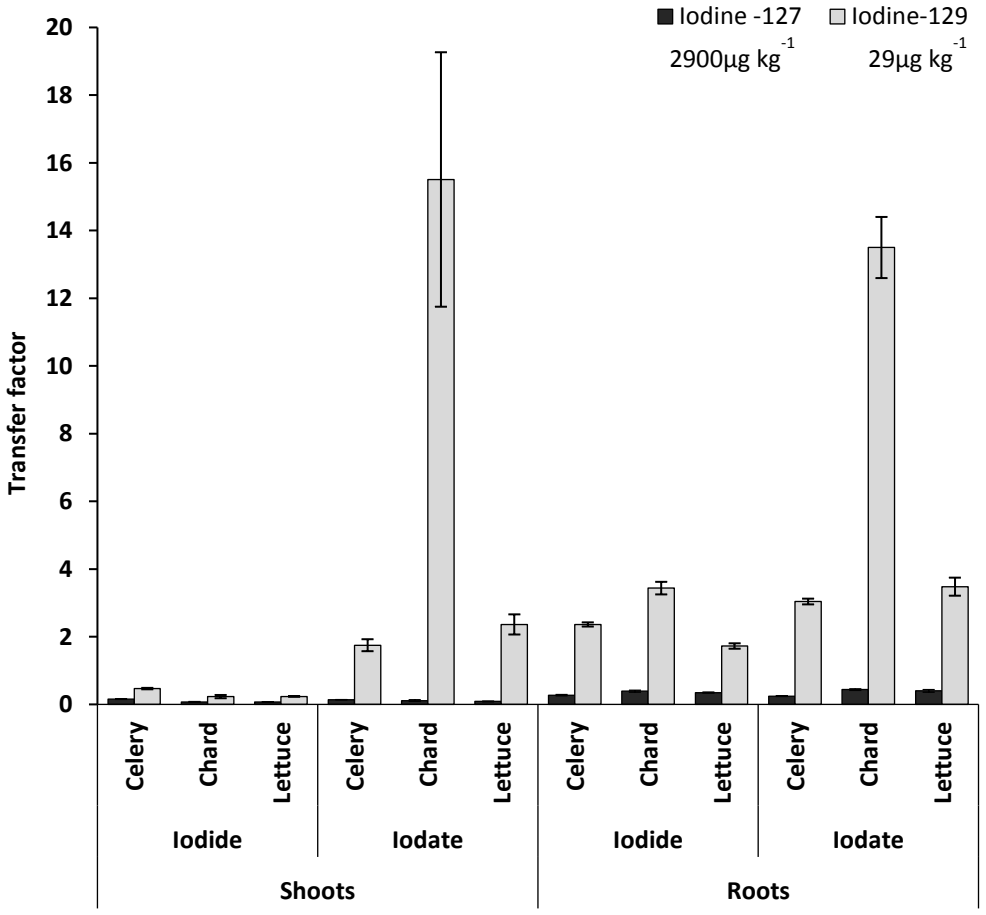


Figure 5-11. Soil to plant transfer factors for soil native iodine (^{127}I) and added ^{129}I in shoots and roots

As in section 3.4.7.3 role of irrigation water iodine content in plant iodine content was demonstrated suggesting iodine from native soil is not the only source of iodine for plant uptake as irrigation water can be an important source of plant iodine. In current experiment due to using milli-Q water as an irrigation water and low iodine background of milli-Q water (mean= 0.163 μg L^{-1}) it doesn't seems to be an influential in plant uptake. However, after calculation of accumulative ^{127}I added during the growing season and the whole ^{127}I taken up by plant biomass found that irrigation water potentially able to support a partial iodine requirement of plant even when milli-Q water used. The amount of ^{127}I taken up by plant biomass was calculated as 0.63, 0.96 and 1.41μg for celery, lettuce and chard respectively whilst the amounts of ^{127}I added in irrigation water during the whole season were 0.40, 0.54 and 0.46 μg for celery, lettuce and chard. It is not clear how much of the plant ^{127}I content originated from irrigation water, rather than the soil, but considering the much greater availability of iodine added in water it seems likely that a considerable proportion of the iodine in irrigation water would be available for plant uptake. This in turn suggests that the disparity in ^{127}TF and ^{129}TF seen may not fully reflect differences in the availability of soil and irrigation water and the availability of soil iodine may be considerably lower than suggested by the value of ^{127}TF.

In agreement with the TF result, Dai et al. (2006) biofortified soil-grown spinach and found that the TF of spinach leaves increased significantly from 0.04 to 2.32 with application of iodate. They also reported that the TF values for spinach leaves over several different iodate treatments were tenfold greater than those for iodide treatments. Weng et al. (2009) also measured

a TF value of of 2.63 for the whole plant and a maximum value of 13.85 for the roots in iodine-biofortified cabbage in a soil-based pot experiment.

5.3.9 The recovery of ^{129}I

The recovery of ^{129}I was determined from the accumulative irrigation water applied during the growing season, the amount of ^{129}I taken up by plant biomass and total ^{129}I soil analysis following the growing season. There were some differences in total recovery of ^{129}I depending on the plant type grown and the iodine species used in the ^{129}I treatments. The apparent recovery rate ranged between 63-95% and 65-97% calculated according to the analysis of moist and dried soil from the pot trial. Details are shown in Table 5.2. The ^{129}I recoveries in pots irrigated by $^{129}IO_3^-$ were less than in $^{129}I^-$ irrigated soils suggesting more missing iodine from iodate irrigated systems. There should not have been any iodine loss from leaching of irrigation water as this was conserved within the pot. Iodide transformation to organic-I could be one reason for this difference if organic matter adsorption of iodine is a mechanism that prevents transformation to gaseous forms and volatilization from the soil surface. Oxidation of I^- to I_2 can occurs in acidic pH and presence of organic acids on the other hand, in aerobic situation reduction of IO_3^- to I_2 occurs relatively easy in the soils or root surface (Fuge and Johnson, 1986).

However, incorporation into soil humus under acidic conditions of moorland woodland soils seem unlikely to apply in the calcareous soils used in this experiment. It may be that the apparent loss of ^{129}I was incorporation into

$CaCO_3$ and, although still present in the soil at harvest, the extraction with 10% TMAH was inadequate to solubilize such fixed forms of iodine.

Soils in which celery had been grown had greater ^{129}I recovery compared to those used to grow chard and lettuce. This suggests lower losses of iodine from soils in which vegetables with relatively small biomass had been grown. One of the reason could be that plants with a greater leaf surface area, such as lettuce and chard, can volatilize iodine from their leaves (Amiro and Johnston, 1989).

The amount of ^{129}I recovered by plant biomass (≈ 1%) was negligible compared to soluble and adsorbed fractions except for chard grown in iodate irrigated system. Also, ^{129}I recovery was slightly higher when calculated from analysis of air-dried soils compared to analysis of moist soil samples. The largest difference was seen in a higher amount of iodine in the soluble and adsorbed fractions after air-drying which translated into a greater overall value of $^{129}I_{Tot}$ compared to analysis of moist soils. In general, in a iodate-irrigated system 8-35% iodine was missing depending on the vegetable varieties; in iodide-irrigated pots this range was 3-25%.

Weng et al. (2009) fortified cabbages grown in potted soils with ^{125}I and found that paddy soils and sandy soils exhibited strong retention for spiked ^{125}I with most of the iodine applied remaining in the surface 10 cm of soil. Kashparov et al.

Kashparov et al. (2005) applied ^{125}I in a field experiment and found that 97% of the radioiodine was absorbed by soil over the course of 30 days. It can be suggested to apply higher concentration of iodine as a bio

fortification in studied calcareous soils as the low concentration of 5 μg L^{-1} of ^{129}I was applied as a tracer in this study.

Ujowundu (2010) applied 10, 40 and 80 μg L^{-1} iodate in irrigation water applied to Nigerian vegetables and obtained the most significant iodine was observed in the 40 μg L^{-1} treatment compared to other two treatments.

Table 5.2. The recovery rate of ^{129}I applied during growing season according to iodine spacious treatments and in wet and air-dried soils

vegetables	^{129}I species treatment	Irrigated water L	Water ^{129}I Con µg L^{-1}	^{129}I Added µg Pot^{-1}	Biomass ^{129}I uptaken µg	Wet Soil $^{129}I_{Tot}$ µg	Dry soil $^{129}I_{Tot}$ µg	Wet Soil recovery %	Dry soil recovery %
Celery	$^{129}IO_3^-$	2.15	6.89	14.8	0.0922	11.7	13.5	79.9	91.6
	$^{129}I^-$		5.56	11.9	0.0291	11.3	11.6	94.9	97.2
Chard	$^{129}IO_3^-$	3.30	6.89	22.7	1.11	13.1	13.6	62.7	64.6
	$^{129}I^-$		5.56	18.4	0.120	13.2	14.4	72.4	79.0
Lettuce	$^{129}IO_3^-$	2.80	6.89	19.3	0.196	12.8	13.5	67.2	70.7
	$^{129}I^-$		5.56	15.6	0.0326	11.2	11.6	72.0	74.8

5.4 Conclusion

The results of this study demonstrate that both plant physiological characteristics, or growth pattern, and speciation of iodine applied will affect plant iodine concentration. Plant ^{127}I concentration originating from soil native iodine and irrigation water varied between different plant types. This was also true for ^{129}I plant concentration originating from spiked ^{129}I added via irrigation water. The ratio of $^{129}I/^{127}I$ in plants also varied which suggests differences in plant affinity for iodine uptake but also differences in timing of uptake during the growth period. The majority of the plant iodine was from soil native ^{127}I and from irrigation water sources, except in the case of chard in which more than half of the shoot iodine content originated from spiked ^{129}iodine. Generally iodine concentrations in roots were greater than in shoots for all treatments and plants. The iodine concentrations in plants irrigated with iodide were significantly lower than in those irrigated with iodate.

The soluble iodine fraction in soil varied with plant type grown. Soluble iodine was greater in soils in which plants had generated a greater root mass suggesting some specific rhizosphere effect on iodine solubility. This effect was also seen for the adsorbed iodine fraction but to a lesser extent.

The concentration of ^{127}I in the soluble fraction was considerably increased as a result of air-drying the soil prior to analysis, compared to analysis of moist soils following harvesting. This difference was also observed in the adsorbed fraction but to a lesser extent. Mineralization of immobilized organic iodine, or microbial biomass, during the drying process could be one

possible reason. The ^{129}I concentration in the soluble fraction, but not the adsorbed fraction, was also found to be affected as a result of soil drying.

