GEOCHEMICAL DYNAMICS AND BIOAVAILABILITY OF IODINE AND SELENIUM

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I dedicate this work to my daughter (Eman) and son (Hashir).

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GLOSSARY OF TERMS

$\%IO_3^-$	percentage of iodate in soluble fraction of soil iodine
$\%I^-_{sol}$	percentage of iodide in soluble fraction of soil iodine
$\%Se_{ads}$	concentration of soil adsorbed selenium as a percentage of soil total selenium
$\%Se_{ads}^{IV}$	percentage of selenite in adsorbed fraction of soil selenium
$\%Se_{ads}^{VI}$	percentage of selenate in adsorbed fraction of soil selenium
$\%Se_{sol}$	concentration of soil soluble selenium as a percentage of soil total selenium
$\%Se_{sol}^{IV}$	percentage of selenite in soluble fraction of soil selenium
$\%Se_{sol}^{VI}$	percentage of selenate in soluble fraction of soil selenium
B_R	bioavailability ratio
CBD	citrate-bicarbonate-dithionite
comb oxides	combined metal oxides of Fem Mn and Al
cps	counts per second
cre	Creatinine
DIC	dissolved inorganic carbon in water
DOC	dissolved organic carbon in water
EC	electric conductivity
EDTA	ethylene diamine tetra acetic acid
GB	Gilgit-Baltistan
HPLC-ICP-MS	high performance liquid chromatography inductively coupled plasma mass spectrometry
I^-	Iodide
ICP-MS	inductively coupled plasma mass spectrometry
IO_3^-	iodate
kd	distribution coefficient
m/z	mass to charge ratio
MW	molecular weight
Org	Organic
PES	Polyethersulfone
PFA	Perfluoroalkoxy alkanes
pznc	point of zero net charge
RDA	recommended daily allowance
SC	soil total carbon
Se_{Fert}	fertlizer derived selenium
Se^{IV}	Selenite
Se_{TMAH}	soil humus selenium
Se^{VI}	Selenate

Se_w	selenium in water
sg	specific gravity
SIC	soil inorganic carbon
SOC	soil organic carbon
TC	total carbon in water
TF	soil to plant trasnfer factor
TMAH	tetra methyl ammonium hydroxide
UIC	urinary iodine concentration
UIC_{cre}	urinary iodine concentration corrected with creatinine
UIC_{sg}	urinary iodine concentration corrected with specific gravity
UIC_{uncor}	uncorrected urinary iodine concentration
UNaC	urinary sodium concentration
$UNaC_{cre}$	urinary sodium concentration corrected with creatinine
$UNaC_{sg}$	urinary sodium concentration corrected with specific gravity
$UNaC_{uncor}$	uncorrected urinary sodium concentration
USeC	urinary selenium concentration
$USeC_{cre}$	urinary selenium concentration corrected with creatinine
$USeC_{sg}$	urinary selenium concentration corrected with specific gravity
$USeC_{uncor}$	uncorrected urinary selenium concentration

ABSTRACT

Iodine (I) and selenium (Se) are vital dietary micronutrients for human and animal health. Approximately 35% of the world population is living at risk of I deficiency and more than half a billion people worldwide are thought to be facing Se deficiency. Historically, I deficiency has been reported in Gilgit-Baltistan (GB), Pakistan. The deficiencies of both I and Se are dietary and people of GB are largely consuming locally grown agricultural produce. Soil characteristics control the availability of I and Se to plants. Therefore, better understanding of I and Se geochemistry is required to enhance the dietary intake of these essential micronutrients via the food chain.

This study aimed to assess the factors controlling I and Se status in the population of GB. The objectives of this study were to: (i) to investigate the status of I and Se in soil, water and plants of GB; (ii) gain greater understanding of I and Se speciation in water and soil, and factors controlling their availability for plant uptake; (iii) assess the feasibility of Se biofortification in a wheat crop with the use isotopically enriched ^{77}Se as a tracer and thereby determine the fate of residual ^{77}Se; (iv) assess the nutrition status of I and Se of the local population with a survey of a human biomarker and use of iodised salt.

A survey was undertaken to collect soil (n = 26), water (n = 107) and plant (n = 281) samples from different districts of GB. Iodine and Se concentration in water ranged from 0 – 10.2 $\mu g\ L^{-1}$ and 0.016-3.04 $\mu g\ L^{-1}$ respectively with iodate (IO_3^-) and selenate (Se^{VI}) being the dominant inorganic species. Average concentrations of I and Se in soil were 685 ± 247 $\mu g\ kg^{-1}$ and 209 ± 96 $\mu g\ kg^{-1}$ respectively, which are much lower than global mean values. Soil samples were alkaline but with a coarse texture and had low

organic carbon; these properties have probably contributed to low retention of I and Se in soil. The potential availability of I and Se for plant uptake was assessed by measuring their reactive fractions ('soluble' and 'adsorbed') and speciation in these fractions. The reactive fractions of I and Se accounted for < 7% and < 5% of their total concentrations in soil respectively. Speciation analysis indicated that I and Se were present both in organic and inorganic forms in soil. More than 90% of the reactive I fractions (soluble and adsorbed) were present as organic species; the inorganic I (<10%) had a higher proportion of iodide (I^-) compared to IO_3^-. Inorganic Se was largely present as selenite (Se^{IV}), which is strongly adsorbed on soil particle surfaces and less available for plant uptake than Se^{VI}. The low concentrations of I and Se in water and soil were also reflected in all plant samples. Typically, all plant samples had low concentrations of I and Se compared to similar species from other parts of the world. Based on a typical Pakistani food basket it was estimated that consumption of locally grown plants and drinking water would only provide 32% and 61% of the recommended daily allowances (RDA) of I (150 µg day^{-1}) and Se (55 µg day^{-1}) respectively.

Selenium biofortification of wheat crop was undertaken in a rotational crop system (wheat, maize, wheat) with a single application of isotopically enriched ^{77}Se species (Se^{IV} and Se^{VI}) at three different levels (0, 10 and 20 g ha^{-1}) at the crop's early stem extension stage. With application of 10 and 20 g ha^{-1} Se^{IV}, grain Se concentration in the first wheat crop was increased by 14 and 32 times while application of Se^{VI} at the same rate as that of Se^{IV} resulted in a 35 and 95 times increase compared to a Se concentration of only 1.42 µg kg^{-1} in grain from control plots. There was no added Se

(^{77}Se) detectable in the second crop (maize) or the third crop (wheat) indicating that fresh applications of Se would be necessary for each crop to adequately biofortify grain. Considering the average daily consumption of wheat, the grains obtained from control plots would provide <1% of Se RDA to an adult.