The ^{129}I concentration in the soluble and adsorbed fractions were low in iodide-irrigated systems compared to soils subject to iodate-spiked irrigation water. Speciation results demonstrated that at the end of the growing season almost half of the $^{129}I^-$ in the soluble fraction was converted to soluble organic forms whilst only 18% of $^{129}IO_3^-$ was in the form of organic iodine. By contrast, in the adsorbed fraction, the majority (>80%) of $^{129}I^-$ was converted to organic forms whilst almost half of the $^{129}IO_3^-$ was organic iodine. There was no conversion of $^{129}I^-$ to $^{129}IO_3^-$ in iodide-irrigated systems but some of the $^{129}IO_3^-$ was converted to iodide ($^{129}I^-$) where iodate had been added in irrigation water. Generally iodate was more efficient in biofortifying crop plants.

The overall recovery rate of applied ^{129}I ranged from 64-97% depending on iodine species and the type of vegetable crop grown. In soils irrigated with iodide more ^{129}I was recovered suggesting greater loss of iodate but it was unclear whether a hidden form of iodine, perhaps included within $CaCO_3$ matrices, remained unaccounted for by TMAH extraction of the soil. Nevertheless, soils in which plants produced less biomass, and less roots, exhibited greater retention of iodine possibly indicating foliar volatilization.

The role of irrigation water ^{127}I should be considered as a source of plant iodine uptake in all trials utilizing enriched isotope spikes of iodine. The current study showed substantial potential for plant uptake of 'incidental' ^{127}I from irrigation water, even when Milli-Q water was used for irrigation.

The ratio of $^{129}I/^{127}I$ differed between plant types and was generally very low. The different in ratio could be because of physiology of plants but the low ratio can be resulted by very low ^{129}I concentration of irrigation water. It could be suggested using iodate species in higher concentration for this soils. The Haigh calcium carbonate content (35%) of studied soil could be a reason to restrict the bioavailability of added iodine during the growing season. More investigation is required to better understanding of physiological role in iodine uptake in other plant species.

6 IODINE AND SELENIUM DIETARY SOURCE APPORTIONMENT AND EVALUATION OF URINARY IODINE AND SELENIUM IN THE IRAQI-KURDISTAN POPULATION.

6.1 Introduction

Iodine and selenium are essential micro-nutrients important for the function of several physiological processes in the body (Fordyce et al., 2000). Iodine is a vital component of thyroid hormones. Iodine deficiency is a significant global public health issue (Kučera, 2009). Low dietary intake of iodine can lead to functional and developmental abnormalities categorized as iodine deficiency disorders (IDD) of which the most well-known are goitre and mental retardation (WHO, 2004). Excessive iodine intake may result in pathological issues, including hypo and hyperthyroidism (Burgi, 2010). Selenium acts as an enzymatic cofactor of glutathione peroxidase and its deficiency has been suggested to increase cardiovascular disease and muscle weakness (Cohen and Avissar, 1994). It has been reported that selenium deficiency may be important in the onset of IDDs (Arthur et al., 1999).

There are several approaches to assessing the micro-nutrient intake of individuals including 24 h dietary recall or history surveys, food amount or frequency questionnaires (FAQ and FFQ), total diet studies, and 'duplicate' portion studies (Kučera, 2009). Nutritional iodine status can also be determined by measuring urinary iodine excretion as over 90% of ingested iodine is excreted in urine (WHO, 2013).

Foods typically contain low concentrations of both iodine and selenium, with the exception of dairy products and seafood which are rich in iodine (Dahl and Meltzer, 2009). To address IDDs in their populations many countries, including Iraq, encourage the use of iodized salt.

Locally generated food composition data to enable the assessment of I and Se status are not available for many countries, including for Iraq. The aim of this study was therefore to assess the I and Se status of the Iraqi population, specifically those living in the mountainous Kurdish controlled areas.

The main aim of this project was:

a) To determine the daily dietary intake of selenium and iodine by people and dietary source apportionment of these elements using a food amount questionnaire according to local and traditional food of that region.

b. To assess iodine and selenium nutrition status using human biomarkers (urine samples) and understand possibly relations with dietary intake or other sources

6.2 Material and methods

6.2.1 Survey of local foods

Vegetable crops, including leafy and non-leafy (fruit vegetables) vegetables and tubers, were collected from 40 farms across Sulaimani province in April-May 2014. A total of 300 plants covering 27 species commonly consumed in Kurdish diet were collected. Upon collection samples were placed in plastic bags and transported to the laboratory where they were weighed,

washed in deionised water, oven dried (50 C°) and then milled (ultra-centrifugal mill, Retch, Model ZM200, Germany). Ground samples (c. 250 mg) were microwave digested (Anton Paar, Multiwave) with conc HNO_3 for determination of total Se or extracted with 5% tetra methyl ammonium hydroxide (TMAH) for iodine prior to analysis using ICP-MS (Thermo Fisher, Model ICap Q) and employing 5 µg L^{-1} ^{72}Ge as an internal standard. The I and Se concentration of commonly consumed food crops from local farms in Kurdistan were measured to generate food composition data for the region

6.2.2 Dietary intake

A semi-quantitative FAQ based on the typical Kurdish diet was constructed and including 80 commonly consumed food items. Ethical approval for the questionnaire was granted by both the University of Nottingham (UK) (Ethics Reference No. OVS10022017; Feb-2017) and Sulaimani Polytechnic University (Iraq). Healthy participants (n = 410) were interviewed from volunteers family (n = 115) during March-April 2017. All members of the family older than 6 years were included. The questionnaire recorded the frequency and amount of food purchased, cooked and eaten by the family. The daily intake of each food item was calculated (g d^{-1}) for each family member.

Food waste and seasonal availability of some foods was also accounted for. From each family a sample of drinking water, rice (a widely consumed staple food) and salt were also collected for analysis of I and Se content. Salt iodine content was determined in 0.4 % a w/v salt solution by ICP-MS.

Samples of bread and yoghurt were purchased from shops in the area and analysed as described above. Dietary (μg d^{-1}) of Se and I and energy (kcal d^{-1}) intakes of food items were calculated for each survey participant, excluding school aged children under 14 years of age (n = 88) where information on intake was unreliable, using micro-nutrient concentrations in the locally collected foods and, where data was unavailable, food composition information compiled from literature databases .

6.2.3 Urine sampling

Each participant provided a first void urine sample on the morning after the dietary questionnaire was completed. Samples were collected, transported to the laboratory and frozen (-18 °C) pending analysis. Urinary creatinine, a breakdown product of creatine which is typically excreted at a relatively constant rate in urine (Soldin, 2002), was measured using (Randox RX-imola) spectrophotometrically.

Correction of measured urinary concentrations for hydration and adequacy of 24 h urine was tested using both creatinine and osmolality (Middleton et al., 2016) For creatinine: Eq 6.1

$$C_{crt-adj} = C_m / C_{crt}$$

Where C_m is urinary analyte concentration (μg L^{-1}) and C_{crt} is the corresponding urinary creatinine concentration (g L^{-1}). Urinary osmolality was determined by freezing-point depression (Cryoscopic Osmometer, Model Osmomat 030 series M). Urine concentrations were corrected for osmolality by applying a Levine-Fahy specific gravity adjustment:

$$C_{osm-adj} = C_m \times \left(Osm_{ref}/Osm_m\right)$$

Where Osm_{ref} was the median osmolality for all urine samples (674 mOsmol kg^{-1}; n = 410) and Osm_m was the osmolality measured in an individual sample (mOsmol kg^{-1}) (Middleton et al., 2016). Urinary I, Se and Na concentrations were measured by ICP-MS (0.5ml of urine diluted with 9.5 mL 1% TMAH).

6.2.4 Quality control

Standard reference materials 1573a - Tomato leaves, (NIST), 1567b-Wheat Flour (NIST) were digested/extracted in triplicate alongside crop samples. Seronorm L-1 & L-2 for urine trace element analysis were prepared and analysed alongside the urine samples.

6.3 Results and Discussion

Results for reference standards are given in Table 6.1. Recovery for both I and Se was within the quoted acceptable uncertainty range.

Table 6.1. Analytical results for certified reference materials

		I (µg L^{-1})	Se (µg L^{-1})	Na (mg L^{-1})
1573a - Tomato leaves	Measured:	844	59.0	
	Certified:	850	54.0	
	Recovery:	*99%*	109%	
1567b-Wheat Flour	Measured:		1130	
	Certified:		1140	
	Recovery:		*99%*	
Seronorm L-1	Measured:	90[a]	14.4	2310
	Certified:	105	13.9	2330
	Recovery:	*86%*	*104%*	*99%*
Seronorm L-2	Measured:	263[b]		
	Certified:	297		
	Recovery:	*89%*		

a. (84-126 µg L^{-1}) b. (237-356 µg L^{-1}) analytical uncertainty with 95% confidential interval

6.3.1 Iodine and selenium content of local foods

Iodine and selenium concentrations of local crops and drinking water are given in Table 6.2. The mean concentration of iodine for all leafy vegetables was 92 µg kg^{-1}_{fw} and for fruit vegetables it was 74 µg kg^{-1}_{fw}. Lower concentrations were found in roots and tubers, 25.3 µg kg^{-1}_{fw}. The iodine content of locally grown wheat and imported rice was 12.5 and 17.8 µg kg^{-1}_{dw} respectively and rice iodine concentrations were generally lower than those of the vegetable crops. Mean drinking water iodine was 2.18 µg L^{-1}. The mean of Se concentration of leafy, non-leafy (fruit) vegetables and roots/tubers was 23.1, 13.1 and 32.1 µg kg^{-1}_{fw} respectively. Unlike iodine, selenium content in rice was typically greater (115 µg kg^{-1}) than that in vegetable crops, wheat concentrations were lower than rice (49.4 µg kg^{-1}) but comparable to some vegetable crops e.g. cow pea, tarragon and celery.

The Se concentration in locally sourced bread was 25.6 µg kg^{-1} which is lower than values reported in the literature (MAFF, 1997, Anke et al., 1993, Fordyce, 2003). Mean drinking water Se was 0.308 µg L^{-1}. Table 6.3 gives information on I and Se concentration in other common foods from a range of sources. It demonstrates foods high in protein are richer sources of Se than cereal crops and vegetables.

Table 6.2. Iodine and selenium content of foods and drinking water, this study.