Iodine concentration in iodised salt was 8.85 ($\pm$10.4) μg kg^{-1}, which is less than the minimum threshold of WHO recommended I concentration in iodised salt. The population average salt intake (13.0 ± 5.64 μg kg^{-1} day^{-1}) largely fell within the usual range for daily worldwide salt consumption however it was almost three times higher than the WHO recommended daily salt intake.

Average daily I and Se intake estimated from urinary I and Se concentration was found to indicate that the I and Se RDAs were not being met and that the GB population is living at risk of I and Se deficiency.

1 INTRODUCTION

This chapter is divided into four sections. Sections 1 and 2 describe the role of iodine and selenium in the terrestrial environment and its importance for human nutrition, respectively. Section 3 provides a description of the study area and Section 4 outlines the aim, objectives and hypothesis of the work.

Section 1. Iodine

1.1 Iodine - introduction

Iodine (I) is a non-metallic naturally occurring element present in trace concentration in the environment (Johnson et al., 2003; Saikat et al., 2004; Shetaya et al., 2012). It exists in a variety of valence states and forms in nature including elemental I (I_2), iodide (I^-), iodate (IO_3^-) and organic I such as methyl iodide and humus bound I (Whitehead, 1984; Fuge and Johnson, 1986; Johnson et al., 2003; Seki et al., 2013; Fuge and Johnson, 2015). There is one stable non-radioactive isotope (^{127}I) and several radioactive isotopes of I, most notably ^{129}I and ^{131}I. The half-life of ^{129}I is 15.7 million years while that of ^{131}I is 8 days (Haldimann et al., 2005; Kabata-Pendias, 2010). Iodine is present in different components of the environment such as atmosphere, biosphere and seawater. Therefore, it is categorised as an atmophile, biophile and hydrophile element (Goldschmidt, 1954) cited in (Fuge, 2013). Moreover, Fuge (2013) considered it to be classified as a chalcophile element due to its occurrence in sulphur containing minerals.

Iodine is an essential micronutrient for humans and livestock growth (Saikat et al., 2004) because of its significant role in the normal function of the thyroid gland (Köhrle, 1999; Amachi, 2008; Schomburg and Köhrle, 2008; Leung et al., 2015). The average person needs approximately 0.2 mg of I daily which mostly comes from different types of food (Amachi, 2008). An insufficient amount of I in the diet causes I deficiency (Amachi, 2008) which can result in a number of health complications collectively called I deficiency disorders (IDDs) (Fuge and Johnson, 2015). Iodine deficiency is amongst the most common micronutrients deficiencies in the world (Allen, 2003; Tulchinsky, 2010). According to estimates, approximately 35% of the world population is at risk of I deficiency and this poses a substantial economic and social stress on developing countries (Shetaya et al., 2012; WHO, 2013). Iodine deficiency disorders are more common in poor countries where there is a lack of access to nutrient rich foods or the locally grown food does not provide all the necessary micronutrients (WHO, 2007a; Thurnham, 2014).

1.2 Iodine in the environment

Iodine moves between various environmental compartments but oceans are believed to be its largest reservoir having a mean concentration of 58 μg L^{-1} (Fuge and Johnson, 1986; Johnson et al., 2003; Fuge and Johnson, 2015). Iodate concentrations are greater than I^- in seawater (Fuge, 1996; Fuge and Johnson, 2015). Phytoplankton and seaweeds take up I^- and release it as organic I into the atmosphere in the form of gases (CH_3I and CH_2I_2). The photolytic dissociation of these organic gases produces I in the atmosphere (Whitehead, 1984; Johnson, 2003). The elemental I is transported

by wind and returned to soil and plants through dry and wet precipitation. The principal source of I in the atmosphere is seawater. Areas far away from the sea are usually I deficient, such as Alpine and Himalayan regions (Whitehead, 1984; Fuge and Long, 1989; Saikat et al., 2004).

1.2.1 Iodine in water

1.2.1.1 Iodine in seawater

Oceans are the largest reservoirs of I (Gibson, 1969; Ahad and Ganie, 2010). Its concentration ranges from 45 - 60 µg L^{-1} in seawater (Whitehead, 1984; Fuge, 2013). Iodine is largely present in inorganic forms such as I^- and IO_3^- with a small amount near the surface present as organic I (Butler et al., 1981; Whitehead, 1984; Hou et al., 2001; Carpenter, 2003; Ito and Hirokawa, 2009; Zheng et al., 2011; Fuge and Johnson, 2015). Iodine accumulates in marine phytoplankton and seaweeds (Leblanc et al., 2006; Nitschke et al., 2018). The brown algae seaweed is the highest accumulator of I followed by red and the green algae (Küpper et al., 2008; Verhaeghe et al., 2008; Fuge and Johnson, 2015).

Both micro-algae and macro-algae produce volatile organic I compounds such as CH_3I, C_2H_5I, CH_2I_2, CH_2ClI due to environmental stress (Johnson et al., 2003; Hou et al., 2009; Bravo-Linares et al., 2010; Ooki et al., 2016). Photo dissociation of these compounds produces oxides of I in atmosphere (Fuge and Johnson, 2015) which migrate onshore and are deposited via wet or dry precipitation (Johnson et al., 2003).

1.2.1.2 Iodine in rainwater

Iodine concentration in rainwater ranges from 0.5-2.5 µg L^{-1} (Fuge and Johnson, 2015). Rainwater removes I from atmosphere through interaction of water droplets with gaseous or particulate I in the atmosphere (Whitehead, 1984). The geographical location, intensity, frequency and duration of rainfall affect the I concentration in rainwater. Rainfall in coastal region has a higher concentration of I compared to inland areas (Fuge, 1996; Johnson, 2003). Fuge and Johnson (1986) have reported that intense rainfall will wash out I more quickly from the atmosphere. Several workers have suggested that rainwater has more I at the beginning of precipitation and gradually decreases with time (Whitehead, 1984; Fuge and Johnson, 1986; Fuge and Johnson, 2015).

1.2.1.3 Iodine in surface water

Recent data suggest that rivers and lake water shows a broad range of I concentrations with some rivers in North America having concentrations as large as 212 µg L^{-1} (Fuge and Johnson, 2015). However, previously I concentrations in rivers were reported to be largely less than 20 µg L^{-1} with many values in the range of 0.5-5 µg L^{-1} (Fuge and Johnson, 1986; Fuge and Johnson, 2015). The concentration of I in non-marine surface water is affected by various factors including distance from the sea, precipitation, geology of the watershed and anthropogenic activities such as agriculture practices (Whitehead, 1984; Johnson et al., 2003; Fuge and Johnson, 2015). Surface waters in close proximity to the sea have higher I concentrations than further inland (Fuge and Johnson, 2015). Rivers running through I rich strata of

sedimentary rock usually have higher concentrations of I than rivers in igneous rock areas (Fuge, 1989; Moran et al., 2002). Sludge from large towns and cities and agricultural runoff from fields also contribute to higher I concentrations (15-20 µg L^{-1}) in surface water (Whitehead, 1979; Whitehead, 1984; Fuge, 1989; Moran et al., 2002). Fuge and Johnson (1986) reported that approximately half of the I introduced to the land surface is removed through surface drainage in Japan.

1.2.1.4 Iodine in groundwater

Iodine concentrations are usually higher in groundwaters compared to surface waters (Fuge and Johnson, 2015). This is generally attributed to factors including geographical location and geology of the area, leaching of I from I rich marine strata, evapo-concentration in arid and semi-arid areas and degradation of I rich organic matter (BGS, 2000; Andersen et al., 2009; Wen et al., 2013; Li et al., 2013). Typical concentrations are in the range of 0.01-70 µg L^{-1} (BGS, 2000) while higher values has been recorded in certain provinces of China from deep groundwater with values up to 1890 µg L^{-1} in Datong basin (Li et al., 2013) and up to 4117 µg L^{-1} in villages in Wenshui county of Shanxi province (Tang et al., 2013). Li et al. (2013) suggest that the high I concentrations were linked to reductive dissolution of Fe_2O_3 as sequential extraction of the soil samples revealed that most of the I was bonded to Fe_2O_3. High I concentrations in groundwater have also been reported in other places such as La pampa aquifer in Argentina, Tindouf Basin in Sahara, Somalia and Kenya and eastern Denmark (Fuge and Johnson, 2015).

1.2.2 Iodine in soil

The natural I content of soil is a combination of I accumulation during soil development and the soil's capacity for I retention and fixation (Fuge and Johnson, 1986; Fuge, 1996). Thus, soils are generally rich in I compared to the parent material (Fuge, 1996). The accretion of I in soil comes from numerous sources including weathering of parent rock, atmospheric precipitation, decomposition of plant and animal tissues and some anthropogenic sources including certain pesticides and fertilizers. Rocks are generally low in I and are considered to contribute a very limited amount towards soil I mass (Whitehead, 1984; Johnson, 2003). The distribution of I in metamorphic and igneous rock groups is fairly uniform which exhibit a low concentration of I. The mean concentration of I in igneous rocks is 0.24 mg kg^{-1} (Fuge and Johnson, 1986; Fuge, 1996). Concentration of I in sedimentary rocks covers a wide range. Fuge and Johnson (1986) suggest 0.8 mg kg^{-1} in sandstone, 2.3 mg kg^{-1} in shales, 2.7 mg kg^{-1} in carbonates and as high as 5-200 mg kg^{-1} in recent sediments of marine origin. The concentration is generally higher in clay rich, argillaceous rocks than sand rich arenaceous rocks (Fuge and Johnson, 1986; Fuge, 1996; Fuge, 2005) and usually correlates with the amount of organic matter in sedimentary rocks (Fuge, 2005).

Atmospheric precipitation, dry and wet, is the major source of soil I (Fuge, 1996; Cuevas et al., 2018). Soils in coastal areas generally have higher concentration of I as a consequence of marine inputs (Fuge, 1996; Ahad and Ganie, 2010). Soils in areas that receive high rainfall also have relatively greater inputs of I and greater

concentrations of I, provided the soil can retain it. Conversely, soils in rain shadow areas generally have lower I concentrations (Fuge, 1996).

1.2.3 Factors affecting iodine retention in soil

Many factors including soil texture, types of clay minerals, soil organic matter content, concentrations of Al and Fe oxides, chemical forms of I, soil pH and redox potential (Eh) influence the I 'fixation' potential of a soil (Fuge, 1996; Yu et al., 1996; Johnson, 2003; Johnson et al., 2003).

1.2.3.1 Soil texture

Soils with coarse texture and low organic matter content have a low I adsorption capacity and allow free water infiltration and gaseous movement; they are therefore susceptible to I losses through leaching and volatilization. Fine textured soils, with a high I adsorption capacity, are compact and resist free movement of water and air which reduces the loss of I. Based on the textural classification of soil the order of I fixation potential is normally: peat > clay > silt > sand (Johnson, 2003; Johnson et al., 2003).

1.2.3.2 Clay minerals

There are contradictory findings about the role of clay in I retention in soil. Fuge and Johnson (1986) reported high concentrations in clayey soils in Japan. Similarly, Chilean Iodine Educational Bureau (1950) reported that clay increases the I sorption capacity of soil. This is further supported by other researchers including Vinogradov

and Lapp (1971), De et al., (1971), Proskuryakova et al., (1969a), Yeshchenko and Trofimvo (1970) cited in Fuge and Johnson (1986). However, Yu et al. (1996) found that the negative charge on clay alumino-silicate layers does not facilitate the absorption of negatively charged I species. Fuge (2005) has supported these findings and suggested that the role of clay in I retention is not significant. However finer textured soils are generally associated with a humus and metal oxides coating (Bot and Benites, 2005; Hakeem et al., 2013) and Whitehead (1979) also suggests that I retention in soil is due to the presence of metal (Al and Fe) oxides and organic matter coatings.

1.2.3.3 Organic matter

Organic matter probably plays the most substantial role in soil I retention (Johnson, 2003; Sheppard et al., 1996). However, soils with large concentrations of I will not necessarily be a good source of I to the food chain (Saikat et al., 2004) as I in soil with large concentrations of organic matter is generally fixed and not bioavailable. Thus, organic matter is often found to contribute positively towards the total I contents of soil but is inversely related to the bioavailability of I in soil (Johnson, 2003; Johnson et al., 2003). A diversity of opinion exists among researchers as to how organic matter retains I in soil. It is considered that organic I is bound to fulvic and humic acids (Yu et al., 1996; Yamada et al., 1999; Johnson, 2003). In particular, humic substances appear to play an important role in binding I^- (Behrens, 1985). Sheppard and Thibault (1992) consider that I retention by organic matter is not through electrostatic forces but rather a physical association with the surfaces. Sheppard et al. (1996) reported

that possible mechanisms of I retention in soil included simple anion exchange of I^- or IO_3^- and chemical or biological oxidation of I^- to elemental I followed by reaction with soil organic matter. Huang and Lu (1991) found that anoxic conditions hinder the sorption of I^- by humic acid which may indicate the importance of I^- oxidation as a prequel to bonding to humus.