Food items		n	Iodine		Selenium	
			Mean (SD) $\mu g\ kg^{-1}$ FW	Median $\mu g\ kg^{-1}$ FW	Mean (SD) $\mu g\ kg^{-1}$ FW	Median $\mu g\ kg^{-1}$ FW
Basil	Leaf	4	63.0 (8)	62.8	10.9 (2)	11.60
Celery	Leaf	20	157 (101)	94.9	59.8 (49)	47.7
Chard	Leaf	20	112 (63)	85.5	23.4 (16)	15.0
Cress	Leaf	4	77.2 (24)	57.6	13.2 (6)	11.6
Dill	Leaf	17	93.5 (53)	76.4	36.1 (21)	32.9
Leek	Leaf	16	141 (77)	48.8	31.0 (24)	17.6
Mint	Leaf	2	157 (130)	158	16.7 (8)	
Parsley	Leaf	10	184 (73)	117	35.3 (15)	28.6
Pennyroyal	Leaf	1	55.3		12.0	
Purslane	Leaf	14	83.3 (53)	74.3	6.34 (2)	4.48
Radish Leaves	Leaf	6	101 (43)	64.7	20.2 (9)	20.7
Spinach	Leaf	1	52.7		13.4	
Sp-Onion	Leaf	13	33.9 (23)	28.4	20.3 (10)	18.6
Tarragon	Leaf	4	86.7 (64)	86.4	43.0 (33)	38.7
Thyme	Leaf	2	31.8 (6)	31.8	12.1 (1)	
Courgette	Fruit	6	24.4 (19)	16.3	4.43 (2)	3.96
Cucumber	Fruit	11	25.0 (10)	9.64	11.4 (14)	4.53
Egg plant	Fruit	10	52.2 (36)	42.9	10.1 (13)	3.90
melon	Fruit	2	37.9	37.9	3.26 (3)	
Okra	Fruit	15	86.3 (34)	82.8	13.1 (12)	8.17
Pepper	Fruit	13	72.9 (42)	53.8	10.4 (9)	6.91
Tomato	Fruit	8	65.5 (32)	58.2	13.1 (8)	10.7
Water melon	Fruit	4	17.8 (9)	15.7	6.89 (6)	7.10
Cow pea	Pod	9	270 (128)	253	44.9 (27)	45.1
Fava Bean	Pod	6	84.2 (65)	81.9	13.8 (9)	10.5
Garlic	Tuber	1	18.2		67.7	
Onion	Tuber	5	26.7 (16)	6.64	8.92 (5)	8.43
Radish	Root	5	31.0 (2)	11.4	19.7 (22)	7.68
Bread	Cereal	50			25.6 (10)	23.0
Rice	Cereal	82	14.7 (20)	7.94	115 (100)	94.3
Wheat	Cereal	13	12.5 (12)	9.83	49.4 (27)	40.8
Yogurt	Dairy	9	52.0 (57)	31.8	10.9 (6)	7.94
Water($\mu g\ L^{-1}$)		82	2.18 (1)	2.17	0.308 (0.3)	0.233

Table 6.3 Iodine and selenium content of common foods

Food		I* µg kg^{-1}	Reference(s)	Se*# µg kg^{-1}
Bread	Cereal	31.2	MAFF (1997); Anke et al. (1993); Fordyce (2003)	
Spaghetti	Cereal	44.4	Haldimann et al. (2005)	121
Bulgur	Grain	107	Fordyce (2003)	120
Chickpea	Grain	164	Hou et al. (1997)	118
Lentil	Grain	186	Fordyce (2003)	119
White bean	Grain	104	Fordyce (2003)	117
Red meat	Protein	115	Hou et al. (1997); Haldimann et al. (2005); Fordyce (2003)	133
Chicken	Protein	117	Pennington and Young (1990);Anke et al. (1995); Haldimann et al. (2005); MAFF (1997);	180
Fish	Protein	282	Fordyce (2003); Haldimann et al. (2005) Mahesh et al. (1992)	403
Egg	Protein	398	Dahl and Meltzer (2009); Haldimann et al. (2005); Hou et al. (1997); Fordyce (2003)	219
Fresh fruits	Fruit	32.1	Leufroy et al. (2015); Anke et al. (1993);	8.9
Nuts	Fruit	172	Fordyce (2003); Haldimann et al. (2005)	335
Cheese	Dairy	77.1	Dahl and Meltzer (2009); Fordyce (2003); Anke et al. (1993); H. Larsen et al. (1999)	58.3
Cream	Dairy	78.5	Larsen et al. (1999); Dahl and Meltzer (2009)	16.0
Milk	Dairy	120	Larsen et al. (1999); Dussault (1993); Fordyce (2003); Hou et al. (1997); Haldimann et al. (2005)	18.8
Oil	Additives	67	Fordyce (2003); Dahl and Meltzer (2009)	0
Sugar	Additives	57.3	Fordyce (2003); Hou et al. (1997)	1.0
Tea (µg L^{-1})	Beverage	11	Fordyce (2003)	0.308

*uncooked, #USDA (2017)

6.3.2 Dietary energy intake

Cereals and grains, oils and proteins supplied almost 70% of daily energy intake (DEI). Vegetables (leafy and fruit) collectively supplied only 6% of DEI although 60% of daily food consumption by weight (1019 g) was from this food group. The mean total DEI according to the questionnaire responses was 2432 kcal d^{-1} per person which is in line with the normal range reported by the FAO/WHO of 2000 - 3000 kcal d^{-1} (FAO, 2001).

6.3.3 Dietary iodine and selenium intake

The daily dietary intake of I from all foods excluding salt intake was 119 μg d^{-1} (Table 6.4). Vegetables and fruits supplied 48%, protein sources 25%, cereal and grains 9%, dairy products 8% and water 2% of daily I intake (Figure 6.1a). Excluding salt intake, cereal and grains provide only 9% of total iodine intake whereas the high consumption of fruits and vegetables such as tomato, potato, courgette and egg-plant, provide almost half of the daily iodine intake. Low consumption of dairy products in the Kurdish diet results in only 8% of I intake coming from these sources. The daily intake of salt estimated from the questionnaire was 13.8 g indicating a high consumption. The majority (>90%) of salt samples collected were iodised with a median I concentration of 43 mg kg^{-1} (mean= 40 mg kg^{-1}, range 8.4 - 77 mg kg^{-1}). This is a typical iodine concentration for iodized salt; the U.S. Food and Drug administration (USDFA) recommended 46 - 76 mg I kg^{-1} (Dasgupta et al., 2008). Accounting for this high salt intake, daily iodine intakes increase to 668 μg d^{-1}, considerably greater than the recommended dietary allowance of 150 μg d^{-1}, with salt supplying 82% of the I intake (Table 6.4, Figure 6.1b).

Table 6.4. Iodine and selenium intake by food groups in the Kurdish population.

Food group	Description	Consumption	I - intake	Se-intake
		g d^{-1}	µg d^{-1}	µg d^{-1}
Cereal and grains				
Rice	Long and short grain white rice	114	1.67	13.1
Bread	Naan bread	95.1	2.96	2.43
Beans and grains	Bulgur, Chick pea, Lentil, White bean	45.25	5.71	5.52
Meat and protein				
Red meat	Beef, Lamb, Veal	30.9	3.55	4.11
Poultry	Chicken	91.3	10.6	16.4
Fish	Freshwater fish	19.3	5.44	7.79
Egg	Whole egg	24.8	9.87	5.43
Fruits and vegetables				
Fresh fruits	Apple, Apricot, Banana, Cherry, Date Grape, Grapefruit, Kiwi, Lemon, Melon, Orange, Peach, Pear, Pomegranate, Watermelon	449	13.6	2.99
Leafy vegetables	Basil, Celery, Chard, Cress, Dill, Leek, Lettuce. Mint, Parsley, Purslane, Radish	105	16.2	2.42
Non-leafy vegetables	Carrot, Courgette, Cowpea, Cucumber, Eggplant, Fava bean, Garlic, Okra, Onion,	465	25.1	4.69
Nuts and seeds	Mixed nuts, Sunflower seed, Walnut	15.47	2.17	4.11
Dairy products				
Cheese	All available cheese	11.3	0.873	0.66
Cream	Mostly industrially produced cream	4.08	4.44	0.063
Milk	Mostly industrially produced milk	37.9	0.310	0.696
Yogurt	Industrial and locally made	75.5	3.93	0.824
Additives and flavours				
Oil	Sunflower oil, Corn oil	55.3	3.71	0
Paste tomato	Canned	24.8	1.60	1.29
Salt	>90% Iodised salt	13.78	549	0
Sugar	White Sugar	37.1	2.12	0.037
Beverages				
Water	Tap and bottled water	1154	2.50	0.267
Tea		282	3.04	0.072
Total:	*Excluding iodised salt*		***119***	***72.9***
	Including Iodised salt		***668***	