1.2.3.4 Metal oxides

Several authors have reported that oxides and hydroxides of aluminium, manganese and iron in soil are believed to play a role in adsorption of inorganic I and oxidation of I^-. It has been found that I is concentrated in iron-rich soils (Johnson, 2003; Fuge, 2005). Oxides of iron(III) and aluminium adsorb IO_3^- to a greater extent than I^- (Whitehead, 1984; Sheppard and Thibault, 1992; Johnson, 2003; Shetaya et al., 2012) by binding through the oxygens of IO_3^-. Sorption of I by oxides and hydroxides of iron and aluminium is dependent on pH with higher sorption under acidic condition and almost no sorption under neutral pH condition (Fuge, 1996).

1.2.3.5 Role of pH

Iodine speciation partly depends on the pH of the surrounding environment (Whitehead, 1984; Shetaya et al., 2012). Considering inorganic I species, I^- occurs mostly in acidic soil, particularly in waterlogged soil, while IO_3^- largely exists in alkaline soil under dry oxidizing condition (Whitehead, 1984; Johnson, 2003). Soil pH has an important role in sorption and migration of IO_3^- and I^- in both mineral and organic soils (Söderlund et al., 2011). It is reported that sorption of both IO_3^- and I^- is inversely

related with soil pH (Shetaya et al., 2012). Soil pH also affects the rate of conversion of I species. Under acidic pH I^- is converted to elemental I and when I is added as IO_3^- it converts to I^- (Fukui et al., 1996; Söderlund et al., 2011).

1.2.3.6 Redox potential (Eh)

Changes in soil Eh influences I concentration in soil solution (Sheppard and Hawkins, 1995; Ashworth and Shaw, 2006; Yamaguchi et al., 2006; Ashworth, 2009, Lawson, 2014). Muramatsu et al. (1996) found a negative correlation between redox potential and I desorption and reported that higher desorption occurred when the Eh value dropped to about -100 mV or below. Yuita et al. (2005) studied the availability of I in the soil solution of different soils from forest plots, upland fields and paddy rice fields in Japan. There was no significant variation in I concentration in the soil solutions of forest and upland soils. However, the soil solution of rice paddy soils had a greater concentration of I (50 µg L^{-1}) during flood irrigation in summer compared to pre flood I (< 3 µg L^{-1}). This increase in concentration was attributed to low redox potential (-500 – +200 mV) produced by flooded condition. Flooding curtails the availability of atmospheric oxygen to soil and thus generate anoxic condition which results in I release from soil into soil solution (Yuita, 1994; Muramatsu and Yoshida, 1999).

1.2.3.7 Microbial activity

Microorganisms and the products of microbial metabolism (e.g. enzymes, organic acids) affect I fixation in soil (Muramatsu and Yoshida, 1999; Muramatsu et al., 2004). Koch et al. (1989) studied the role of microorganisms and found that I^- sorption in soil

treated with glucose increased while reduced sorption was recorded in soil treated with thymol (an antiseptic). Similarly soils treated with gamma radiation, fumigated or air dried samples have shown to have decreased sorption of I^- (Koch et al., 1989; Bors and Martens, 1992). Muramatsu and Yoshida (1999) studied the role of microbial activities on the accumulation and loss of I from soil (both sterile and non-sterile). It was observed that sorption of I^- was significantly reduced after autoclaving the soil but sorption then resumed after adding a small amount of fresh soil (1%) as inoculum.

Dissanayake and Chandrajith (2009) reported that microorganism creates reducing conditions (low Eh) in rice paddy soils which facilitates desorption of I from soil. The mechanism of I sorption by microorganisms needs further investigation but Amachi (2008) reported two possible mechanisms of I sorption in soil. First that microbial enzymes oxidise I^- to I_2 in soil or sediment and then incorporate it into organic compounds. Second that I is adsorbed by the bacterial cell envelope or taken up by bacteria. Some bacteria capable of oxidizing I^- to IO_3^- and accumulating I have been identified (Gozlan and Margalith, 1973; Amachi et al., 2005) which supports the stance that microorganisms play an important role in I concentration of soils and sediments and its speciation.

1.3 Iodine uptake by plants

Iodine is freely absorbed both by plant roots and leaves (Whitehead, 1984) but there is no evidence to suggest that it is essential for plant growth (Tensho and Yeh, 1970; Yamada et al., 1999; Zhu et al., 2003; Ashworth, 2009; Thompson and Amoroso, 2011;

Mehra et al., 2014). Large concentrations of I in soil solution have been reported to have detrimental effects on the growth of some plants (Whitehead, 1984; Yuita, 1994; Sheppard and Evenden, 1995; Dai et al., 2004; Ashworth, 2009). For example Zhu et al. (2003) and Dai et al. (2004) reported that high intake of I adversely affected the yield of spinach. Similarly excessive intake of I is believed to cause Akagare disease in rice plants (Watanabe and Tensho, 1970; Yamada et al., 1999; Fuge, 2005) and high concentrations of I in solution results in negative effect on the growth of tomato plants (Kiferle et al., 2013). Zhu et al. (2003) suggested that the adverse effects on plant growth could possibly be a result of excessive accumulation of I^- in plant tissues.

Uptake of I by plant roots can be affected by several environmental factors during the growth period (Muramatsu et al., 1983; Lawson, 2014). The chemical form of I is considered to be one of the most important factors because of its presence in a variety of valence states and chemical instability of different species in soil solution (Muramatsu et al., 1983; Burte et al., 1991). Iodide is taken up more rapidly by plants than IO_3^- (Whitehead, 1984). Kato et al. (2013) suggested that IO_3^- is biologically or electrochemically reduced to I^- by plants roots before being taken up. Therefore, it is possible that the slower uptake rate of IO_3^- is a consequence of a slow reduction process (Zhu et al., 2003).

The uptake of I by plants increases with increasing I concentration in soil but the rate of accumulation differs and depends on the type of plant (Whitehead, 1984; Hong et al., 2007). Generally the concentration of I is higher in roots than in shoots (Whitehead, 1973; Hong et al., 2007) probably due to its adsorption on root surfaces

(Muramatsu et al., 1983). Kiferle et al. (2013) reported that I is mostly translocated through the xylem in plants. Therefore, leafy vegetables such as spinach, where edible parts largely rely on I supply through the xylem, accumulate higher concentrations of I (Zhu et al., 2003) whereas, in radish, higher amounts of I accumulate in the rhizome compared to the shoots (Hong et al., 2007). Iodine accumulation in cereal crops is low as I does not appear to be phloem mobile (Zhu et al., 2003; Caffagni et al., 2011).

Uptake of I by plants is also affected by soil properties including retention of I by solid phase components, oxidation/reduction and volatilization (Whitehead, 1984). Strongly sorbed forms of I are not bioavailable (Kabata-Pendias, 2010; Fuge, 2013), therefore large soil I concentrations do not necessarily mean that a plant will accumulate large I contents (Fuge, 2005).

Plants are able to absorb gaseous forms of I from the atmosphere through stomatal and leaf surface absorption (Whitehead, 1984; Fuge and Johnson, 2015). Sorption of I through leaf stomata is greater in humid environments as humidity affects the stomatal movement of leaves (Barry and Chamberlain, 1963; Fuge, 2005). The aerial parts of the plants are considered to contribute significantly to the total I content of plants (Fuge, 2013). Mosses and lichens, which do not have a root system and depend entirely on atmospheric inputs for nutrients, have nearly the same concentration of I as plants with roots growing in the same area (Fuge and Johnson, 2015).

1.4 Dietary iodine sources

Seafood is generally a rich source of dietary I intake (Vought and London, 1964; Bourre and Paquotte, 2008; Nerhus et al., 2018). Marine fish and several seaweeds have higher concentrations of I ranging from 0.39-6.9 mg kg^{-1} in marine fish (Haldimann et al., 2005) and more than 8 mg kg^{-1} in certain edible seaweeds (Zava and Zava, 2011). Therefore, a population using seafood as a major dietary component will usually have adequate I intake (Fuge and Johnson, 2015). However, it is important to mention that excessive intake of I can lead to serval health complications (Zava and Zava, 2011; Leung and Braverman, 2014). It is reported that leafy vegetables, such as lettuce and spinach, accumulate relatively high I concentrations compared to root vegetables, such as carrot, and hence consumption of leafy vegetables effectively supplement the daily intake of I (Dai et al., 2004; Blasco et al., 2008).

A significant component of the human diet includes animal products such as milk, eggs and meat. The consumption of cow's milk is a good source of dietary I for humans although its concentration varies in different areas and seasons (Bowley, 2013). It has been found that the I content of milk from cows grazing near coastal areas is higher than that from remote mountainous areas (Fuge and Johnson, 2015). Eggs are also an important source of dietary I intake (Fordyce, 2003; Haldimann et al., 2005). The high content of I in eggs is possibly due to I-enriched poultry feed (Fordyce, 2003). Intake of dietary I can also be enhanced through adventitious sources such as using iodised salt, iodised vegetable oil, iodised water, high dose I injection and capsules to curb I deficiency problems (Girerd-Barclay and Tiwari,

2002). Otherwise most diets particularly in developing countries would fail to provide the WHO recommended adult daily intake of 150 μg day^{-1} (Fordyce, 2003).

1.5 Consequences of limited or extreme iodine intake

The recommended intake of I varies with age and sex. The WHO (2007a) recommend a daily intake of I of:

"90 μg for preschool children (0 to 59 months), 120 μg for schoolchildren (6 to 12 years), 150 μg for adolescents (above 12 years) and adults and 250 μg for pregnant and lactating women".

When I consumption decreases below recommended levels, it causes I deficiency which is a major public health problem in some areas of the world (Hess, 2010). Iodine is an essential component of thyroid hormones (McGuire and Beerman, 2012; Kato et al., 2013) and plays an important role in the function of the thyroid gland (Triggiani et al., 2009). It is required for the synthesis of thyroid hormones, thyroxine and tri-iodothyronine (Girerd-Barclay and Tiwari, 2002; Kapil, 2007). Iodine deficiency causes numerous health disorders, collectively called I deficiency disorders (IDDs) (Zimmermann, 2008). These include goitre (enlarged thyroid gland), cretinism, mental impairment, stillbirth, abortion, reduced IQ, decreased fertility, congenital abnormalities and foetal brain damage (Stewart and Pharoah, 1996; Johnson et al., 2003; Zimmermann, 2008). The spectrum of I deficiency is quite wide and affects human development at different stages including; foetus, neonate, child and adolescent and adults (Hetzel, 1983; WHO, 2007b). Foetal brain development during second and third trimester of pregnancy and up to three years after childbirth is very

sensitive to I deficiency (Velasco et al., 2018). Iodine deficiency can cause brain damage and in extreme cases results in cretinism (Qian et al., 2005; WHO, 2007a). Iodine deficiency has been recognised as the single most common but preventable cause of mental health retardation (Delange, 2001; Slavin, 2005; Kapil, 2007; Eastman and Zimmermann, 2018). Delange (2001) suggested that I deficiency results in a loss of 10-15 IQ points in the global population. Boyages et al. (1989) from their study of I deficiency in north-central China found that I deficiency has resulted in the displacement of cognitive skill in the whole population of the area to a lower level. Thus, the effects of IDDs are serious and severe, affecting intellectual ability, achievement, development and overall potential of the whole community (WHO, 2007a; Zimmermann, 2011). It also affects animals and dramatically reduces livestock productivity (Cabello et al., 2003; Zimmermann, 2011).

Before 1950, IDDs affected almost every country of the world (Fuge, 2005). The deficiency is dietary in nature and reduces when I is supplied to affected populations (Hetzel, 1983; Bath et al., 2014; Fuge and Johnson, 2015). By 1970, IDDs were eliminated from most developed countries by introducing dietary I supplementation (Fuge, 2005). Currently IDDs are a problem in poor parts of the world where the population is largely dependent on locally grown food, however parts of Europe are also vulnerable with IDDs being reported in some countries of Western Europe (Fuge, 2005; Kapil, 2007; Velasco et al., 2018).

Iodine deficiency is generally restricted to high mountain ranges, central continental areas and areas of high rainfall (Kelly and Snedden, 1958; Fuge, 1987; Fuge, 1996). It is easy to explain IDDs in remote areas of the world with the conventional explanation

of low I contents of soil and low aerial I availability (Fuge, 2005). However, endemic goitre has also been recorded in some coastal areas of Malaysia, Sri Lanka and China (Osman et al., 1995; Fordyce et al., 1998) despite higher concentrations of I in soil, water and crops (Fuge, 1996; BGS, 2000; Johnson, 2003). Similarly the Derbyshire area of the UK, which is not a coastal area but has a relatively high marine influence, has shown a prevalence of endemic goitre in the past because most of the I is locked up in the limestone parent material (Saikat et al., 2004; Fuge, 2005). Thus, it is suggested that IDD is not simply determined by the total I content of soil but rather by the bioavailability of I (Fuge, 2005; Mehra et al., 2014).