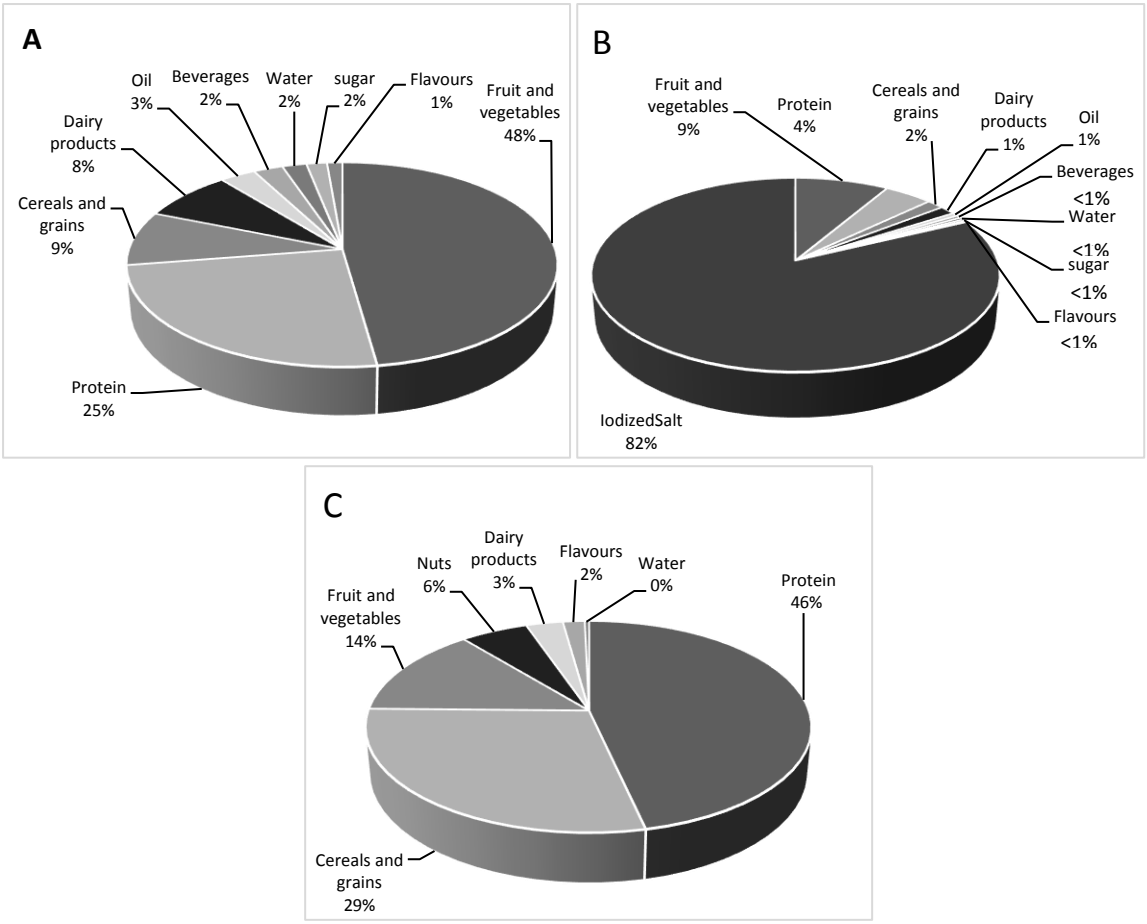


Figure 6-1. Iodine and selenium intake from different food groups; (a) Iodine intake excluding salt (b) Iodine intake including salt I water, sugar and flavour value sare <1%(c) Selenium intake

Mean total daily intake of Se according to the questionnaire responses was 72.9 μg d^{-1} (range from 25.6 to 170 μg d^{-1}) with a median daily intake of 65.3 μg d^{-1}. These figures fall close to the recommended dietary allowance (RDA) of 55 μg d^{-1} (DRI, 2000) (Table 6.4). Twenty nine percent of participants had a Se intake below the RDA. Protein sources, cereals and grains supply c.75% of daily intake (46 and 29% respectively). Fruit and vegetables, nuts and dairy products supply 14, 6 and 3% of daily intake

while the amount of Se supplied by drinking water was negligible (0.4 %) (Figure 6.1c).

6.3.4 Urinary iodine

A good correlation (r = 0.70, p <0.0001) was observed between urinary creatinine and osmolality (Fig 6.2a) and adjusted urinary I concentrations, UIC_{CRT} and $UIC_{OSM,}$ (r = 0.62; P<0.0001) (Fig 6.2b). Creatinine adjusted UIC_{CRT} (μg g^{-1}), for all participants gave a mean and median of 417 and 379 μg L^{-1} respectively (range 58-1380 μg L^{-1}; n = 410) slightly lower than osmolality adjusted mean and median values, $UIC_{OSM,}$ 470 and 424 μg L^{-1} respectively (range 71-1990 μg L^{-1}; n= 410) (Table 6.5) while mean and median uncorrected UIC was 489 and 411 μg L^{-1} (20 - 2380 μg L^{-1} n=410) . A relationship was also observed between uncorrected UIC with both UIC_{CRT} and UIC_{OSM} (r = 0.50; P<0.0001) (r = 0.80; P<0.0001) (Fig 6.2c, 6.2d). According to (Mizéhoun-Adissoda et al., 2016, Jooste and Strydom, 2010) 90% of ingested iodine is excreted in urine. Accounting for the 10% non-renal loss and assuming a total urine volume over 24 h of 1.4 L, the estimated daily iodine intake was in the range 648-731 μg d^{-1} (according to creatinine and osmolality adjustment). This range is in a good agreement with the estimated daily iodine intake predicted from the dietary survey (668 μg d^{-1}).

School age children typically had the highest UIC and participants >60 years old had the lowest UIC (Table 6.5). Mean UIC_{OSM} suggested a decreasing UIC with increasing age which was not observed in mean UIC_{CRT}; overall no

correlation between age and UIC was observed when all participants were considered (Table 6.5).

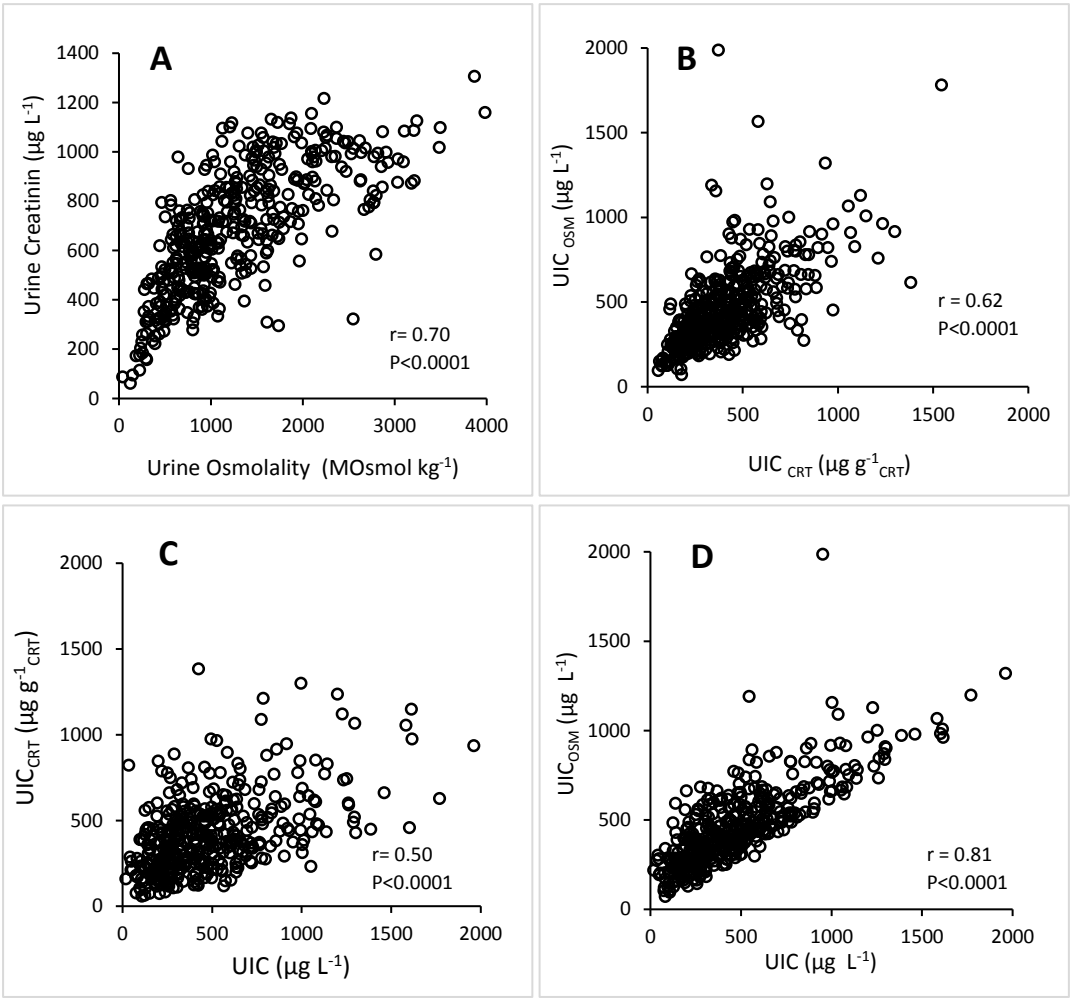


Figure 6-2 . (a) Correlation of urine creatinine and osmolality values (b) Correlation of UIC between both justification method of creatinine and osmolality (C) Correlation of uncorrected UIC and creatinine corrected values (d) Correlation of uncorrected UIC and osmolality corrected values

According to the WHO classification a UIC level in the range 100-199 µg L^{-1} indicates adequate I nutrition with values of UIC ≥300 µg L^{-1} classified as excessive iodine intake which may result in consequences for health (WHO, 2013). Based upon these guidelines c. 90% of school age children had excessive I intake and none had an adequate intake (Table 6.6). Less

than 13% of participants had UIC in the adequate range with 80-90% classed as having intakes that were above requirements or excessive. Over 55% of participants had iodine intakes classed as excessive (Table 6.6).

Table 6.5. Summary of urinary iodine concentration (UIC) by age group.

Age group	Population	UIC_{CRT}		UIC_{OSM}	
		Mean (SD)	Median	Mean (SD)	Median
	%	μg L^{-1}	μg L^{-1}	μg L^{-1}	μg L^{-1}
6-14	20.3	565 (250)	503	533 (268)	469
15-24	18.1	370 (200)	331	522 (256)	480
25-39	29.8	393 (189)	363	470 (209)	434
40-60	18.1	418 (204)	390	446 (205)	394
>60	13.7	351 (173)	320	344 (139)	312
All	100	417 (221)	379	470 (233)	424

Table 6.6. Iodine intake status based on measured UIC level and WHO classification for each age group.

		Percentage (%)			
Age (yr)	UIC (μg L^{-1}):	< 100 Insufficient	100-199 Adequate	200-299 Above Requirements	≥ 300 Excessive
Creatinine Corrected:					
6-14		0	3	4	92
15-24		5	13	27	55
25-39		1	13	22	65
40-59		0	11	16	72
≥60		4	7	33	56
Osmolality Corrected:					
6-14		0	1	12	87
15-24		1	7	8	84
25-39		0	5	18	77
40-59		0	6	14	79
≥60		4	4	37	56

Iodine Global Network (2017) reported on the iodine status of 177 countries worldwide and found that in 63% of countries iodine intake was adequate, 11% had insufficient intake and 6% had excessive intakes. There was no data on which to assess the iodine status of the populations in 20% of the countries, including Iraq. The lowest and highest median UIC recorded were for Angola and Uganda; 29 and 464 μg L^{-1} respectively. The median UIC recorded in the current study would place Iraq third in a list of countries ranked according to UIC after Uganda and Somalia.