Several authors have reported the role of goitrogens in IDDs. Goitrogens are substances that interfere with I uptake in the thyroid gland and inhibit the production of thyroid hormones (Wotango, 2008). Stewart (1990) from his studies on endemic goitre in North East Pakistan suggested the existence of possible geological goitrogens such as lithium, boron, molybdenum and calcium which are present in rocks of that area. It is reported that certain foods have potential goitrogenic substances such as apricot kernels, brassica seeds, turnips, brussels sprouts, millet, cassava and rapeseeds (Gaitan et al., 1989; Akindahunsi et al., 1998; Stewart, 1990; Fuge, 1996; Lee and Kwon, 2009).

Excess I intake can also lead to endemic goitre. Medani et al. (2013) found that in coastal areas of Sudan excess I is the possible cause of endemic goitre. Zimmermann et al. (2005) also noted that excess dietary intake of I can increase the risk of goitre and reported that excessive intake of I can result in certain health problems such as gastrointestinal irritation, vomiting, abdominal pain, diarrhoea and skin disorders

(Zimmermann, 2008). Sun et al. (2014b) noted that excess intake of I can result in hypothyroidism. Similarly Mu et al. (1987) attributed goitre in Shanxi province of China to high I concentrations in the water.

In summary both deficiency and excess of I is harmful for human and animal health. Any approach to correct the problem of I deficiency should be carefully monitored both for deficiency and excess (Zimmermann, 2008). Overall, the problems associated with I deficiency are far more serious and complicated than excessive I intake.

1.6 Iodine biomarkers

According to Potischman and Freudenheim (2003):

"A nutritional biomarker can be any biological specimen that is an indicator of nutritional status with respect to intake or metabolism of dietary constituents".

There are several possible biomarkers, including urinary I (UI), thyroglobulin (Tg), thyroid stimulating hormones (TSH), thyroxine (T4) and goitre for determining human I status (Ristic-Medic et al., 2009; Zimmermann and Andersson, 2012). All of the above biomarkers have their advantages and disadvantages; however, UI is considered a well-established and cost-efficient biomarker for assessing the I status of a population (WHO, 2013; Pearce and Caldwell, 2016). It is estimated, that the majority of dietary I (~90%) is excreted in urine (Zimmermann and Andersson, 2012; Rohner et al., 2014; Pearce and Caldwell, 2016; Beckford et al., 2017). Furthermore, urine sampling is relatively convenient to collect and median UI concentration (UIC)

can be used to represent the I status of populations (Rohner et al., 2014). A median UIC of < 100 μg L^{-1} in a population indicates mild I deficiency (WHO, 2013).

Section 2. Selenium

1.7 Selenium - introduction

Selenium (Se) is a naturally occurring metalloid and belongs to group 16 (VIA) of the periodic table (Martens, 2003; Fordyce, 2013). It has five naturally occurring stable isotopes, including ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se and ^{80}Se, and a short lived radio-isotope ^{75}Se (Johnson et al., 2004; Mathers, 2015). In nature, it exists in four oxidation states: selenate (+6), selenite (+4), elemental Se (0) and selenide (-2) (Fishbein, 1983; Rosen and Liu, 2009; Hooda, 2010; Sun et al., 2014a).

It originates in the environment both from natural and anthropogenic sources. However, anthropogenic activities are believed to be the major sources of Se release in the environment (Haygarth, 1994; Fordyce, 2013).

1.8 Selenium in the Environment

Selenium is one of the most widely but unevenly distributed elements of the Earth's crust. The average concentration of Se in the Earth's crust ranges from 0.05 to 0.09 mg kg^{-1} (Shamberger, 1981; Högberg and Alexander, 2007; Dissanayake and Chandrajith, 2009). Selenium is a chalcophile element and occurs mostly associated with sulphide minerals (Johnson et al., 2010). It is found in all natural materials on the Earth's surface including rocks, soil, air and water and in both plant and animals tissues. It is continuously recycled through terrestrial, atmospheric and marine systems (Fordyce, 2013).

1.8.1 Selenium in water

It has been estimated that the annual global loss of Se from terrestrial to aquatic environments is 14,000 tonnes per year (Nriagu, 1989; Fordyce, 2013). There are diverse figures reported in the literature concerning the average concentration of Se in the marine environment. Cutter and Bruland (1984) have estimated the average concentration of Se in ocean water at 0.9 μg L^{-1} (Fordyce, 2013). However Kabata-Pendias and Mukherjee (2007) have reported that the mean Se concentration in the marine environment varies between 0.1 and 0.35 μg L^{-1}. The concentration of total Se increases with depth in the ocean (Conde and Alaejos, 1997; Atwood, 2013). It is reported that that concentration of selenate (Se^{VI}) is invariant in both the upper and deep layers of ocean while selenite (Se^{IV}) concentration increases in deep water (Sugimura et al., 1976). The mean residence time for Se is believed to be 70 years in mixed layers and 1100 years in the deep ocean (Fordyce, 2013). Therefore, the marine environment constitutes a major environmental sink for Se (Haygarth, 1994). The range of total Se reported in natural water is 1-400 μg L^{-1} (Conde and Alaejos, 1997). The average concentration of Se in river water is 0.07 μg L^{-1} while some rivers (e.g. the Colorado River, USA) have been reported to have Se concentrations as high as 400 μg L^{-1} (El-Ramady et al., 2014). Most of the Se (about 85%) in river water is believed to be in particulate form rather than dissolved (Haygarth, 1994; Fordyce, 2013). Groundwater usually has a higher concentration of Se; concentrations as high as 1000 μg L^{-1} have been reported in the groundwater of certain areas in China (Kabata-Pendias and Mukherjee, 2007).

1.8.2 Selenium in the atmosphere

Selenium is present in both the particulate and gaseous phases of the atmosphere (Ross, 1985; Wen and Carignan, 2007) and comes from a variety of natural and anthropogenic sources (Herring, 1991; Kieliszek, 2019). The natural sources comprise volcanic eruptions, volatilisation of Se from soil, sediment and oceans (El-Ramady et al., 2015; Winkel et al., 2015). The anthropogenic sources include combustion (fossil fuel, biomass, incineration etc.), metal smelting, manufacturing and utilization of agriculture products (Fordyce, 2007; Winkel et al., 2015). The atmosphere plays a significant role in the transportation, transformation and dispersion of Se in the environment (Wen and Carignan, 2007). The short residence time of Se in the atmosphere (Herring, 1991; Blazina et al., 2016) makes it a rapid transport route for Se in the environment (Fordyce, 2013). It is reported that approximately 13-19 kt of Se is cycled through the troposphere annually (Mosher et al., 1987; Wen and Carignan, 2007; Winkel et al., 2012). Within the total atmospheric Se budget, 70% is believed to be from anthropogenic sources, particularly in the northern hemisphere where most of the industrial activities occur (Ross, 1985). Coal combustion is believed to be the major anthropogenic source while among the natural sources marine biogenic Se is reported to be the largest source (Wen and Carignan, 2007; Winkel et al., 2015).

In the atmosphere Se undergoes numerous physical, chemical and photochemical processes before deposition to the Earth's surface. Three different phases of Se, including organic, inorganic and particulate Se, are reported in the atmosphere (Wen and Carignan, 2007). Organic Se is believed to be released from soil and marine

systems due to microbial action either in the form of dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) (Winkel et al., 2015; Ivanenko, 2018). It is reported that due to the rapid sorption and instability of DMDSe most of the gaseous organic Se reaching the atmosphere is DMSe (Wen and Carignan, 2007).

In addition to organic Se, inorganic Se species are also available in the atmosphere (Wen and Carignan, 2007; Winkel et al., 2015). The inorganic Se species present in the atmosphere are hydrogen selenide (H_2Se) and selenium dioxide (SeO_2). They are very unstable, with a very short lifetime, and are easily transformed into particulate phases. Inorganic Se comes largely from anthropogenic sources such as coal combustion (Wen and Carignan, 2007).

Particulate Se in the atmosphere is believed to come from sea salt, windblown dust, combustion and incineration. However, a large proportion of particulate atmospheric Se is secondary and formed by conversion of gaseous phase to particulate form. For example, volatile organic or inorganic Se is transformed into solid aerosols by atmospheric reactions or is adsorbed onto atmospheric aerosols (Mosher et al., 1987; Cahill and Eldred, 1998; Wen and Carignan, 2007).

Selenium is returned to the Earth's surface through dry and wet deposition processes (Glooschenko and Arafat, 1988; Martens, 2003; Pan and Wang, 2015). As mentioned earlier, Se has a short residence time in the atmosphere and does not travel long distances. Therefore, the removal of atmospheric Se is usually considered on regional and local scales (Cahill and Eldred, 1998; Wen and Carignan, 2007).

1.8.3 Selenium in soil

The world mean Se concentration in soil is thought to be about 0.4 mg kg^{-1} but concentrations vary from as low as 0.01 – 2.0 mg kg^{-1} in Se deficient soils to 1200 mg kg^{-1} in seleniferous soils (Fordyce, 2007; Fernández-Martínez and Charlet, 2009; Winkel et al., 2011). The concentration of Se in soil largely depends on the Se concentration in parent material and processes subsequent to soil development (Shamberger, 1981; Fordyce et al., 2010). Different types of rocks have diverse concentrations of Se. Sedimentary rocks generally contain higher concentrations of Se than other rocks but levels in some sedimentary rocks, such as limestone and sandstones, barely exceed 0.1 mg kg^{-1} (Fordyce, 2007). As these rocks cover most of the surface of the Earth's crust it is suggested that more areas are Se deficient than Se sufficient or Se toxic (Johnson et al., 2010; Fordyce, 2013). The Se rich soils are usually derived from Se rich rocks such as carbonaceous limestones and cherts, mudstones, black shales and seleniferous coal (Winkel et al., 2011; Yu et al., 2015).

1.8.4 Factors affecting selenium retention in soil

The underlying geology of the area largely controls the total concentration of Se in soil. However, there are other factors that determine the strength of sorption and retention of Se in soils and thereby affect the total concentration and bioavailability of Se in soil. These include organic matter content, the size of the clay fraction, metal oxide concentration, pH, redox potential, chemical forms of Se and the presence of anions, such as PO_4^{3-} and SO_4^{2-}, that compete for adsorption in the soil and uptake by plants (Goh and Lim, 2004; Fordyce, 2007, Fordyce, 2013).

1.8.4.1 Organic matter

Selenium has a strong affinity for soil organic matter, clay minerals and metal oxides (such as iron oxide), which reduce Se availability to plants (Johnson et al., 1996; Fordyce et al., 2000; Čuvardić, 2003; Winkel et al., 2011; Fordyce, 2013). The presence of organic matter in soil removes Se from the soil solution by strong and poorly reversible binding mechanisms. Selenium uptake by plants from organic rich soil may be an order of magnitude less than from a mineral soil (Gissel-Nielsen and Bisbjerg, 1970; Fordyce, 2013). Johnsson (1991) confirmed in a pot experiment that with increasing soil organic matter content the Se concentration of wheat grain decreased significantly; a similar correlation was observed for the clay content of soil and plant Se uptake.

1.8.4.2 Soil texture

Selenium adsorption on fine textured soil particles reduces its bioavailability (Fordyce, 2013). Adsorption of Se on clay sized particles such as iron oxides is strongly pH dependent; adsorption increases with decreasing pH and reaches maximum between pH 3 and 5 (Mikkelsen et al., 1989; Johnson et al., 1996; Fordyce, 2013).

1.8.4.3 Soil pH and Eh

Soil pH and redox potential largely determine the speciation of Se (Weres et al., 1989; Johnsson, 1991). Selenium speciation affects its mobility and bioavailability (Oram et al., 2011). Elemental Se^0 and Se^{2-} are stable under acidic and reducing conditions only and mostly unavailable to plants (Plant et al., 2003; Fordyce, 2013). Selenite and Se^{VI}

exist under most natural redox conditions with Se^{IV} being more stable, but less soluble and bioavailable, than Se^{VI} (Fordyce, 2013). The Eh-pH diagram (Figure 1.1) for Se speciation shows that Se^{IV} is present as SeO_3^{2-}, $HSeO_3^-$ and H_2SeO_3 (aq) in soil solution depending on pH (Twidwell et al., 2005; Zhang et al., 2017).