Figure 6.3 demonstrates a correlation between measured iodine concentrations in salt and the mean UIC_{CRT} of the family consuming that salt. This suggests that iodized salt is likely to be the main source of dietary iodine. Salt iodine concentrations have been shown to vary due to storage time. Dasgupta et al. (2008) reported a 50% and >90% loss of iodine from

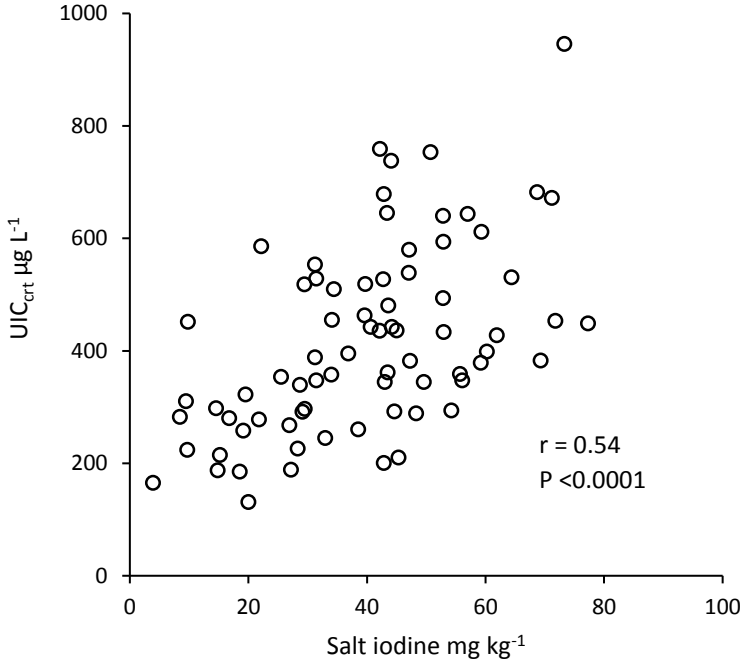


Figure 6-3. Correlation of salt iodine concentration and mean UIC of each family

iodized salt after 20 days exposure to air with 50% and 85% humidity respectively.

6.3.5 Urinary Sodium

Urinary Na concentration (UNaC) was analysed and adjusted using measured creatinine or osmolality (Table 6.7). A normal of urinary Na concentration has been reported as 20 mmol L^{-1} in spot samples and humans can excrete urinary Na within the range 40 - 220 mmol d^{-1} (MedlinePlus, 2017). The mean concentration of UNaC in this study was c.160 mmol L^{-1}. Assuming a total daily urine volume of 1.4 L this suggests that the daily Na excretion is c. 224 mmol L^{-1}, which is at the high end of the normal range. The volume of urine excreted (1.4 L d^{-1}) is estimated based upon the observation that normal urinary excretion is in the range 0.8 - 2 L or 1 mL kg (body weight)$^{-1}$ h^{-1} and from other studies where 24 h urine volume was measured. The average volume of urine excreted in a 24 h period by 711 participants in a Mexican study was 1.56 L(Vallejo et al., 2017). A study of 119 Moroccan adults reported a 24 h urine values of1.12 L (Derouiche et al., 2017) and in similar study the average of 242 sample 24h urine volume reported as 1.7L (Kim et al., 2014).

Table 6.7. Summary of urinary sodium concentration (UNaC) by age group.

Age group	Population	$UNaC_{CRT}$		$UNaC_{OSM}$	
		Mean (SD)	Median	Mean (SD)	Median
	%	mmol g^{-1}	mmol g^{-1}	mmol g^{-1}	mmol g^{-1}
6-14	20.3	193 (96)	186	167 (54)	170
15-24	18.1	110 (70)	92	144 (51)	138
25-39	29.8	145 (86)	130	159 (49)	158
40-59	18.1	174 (88)	168	172 (48)	184
≥ 60	13.7	167 (83)	146	154 (45)	161
All	100	158 (107)	136	161 (60)	160

6.3.6 Relationship between iodine and sodium

A significant correlation was observed between UIC_{CRT} and $UNaC_{CRT}$ for all participants and within each age group (Fig 6.4). Du et al. (2014) reported that salt is a major source of dietary sodium with only 6.8% of sodium originated from processed food which are uncommon in Iraq. The correlation between UIC_{CRT} and $UNaC_{CRT}$ supports the suggestion that iodized salt intake is the main source of iodine to this population. If this is the case then dietary iodine intake calculated from UNaC might be expected to correlate with dietary intake calculated from measured UIC. Figure 6.5 demonstrates that a good correlation does exist between these variables. In this figure the daily salt intake of individual participants has been calculated according to UNaC of individuals and consequently the daily iodine intake estimated according to iodine concentration of salt consumed by correspond individuals. A good correlation in this graph can implies that estimation of iodine intake using urinary Na is an acceptable and efficient method in the areas where their iodine intake dependant on iodized salt due to urinary Na gives an estimation of salt intake in very recent time to urine samples whilst salt intake from questionnaire gives an average for longer time.

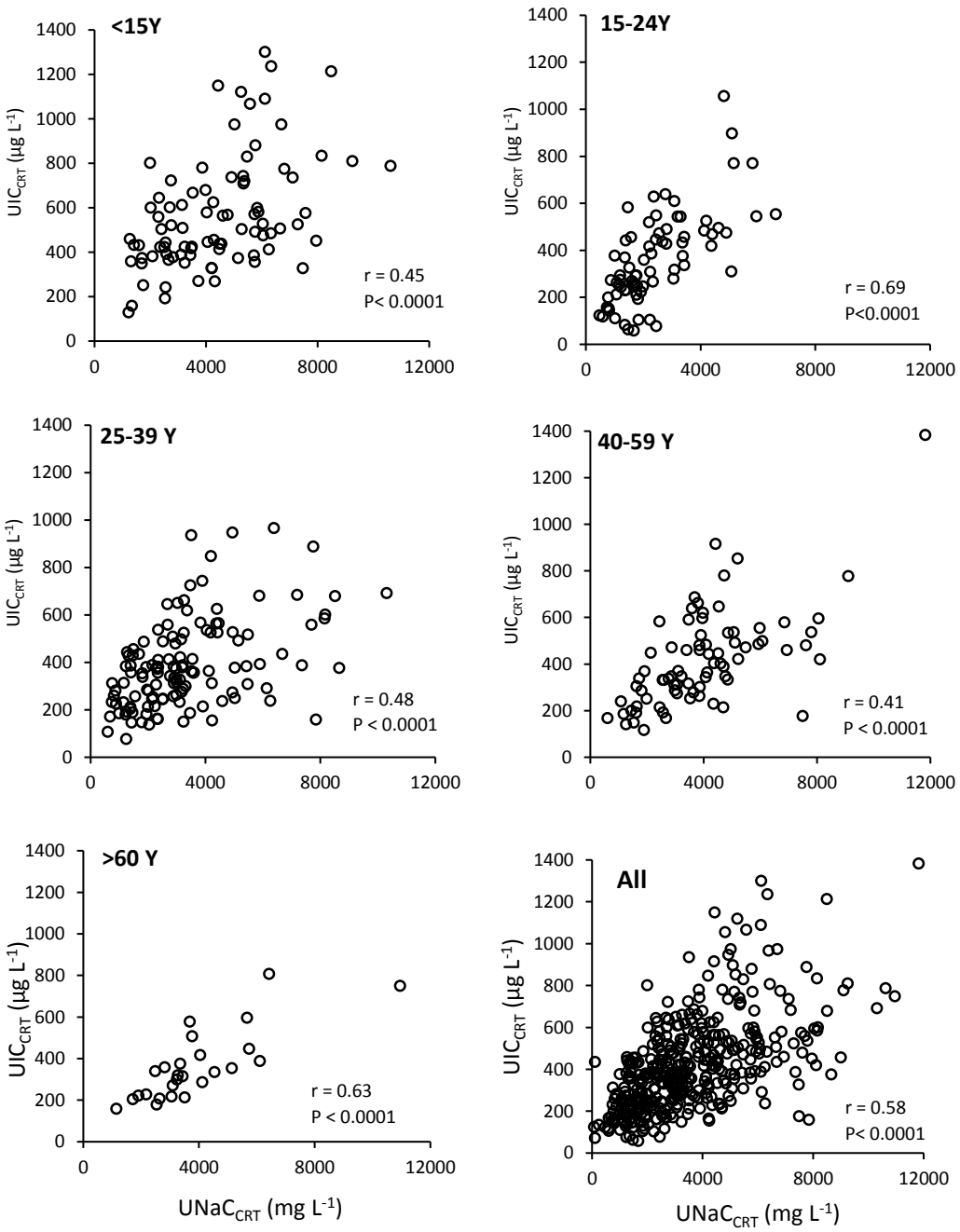


Figure 6-4. Correlation between of urinary sodium and iodine in different age groups and the population as a whole.

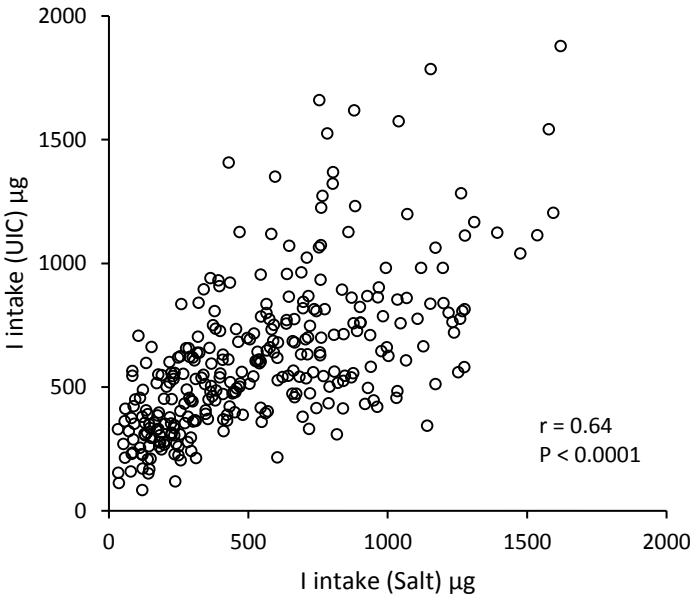


Figure 6-5. The X axis is iodine intake predicted according to salt intake estimated from urinary sodium; Y axis is iodine intake estimated from UIC

Both $UNaC_{CRT}$ and $UNaC_{OSM}$ averages were close to 160 mmol g^{-1} (3680 mg L^{-1}) (Table 6.7). Salt consumption was estimated considering all sodium originated from NaCl (Mill et al., 2012). Measurement of 24 h sodium excretion in urine is estimated to account for 85-90% of dietary intake of Na from all sources (Derouiche et al., 2017). Accounting for a 15% non-renal loss and using 1.4 L as the 24 h urine volume, the mean estimated Na intake of participnats was 263 mmol d^{-1} which equates to a salt intake of 15.3 g d^{-1}. This amount is comparable to the daily salt intake established in the dietary survey (13.78 g d^{-1}). This intake is considerably higher than the WHO recommended salt intake 5 g d^{-1} equivalent to < 2000 mg d^{-1} Na (WHO, 2012). The reported salt intake in this study is comparable to values reported for other countries in the region which share similar diets and culinary practices; for example Turkey, Iran and Kuwait have reported

dietary salt intakes of 15, 10.6 and 8.8 g d^{-1} , the UK, US and France have intakes of 8.1, 8.8 and 8.4 gd^{-1} with the lowest reported salt intakes in Cyprus and Malaysia; 5 and 6.4 g d^{-1} respectively (Trieu et al., 2015).

High salt intake may predispose a population to disease. Du et al. (2014) found that adults (n = 16869) with greatest sodium intake had the most rapid increase of hypertension. Mozaffarian et al. (2014) estimated the mean level of global salt consumption was 9.9 g d^{-1} (3.95 g d^{-1} Na). They also reported that globally there are 1.65 million cardiovascular related death annually attributed to consumption of more than 5 g d^{-1} salt intake (2 g d^{-1} Na), representing 1 of 10 death from cardiovascular disorders. Similar studies have estimated that a reduction in salt intake from 10 to 5 g d^{-1} would decrease overall cardiovascular and stroke rate by 17 and 23% respectively (Cook et al., 2007).

6.3.7 Urinary Selenium

Mean and median creatinine adjusted urinary selenium concentration ($USeC_{CRT}$) for all participants was 21.2 and 19.7 µg $g^{-1}{}_{CRT}$ respectively. Osmolality adjusted values ($USeC_{OSM}$) were 24.8 and 23.2 µg L^{-1} respectively. The $USeC_{CRT}$ of school age children (6-14 yr) was significantly higher than for other age groups ($P < 0.0001$) which all had similar $USeC_{CRT}$. A different pattern was suggested by $USeC_{OSM}$ with a general decline in USe_{OSM} with increasing age (Table 6.8).

Table 6.8. Summary of urinary selenium concentration (USeC) by age group.

Age group	Population	$USeC_{CRT}$		$USeC_{OSM}$	
		Mean (SD)	Median	Mean (SD)	Median
	%	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
6-14	20.3	28.8 (9)	27.5	27.4 (11)	24.6
15-24	18.1	19.3 (8)	18.4	28.3 (9)	27.0
25-39	29.8	19.8 (6)	18.3	24.6 (9)	23.3
40-60	18.1	19.2 (6)	17.3	21.4 (8)	19.8
≥ 60	13.7	18.8 (4)	18.2	19.1 (7)	18.4
All	100	21.2 (7)	19.7	24.8 (10)	23.2

A correlation was observed between UIC and USeC ($p<0.0001$; $r = 50$) (Figure 6.6) but not when creatinine or osmolality adjusted values were considered. Thomson et al. (1996) observed a similar correlation in both spot samples and 24 hr urine samples when they analysed UIC and USeC in samples from 62 adults but not when values were creatinine adjusted. Urinary Se is not the approved biomarker for assessing Se status (blood and serum Se are recommended) however a strong correlation has been observed between urinary Se and daily dietary Se intake (Sanz Alaejos and Diaz Romero, 1993, Hurst et al., 2013a). Doubling measured 24 h urinary

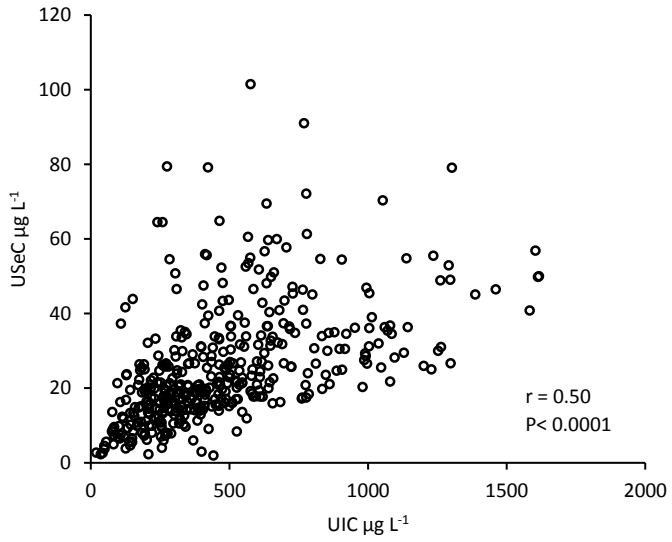


Figure 6-6. Correlation between uncorrected urinary iodine and selenium

excreted Se can give a reasonable estimation of Se intake which may be more accurate than food recording approaches (Robinson et al., 1989, Thomson et al., 1996). Using this approach the estimated dietary Se intake in this study is 59.4 μg d^{-1} which is in line with recommended levels of Se intake (55 μg d^{-1}) but less than the Se intake estimated from the dietary survey of 79 μg d^{-1}. Hurst et al. (2013b) in a study of dietary Se in Malawian population observed a linear relationship between Se dietary intake and USeC and developed an equation to predict daily Se intake from USeC. According to their equation (USeC (μg L^{-1}) = 7.8 + 318 (Se mg d^{-1})) estimated Se intake in this study is 42 μg d^{-1} when all participants considered which is lower than the WHO recommended value and that estimated from the dietary survey also 59-76% (according to methods justification) of people had Se intake less than recommended value. Therefore, to determine more accurate level of Se status taking blood samples could be suggested.

6.4 Conclusions

The main source of dietary iodine in Iraqi-Kurdistan is iodized salt. Due to high salt consumption estimated dietary iodine intake is typically excessive and this was confirmed by elevated UIC. School age children had the highest UIC. Salt intake estimated from urinary Na confirmed the salt intake calculated from the dietary survey and established that dietary intake is considerably higher than WHO recommended amounts which may result in high blood pressure and cardiovascular problem.

To address the issue of high salt intake education of the population to increase awareness of the long term health effects of high salt consumption is recommended. As changing dietary habits is a long term goal another option is to modify the salt composition to include KCl and reduce NaCl intake. As iodine intakes are currently excessive reducing salt intake would also reduce iodine intake.

Average daily selenium intake estimated from the questionnaire suggests that intake may be adequate in the majority of the population. Daily Se intake estimated from USeC differed depending upon the method selected and in one of the methods showed a se deficiency of population. Therefore confirmation of Se status from blood samples is recommended.

7 SUMMARY, CONCLUSION AND FUTURE WORK

Overview

This project was undertaken to provide an overview of the current status of Se and I in the terrestrial environment and the population of the Kurdistan region of Iraq. Data about these two important trace elements in this region was very limited with no national or local data available. A survey of soil, plants and irrigation water from arable farms was therefore undertaken and factors controlling bioavailability and plant uptake in the calcareous soils of the region were investigated in a pot trial utilizing ^{77}Se and ^{129}I as tracers. A semi-quantitative dietary survey was also conducted to establish the daily intake of Se and I from the common traditional diet. Human biomarker samples were also taken and analysed as an index of nutritional status of population. The main conclusions of the work can be summarised in the following sections.

7.1 Selenium status in Kurdistan region of Iraq

The arable calcareous soil of the Kurdish region of Iraq can be classified as a slightly deficient in selenium (Se_{Tot} =309 µg kg^{-1}). Some areas had Se_{Tot} within the region were close to global average values but in other areas $Se_{Tot\ was}$ comparable with Se deficient areas reported in other countries. Total soil Se is however not an efficient measure of the availability of Se for plant uptake and no association between Se_{Tot} and plant Se concentration was observed. The mean of Labile Se (soluble+adsorbed) accounted for a minor proportion of Se_{Tot} (2.57%) and was expected to be more available for plant uptake. Organic carbon influenced retention of Se and was

correlated with Se_{TMAH}. However, the extractability of organic –bound Se (Se_{TMAH}) has been shown to be vary with soil pH (Section 3.4.3.2). The $\%Se_{TMAH}$ fell in range of 20.3-89% (mean 41%). Greater than 50% of Se was not extractable by TMAH and deemed recalcitrant. The form of Se that resists extraction by TMAH is not known but may involve elemental Se, selenides or possibly occluded forms within Fe oxides or other (possibly sulphide) minerals. In some of the studied areas pH was negatively correlated with $\%Se_{TMAH}$ and positively correlated with labile Se. The reason for the areas where not correlated positively could be attributed to narrow range of soil pH in those samples. Overall soil pH and %OC were the influencing factors on Se availability.

Locally crops grown exhibited a wide range of Se concentrations across different areas and plant varieties (4.34 – 678 µg kg^{-1}). Different plant varieties grown in the same field and identical varieties grown in different fields had wide variation in Se concentration. The mean concentrations of Se grouped by crop type were 113, 112, 69 and 49 µg kg^{-1}_{dw} for leafy vegetables, tubers, fruit vegetables and wheat respectively. Soil pH was the most important factor controlling Se availability for plant uptake. Plants grown in soils with pH>8 generally had higher Se concentrations compared to those grown in soils with lower pH values. Overall no strong correlation between $Se_{Tot,}$ $Se_{TMAH,}$ Se_P and Se_N with plant Se content was observed when all plants were considered. However there was a correlation between Se_N and Se_P with plant Se in a few vegetable varieties. The irrigation water Se concentration was in the range 0.05 to 0.859 µg L^{-1} (mean = 0.495 µg L^{-1}) across all areas. Plant Se content does not seem to be affected by Se_{water}

concentrations perhaps due to the low Se concentration of the irrigation water.

Having established the source apportionment of Se in the Kurdistan region and its availability it is important to consider if the Se supplied by crops meet the Se requirement of population and if not what portion of daily Se intake is supplied by these crops? The food amount questionnaire indicates that the Se daily intake from all food items was 72.9 which is sufficient and in line with the WHO recommendation of 55 μg d^{-1}. Of the 72.9 μg d^{-1} 10 μg d^{-1} was supplied by leafy and fruit vegetables (13.7% of daily intake or 18% of the WHO recommended value). Protein sources, cereal and grains supplied 46 and 29% of daily Se intake respectively. Dairy products supplied 3% of daily intake and water only 0.3%.

Urinary Se analysis gave a mean values of 21.2 or 24.8 μg L^{-1} when creatinine or osmolality corrected. Urinary Se is not the recommended biomarker for Se status assessment however a linear positive relationship with dietary intake of Se has been demonstrated and approaches have been suggested to estimate the Se intake from urinary Se. (see section 6.3.7). The mean estimated Se intake of participants was 59.4 μg d^{-1} and 42 μg d^{-1} depending upon whether creatinine or osmolality correction was applied. This suggests that 59-76 % participants had a Se intake less than recommended level by WHO. This can be compared to an estimated 29% of the population having a less than optimum Se intake suggested by the food frequency questionnaire. As both approaches suggest that a significant proportion of the population may have a sub-optimal Se intake a Se biofortification program might be considered for the region. .

The feasibility of Se biofortification of a few local vegetable varieties was examined using a typical calcareous soil from the region. Application of 10 g ha^{-1} of ^{77}Se increased the Se concentration of vegetables. The success of biofortification was different for different species and for different ratios of fertilizer/soil Se. between 1 and 25% of added Se was recovered in the plants. Fractionation of soil $^{77}Se_f$ at the end of growing season showed that 55 and 40% of $^{77}Se_f$ transformed to the organically-bound and recalcitrant $^{77}Se_f$ respectively and only 5% of $^{77}Se_f$ remained available for plant uptake (Se_{N+P}). To reduce fixation of Se in a non-bioavailable form the timing of the Se application could be adjusted e.g. soil application when the crops are actively growing or foliar application may increase Se uptake.

7.2 Iodine status in Kurdistan region of Iraq

Mean I concentration of soil (I_{Tot}) in the areas was 4140 µg kg^{-1}, comparable with reported global mean values. The average concentration of labile iodine (I_{N+P}) was typically 10% of soil I_{Tot} and mainly comprised of soluble and adsorbed organic iodine. No clear relationship between I_{Tot} and %OC was observed; possibly due to the relatively narrow range of %OC. Soil I_{Tot} or soil iodine fractions did not appear to be affected by soil pH.

The iodine concentration of plants grown in these soils varied according to plant species and between areas. Iodine concentration was greatest in leafy vegetables (439 µg kg^{-1}_{dw}) compared to fruit vegetables, tubers and wheat which were 368, 140 and 12 µg kg^{-1}_{dw} respectively. These values are comparable or in some cases higher than concentrations reported in other studies with the exception of iodine in grains which was found to be very low. In a limited number of fruit vegetables a correlation was obsevred

between soil I_{Tot} and plant iodine concentration. In the majority of plants this relationship was very weak or absent. However, labile I_{N+P} typically correlated with mean plant iodine content. Water I concentration varied from 2.49-36.21 μg L^{-1} (mean= 11.9 μg L^{-1}). The geology of the region was an important influence on water iodine content as a strong correlation between soil %$CaCO_3$ and groundwater iodine content was observed This may reflect a geogenic source of I in the underlying rocks e.g. trapped saline water. A strong relationship between water iodine content and mean I concentration in the plants species that were irrigated with groundwater was also observed. Irrigation water may therefore be a significant contributor to plant iodine content in this region.

The estimated daily intake of I supplied by food was 119 μg d^{-1} (excluding intake of iodized salt). Unlike Se, where the majority in the diet was supplied by dietary protein the majority of I was from vegetables with vegetables and fruits suppying 48% of the I, proteins 25%, cereal and grains 9%, dairy products 8% and water 2%. The typical Kurdish diet contains little sea food which would be an important source of I in other parts of the world. However when consumption of iodized salt was considered the daily I intake increased to 668 μg d^{-1}, considerably greater than the WHO recommended dietary allowance of 150 μg d^{-1}. Iodized salt therefore supplies 82% of total dietary I intake from an estimated salt intake of 13.5 g d^{-1} of iodized salt. The mean concentration of I in iodized salt collected from households was 40 mg kg^{-1} which is in the recommended range.

This excessive I intake was confirmed by measurement of urinary iodine concentration (UIC).. Median UIC was 379 μg $g^{-1}{}_{CRT}$ (range 58-1380 μg g^{-}

$^{1}_{CRT}$) or 424 μg L^{-1} (range 71-1990 μg L^{-1} osmolality corrected). This concentration equates to an iodine intake of 648-731 μg d^{-1} which is in good agreement with estimated I intake established from the food frequency questionnaire (see section 6.3.4). According to the WHO recommended intakes, 90% of school age children and 55% of all participants had I intakes classed as excessive. Iodized salt was the main source of this excessive I intake confirmed by i) correlations between iodized salt I concentration for each household and mean UIC of the household and ii) a strong correlation between urinary sodium (originating from salt) and UIC. The high salt intake of the population estimated by the questionnaire was confirmed by calculation of I intake from urinary Na measurements (see sections 6.3.5 and 6.3.6) and was 6 g d^{-1} sodium intake, equivalent to 15.3 g d^{-1} salt, considerable higher than the WHO recommended Na intake. High consumption of iodized salt in this population therefore indicates that they may be susceptible to high I intake disorders and diseases resulting from high sodium intake including hypertension (high blood pressure) and cardiovascular problems. Reducing salt intake through education of the population to increase awareness of the long term health effects of high salt consumption is therefore recommended and would also reduce the I intake from 'excessive' to 'sufficient'.

Individuals who do not consume iodized salt, perhaps because of problems such as hypertension, are unlikely to have sufficient I intake and may require supplementation. Iodine biofortification could be considered as an alternative to iodized salt consumption. A pot trial where ^{129}I was supplied in irrigation water demonstrated that at the end of growing season 64-97% of applied iodine was recovered from soil. The recovery amount varied

according to the iodine species applied and the plant variety. More ^{129}I was recovered from soils irrigated with iodide suggesting greater retention of I in the soil and consequently less availability for plant uptake. In this system the majority of plant I originated from native soil I rather than the added ^{129}I. Plants grown in the iodate irrigated system had greater I uptake and the majority of I in the plant was supplied by the added ^{129}I. Variation in the ratio of $^{129}I/^{127}I$ in the plant can be attributed to an innate affinity of the plant species and low ratio of $^{129}I/^{127}I$ could be because of applied very low concentration of ^{129}I compared to those applied in normal biofortification programs (see section 5.36 to 5.3.9). An increase in labile I in air-dried soils at the end of growing season compared to similar soils at field capacity implies mineralization of I which has become immobilized during the growing season. This was partially confirmed when the majority of iodine was observed to be inorganic. This mineralization of I may be important in the field when soils dry out between irrigation events.

7.3 Limitation

The limitation factors during this project can be accounted as listed below:

a) Due to difficulties of earning licence and need for trained team to collect the blood samples as a main biomarker for Se and TSH hormones, only urine sampling was carried out.
b) The pot experiment cannot represent the field work in case of Se and I behaviour due to limitation of soil amount and other environmental factors.

7.4 Future work

From the results of the work presented here, there are few areas of research that would benefit from further investigation. First a wider survey of soil and water, covering of the entire Kurdish region of Iraq would be useful in order to generate soil I and Se geochemical maps. This, together with soil maps of the area will enable predictions to be made of areas where e.g. biofortification may be beneficial. Alongside this survey approach an investigation of the changing fractionation of I in the plant rhizosphere as a result of wetting and drying cycles the field study is recommended. This will provide information on the changing availability of I for plant uptake and inform biofortification/irrigation approaches

Second, in the current study the Se and I content of locally grown crops and other staple foods was measured but as a significant proportion of food items are imported from other countries building a database of Se and I content of all foods consumed in the region would be beneficial to enable better estimation of daily intake of trace elements would be possible. Also, due to the seasonal availability of food consumed in the region it is recommended that additional dietary questionnaires and biomarker samples (urine and blood) are collected at other times of the year. This would enable an overall picture of I and Se status of the population to be established. Future survey and biomarker collection would be particularly important alongside a recommendation to educate the population to reduce dietary salt intake to ensure I nutrition was sufficient. In addition, other dietary survey methods such as cooking and analysing a duplicate portion

of foods consumed by households would improve reliability of the data obtained.

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APPENDICIES

Appendix 1 . Concentration of common major and minor element in the soil samples of four main areas

Sub areas	Na mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Al mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Ni mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	Mo mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}	I µg kg^{-1}	Se µg kg^{-1}
Tanjaro	2260	19800	8590	114000	39500	562	36400	174	28.1	83.1	0.417	0.303	10.5	4560	241
	2500	19600	8910	112000	38700	644	35800	172	28.9	29.9	0.516	0.301	10.3	4720	253
	2350	20000	8790	110000	39900	566	37300	183	28.9	78.9	0.513	0.287	10.5	4290	252
	2620	20400	9040	106000	40800	616	37700	186	29.3	76.1	0.468	0.306	10.8	4680	269
	2400	19800	8800	101000	39500	512	36800	181	28.6	68.5	0.445	0.313	10.7	3770	252
	2780	17500	9050	110000	38100	646	34900	141	33.2	84.1	0.522	0.336	15.0	4180	275
	2680	12500	7280	156000	29000	644	26900	94.4	27.7	70.1	0.510	0.293	11.1	2150	204
	2660	15400	8130	146000	32400	748	31100	119	31.1	73.8	0.449	0.326	12.7	4440	266
	2710	19800	9190	106000	40400	595	36900	171	30.9	74.5	0.430	0.322	12.0	3190	273
	2680	17700	9310	109000	39400	706	35700	142	34.8	98.9	0.520	0.340	19.4	5020	309
	2790	18200	9030	117000	39000	595	35400	151	29.8	70.6	0.434	0.300	11.2	3390	253
	2640	16700	9910	127000	46100	831	34700	126	35.5	131	0.585	0.405	15.5	3230	283
	3100	17400	12100	107000	52900	844	37900	134	32.6	99.5	0.769	0.564	14.2	2870	279
	2740	16800	9620	125000	41800	742	32500	135	32.5	102	0.504	0.351	17.0	2760	272
	2920	16700	9710	129000	41700	774	32100	134	32.1	91.4	0.498	0.350	16.0	2330	239
Baziyan	2640	16800	9910	127000	46100	831	34700	126	35.5	131	0.585	0.405	15.5	2800	246
	3100	17400	12100	107000	52900	844	37900	134	32.6	99.5	0.769	0.564	14.2	3800	219
Zhalla	2740	16800	9620	125000	41800	742	32500	135	32.5	101	0.504	0.351	17.0	2460	187
	2920	16700	9710	129000	41700	774	32100	134	32.1	91.4	0.498	0.350	16.0	2810	165
Dukan	3560	17900	11800	77700	54700	894	39600	158	30.3	88.5	1.00	2.00	14.4	1200	170
	4060	22500	11700	65600	57600	941	43100	226	30.6	87.7	0.744	0.522	14.0	2860	383
SUL - west area	3050	16100	8970	118000	35100	759	33500	127	33.2	114	0.479	0.298	13.7	4310	246
	2910	14600	8270	119000	34100	708	31900	120	31.8	87.0	0.433	0.256	12.4	3610	254
	2880	14600	7750	135000	31800	639	30500	110	34.1	98.0	0.497	0.316	19.6	2470	236
Industrial	2960	11600	7260	116000	27300	864	74900	123	100	537	6.5	6.50	66.8	2270	218
	2940	11600	6830	111000	27900	1120	117000	152	225	471	11.1	11.1	66.4	2250	226
Kana Sura	2420	17000	10700	87800	45200	672	39800	130	34.6	102	0.777	0.777	14.3	4100	227
	5280	30900	22200	66400	88900	1450	77200	250	60.4	150	0.153	0.153	29.7	5230	432
	3750	18600	13100	50200	53600	916	45300	149	34.9	85.6	0.771	0.771	14.4	7540	269
	3120	17500	12000	60000	48900	745	42900	142	35.9	113	0.751	0.751	15.2	4030	277
	3040	17100	11600	69600	47700	774	42300	143	32.8	90.8	0.599	0.599	13.4	4320	228
	2450	16000	8960	127000	42100	628	35700	117	25.7	91.7	4.29	4.29	10.8	4690	211
	2860	18200	11300	85800	49000	727	42000	143	31.1	77.7	0.961	0.961	12.7	4900	228
	2930	17700	11100	84800	47500	736	41000	139	30.6	76.2	0.644	0.644	12.5	4960	223
	2210	15500	8250	137000	39900	575	33500	108	23.6	61.9	0.545	0.545	10.4	4730	179
Kani Goma	2990	18400	10100	119000	37200	642	31800	111	26.8	75.4	0.463	0.298	13.7	4820	223
	2980	18800	10100	115000	38400	654	32500	112	26.9	73.1	0.458	0.310	13.1	5520	232
	3100	18200	9690	119000	36900	632	31400	109	26.5	79.7	0.483	0.333	13.4	5700	232

	3000	19000	10000	118000	38700	649	32400	112	26.9	27.9	0.465	0.304	13.3	5620	234	
	3290	18800	10700	119000	38700	609	32900	109	26.2	65.2	0.742	0.295	11.3	5730	197	
	2840	17800	9500	115000	37100	551	30500	99.0	22.1	56.0	0.701	0.274	9.60	5010	192	

Sub areas	Na	Mg	K	Ca	Al	Mn	Fe	Ni	Cu	Zn	Mo	Cd	Pb	I	Se
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	µg kg^{-1}	µg kg^{-1}
Sharazoor	2800	14700	13800	52400	67800	1150	45700	136	32.9	114	1.87	0.568	19.0	2780	368
	2140	15000	11200	103000	54000	857	39000	124	29.5	93.7	1.3	0.470	16.2	2700	290
	2660	16800	12900	91000	57600	892	40700	131	30.9	88.3	1.12	0.516	16.1	2770	253
	3590	16400	14400	64500	62300	1000	44300	144	33.9	105	1.07	0.548	19.8	3140	264
	2540	16300	11800	104000	53400	841	38800	133	28.2	80.3	0.980	0.443	13.6	3330	231
	2410	21200	9900	101000	43400	888	36900	224	29.8	66.7	0.658	0.296	9.70	2910	252
	3080	17700	12200	63200	60600	968	43000	145	32.3	94.3	0.795	0.404	15.6	3480	232
New Halabja	2560	16600	11100	98100	47400	905	35400	133	33.5	134	0.819	0.473	17.8	2470	260

Sub areas	Na	Mg	K	Ca	Al	Mn	Fe	Ni	Cu	Zn	Mo	Cd	Pb	I	Se
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	µg kg^{-1}	µg kg^{-1}
Kalar	3800	15000	9420	132000	38200	651	26400	85.1	21.8	61.2	0.874	0.289	9.43	2520	302
	4250	16700	8850	137000	38300	621	26700	86.4	20.5	56.3	0.888	0.220	8.59	2860	206
	3890	15000	9280	134000	38900	658	27400	84.1	19.9	20.9	0.839	0.244	9.66	2810	198
	4190	17400	9480	138000	40400	660	28300	88.2	20.6	57.9	0.829	0.276	9.17	2290	207
	3270	15400	10100	123000	39900	660	27200	89.0	22.4	59.7	0.911	0.275	9.00	2190	211
	3670	16000	10100	124000	40400	682	28000	89.7	23.3	64.0	0.891	0.326	9.52	2250	271
	2760	12300	8620	131000	32200	530	22000	68.9	19.5	52.8	0.742	0.272	7.97	2570	205
	3030	14000	9230	118000	35800	616	24800	77.8	22.3	63.3	0.787	0.736	9.96	2550	245
	3210	14200	8800	130000	35500	588	24500	79.9	19.8	54.3	0.880	0.354	8.36	2490	226
	3350	15000	9490	143000	38000	624	26100	85.7	21.9	56.2	0.878	0.308	8.76	2780	215
	4510	15500	11200	96000	46300	786	32700	98.3	24.1	66.8	0.891	0.242	11.8	1860	229
	6060	17100	13200	99000	50500	845	32800	77.1	25.2	69.2	0.535	0.212	12.2	1840	331
	3720	15400	12000	98300	45500	883	31300	83.7	28.7	64.0	0.725	0.179	9.96	1530	209

Sub areas	Na	Mg	K	Ca	Al	Mn	Fe	Ni	Cu	Zn	Mo	Cd	Pb	I	Se
	3535	19547	4780	52656	23144	768	31501	116.0	39.11	213.59	2.4924	0.5987	31.0780	5055	528
	3268	19071	10429	50425	11383	655	31289	116.7	38.18	196.17	2.6552	0.5971	29.4823	5286	522
	3491	21573	7915	31750	18020	732	35277	135.3	37.67	142.05	2.8660	0.5785	21.4287	5512	480
	3450	21221	11078	37166	20394	735	34101	129.3	38.24	165.77	2.9189	0.5660	21.8270	5284	494
	3475	22650	11119	34421	33660	508	36099	138.7	36.26	106.85	3.1433	0.5583	17.3619	4674	433
	3771	20576	11499	37755	15583	786	33742	130.8	39.24	161.21	2.4052	0.5340	22.0955	5070	458
Kani Kolka	3737	21665	11578	32927	32762	548	35815	142.9	35.84	94.61	2.4374	0.4898	15.5624	5701	383
	3589	20106	10915	59660	11335	797	31462	123.2	34.96	131.68	2.3472	0.5325	18.9033	5787	428
	3784	21480	8304	52813	15015	851	33697	132.1	38.51	119.46	2.4787	0.5929	17.0513	5684	402
	3688	21560	11446	28225	33110	462	35617	137.6	41.25	162.77	2.8181	0.5710	25.8316	5692	525
	3848	22611	12081	27602	25526	626	36717	144.4	40.15	145.90	2.8018	0.5380	20.3185	5986	476
	3435	19237	8311	59849	27232	716	33630	130.2	37.42	112.10	4.5529	0.6478	17.2596	4666	574
	3823	15741	8287	91011	33788	1081	37324	139.0	46.09	186.15	4.0688	0.7104	22.6632	4676	617
	2043	13798	6663	101038	27940	505	21419	70.4	27.26	75.59	0.6125	0.2613	11.4520	7171	388
	1862	12615	7120	92004	24919	430	18931	60.9	23.40	68.83	0.5188	0.2208	10.1872	5984	403
	1527	12054	6797	96867	24345	421	18398	54.9	24.61	76.51	0.5960	0.2403	11.2594	6408	485
Anab	2418	16323	7065	95024	31462	548	23487	84.3	28.11	75.16	0.6404	0.2758	12.3942	7802	367
	2375	15617	5047	52596	33727	545	24364	81.8	37.97	132.91	0.8394	0.3271	33.4126	5572	568
	3264	21761	1851	71086	11839	823	28201	105.5	31.47	78.20	0.8894	0.3163	13.0321	7009	388
	1809	13691	8739	99778	41005	906	34755	99.0	29.33	80.30	0.8998	0.3770	13.9499	9311	366
	1978	14106	9589	90462	40437	849	34444	98.2	32.16	96.22	1.4355	0.3730	17.4855	7339	414
	2809	16471	13969	69340	50299	871	42851	134.7	33.40	90.75	2.2269	0.5057	15.5297	5604	403
	2456	15933	12547	60396	51982	955	44829	138.6	34.79	120.35	2.8254	0.5754	19.0277	5627	432
Kani Spi	2466	15456	12004	63607	49659	948	43306	131.7	36.87	172.49	2.8875	0.5755	23.7306	5491	482
	2436	16501	11991	61960	53899	1002	46383	143.3	33.74	92.81	2.9074	0.5631	16.3486	7005	432
	2684	16609	13257	53694	53859	982	46479	142.2	36.21	133.34	2.7928	0.5790	19.5182	6074	457
	2890	16785	13693	31522	64840	1065	46813	150.8	36.68	113.66	1.4618	0.6275	18.1608	2743	308
Bakrawa	2816	16508	12719	47636	62344	1007	44625	143.2	39.45	112.96	1.3201	0.4904	18.2808	2686	301
	2632	16374	12970	39513	62996	1046	45548	149.5	33.81	105.36	1.2351	0.4905	17.1165	2968	285
	2601	16141	12591	43294	61877	1001	44405	145.8	33.64	106.07	1.2261	0.4707	17.3017	2932	280
Zamaqi	2794	15983	13284	85907	53764	962	38416	124.5	32.61	114.28	1.9936	0.5006	17.0166	3936	337
	2956	15638	12884	88440	52430	932	37738	124.0	30.22	96.31	1.8334	0.5712	16.9697	4003	312
Ka Grdal	2643	12059	13001	105490	45139	828	32299	100.4	31.72	118.48	1.1358	0.4474	54.5180	3316	402
Bia Wella	1599	12473	8347	124934	44947	836	32158	96.9	27.28	77.85	0.7154	0.3788	13.3336	4135	267
Khormal	2685	13796	12990	17900	63169	1055	45828	135.5	38.72	101.90	0.8730	0.4603	16.6947	2161	283