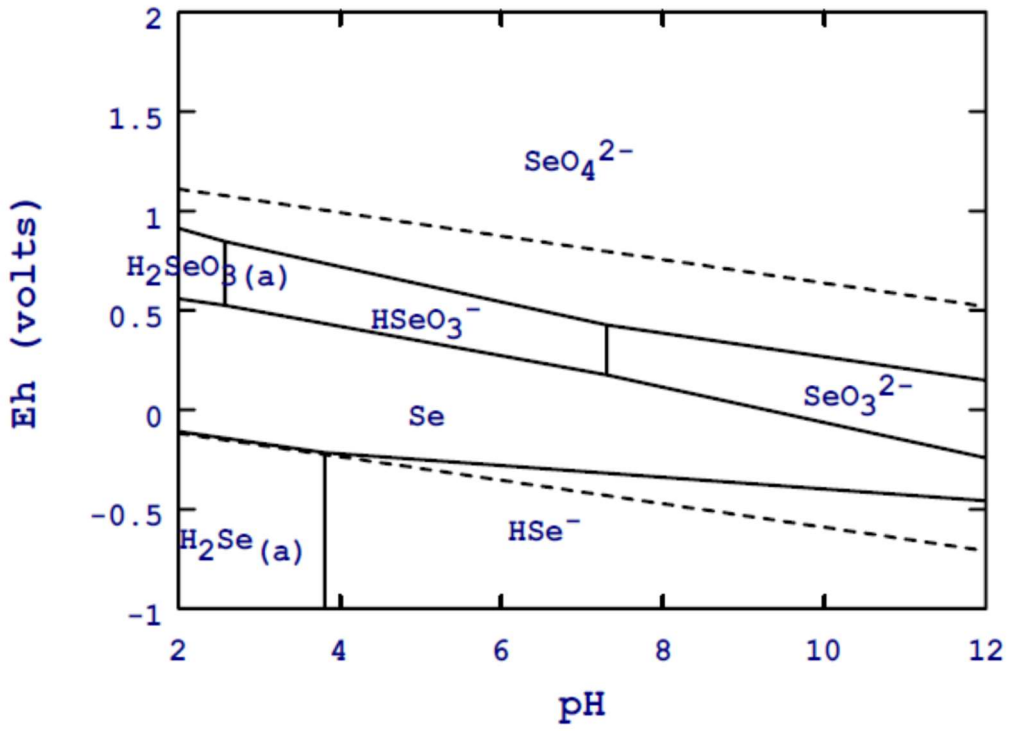


Figure 1.1. Eh-pH predominance diagram showing the stability of selenium species at different pH and Eh values. Diagram obtained from Twidwell and Joyce, 2005 with no amendments.

Selenite is a diprotic weak acid with two pKa values of 2.46 and 7.31 respectively (Li et al., 2008). Selenite is largely present as $H_2SeO_3^0$ at pH values <2.46 while $HSeO_3^-$ is the dominant species in the pH range 2.46-7.31; at higher pH (>7.31) SeO_3^{2-} prevails in the soil solution. Selenate is a monoprotic weak acid and present as $HSeO_4^-$ and SeO_4^{2-} with a pKa value of ~1.9 (Peak and Sparks, 2002). Under realistic soil conditions (pH >2), SeO_4^{2-} is the dominant species while at a very low pH values seldom seen in soils (pH <2) $HSeO_4^-$ is present (Zhang et al., 2017). Soil pH affects the adsorption of

Se oxyanions onto surfaces of iron (Fe), manganese (Mn) and aluminium (Al) hydroxides. Selenite adsorption on hydrous oxides surface is greater at low pH because the amphoteric surfaces of oxides of Fe, Mn and Al are positively charged under those conditions. The surface charge on hydrous metal oxides is positive at pH below their point of zero net charge (pznc). Typical values of the pznc for MnO_2, $Al(OH)_3$ and $Fe(OH)_2$ are 2-4, 7.5-9.5 and 6-8 respectively (Mathers, 2015).

In common with other weak acid anions, the 'adsorption envelope' of Se^{IV} shows a pattern that is determined by several factors. At pH values below pKa_2 for Se^{IV} an increase in pH produces a relatively shallow decline in Se adsorption because the effect of declining positive charge on the oxide surfaces is offset by an increase in the proportion of Se in solution present as SeO_3^{2-} (the main adsorbing species). However, at pH values above pKa_2 (7.31) while the decline in positive charge continues almost linearly with pH, the increase in the proportion of Se present as SeO_3^{2-} slows as most of the Se^{IV} is already converted to the doubly-charged oxyanion. Therefore, a sharp decline in Se adsorption is seen at pH > pKa_2; this is a very common feature on adsorption envelopes. Furthermore, as pH rises there is greater competition from other anions (OH^-, HCO_3^-, PO_4^{3-}) which also reduces Se adsorption on hydrous oxide surfaces.

Selenium is adsorbed on hydrous metal oxides in two ways: (i) specific adsorption (inner sphere complexation) and (ii) non-specific adsorption (outer sphere complexation). In specific adsorption, Se is adsorbed on oxide surfaces by ligand exchange resulting in the loss of the anion hydration sphere and chemical bond formation between the oxide cation (Fe, Al, Mn) and the oxygens of the Se oxyanion.

In non-specific adsorption Se oxyanions are retained on oxide surfaces by electrostatic attraction with water molecules between the Se anion and the adsorbing surface. It is widely accepted that Se^{IV} is more strongly adsorbed onto soil particle surfaces by ligand exchange, forming inner-sphere complexes, while non-specific anion exchange creates outer-sphere complexes with Se^{VI} (Lopes et al., 2017). Against this simple model, Peak and Sparks (2002) reported that Se^{VI} can form a mixture of inner and outer sphere complexes on the Fe oxide, goethite.

1.8.4.4 Competitive ions

The presence of competitive anions such as PO_4^{3-} and SO_4^{2-} affects Se bioavailability in quite complex ways by (i) competing for fixation sites in soils and thereby influencing the solubility of Se but also competing for root transporters (Hopper and Parker, 1999; Dhillon and Dhillon, 2000; Oram et al., 2011). Thus, for example, sulphate ions (SO_4^{2-}) reduce Se^{IV} uptake by plants by competing for root absorption sites while PO_4^{3-} facilitates Se^{IV} uptake by plant by displacing Se^{IV} from soil adsorption sites thus making it more bioavailable (Fordyce, 2013).

1.9 Selenium uptake by plants

As mentioned earlier, Se is not known to be essential for plant growth and development (Ellis and Salt, 2003; Kabata-Pendias, 2011; Winkel et al., 2015) but it is taken up and assimilated into plant structures (Fordyce, 2013). Plants can take up Se both in inorganic (Se^{IV} or Se^{VI}) and organic forms (selenomethionine or selenocystine (Ellis and Salt, 2003; Kikkert and Berkelaar, 2013; Winkel et al., 2015). The uptake of

Se is affected by several factors, such as soil pH, texture, organic matter content, Se species and the presence of ions such as SO_4^{2-} and PO_4^{3-} (Mikkelsen et al., 1989; Kabata-Pendias, 2011; Winkel et al., 2015). Soil pH is found to be positively correlated with uptake (De Temmerman et al., 2014). However this relationship is not simple and there are other interactive factors (e.g. soil texture and organic matter) which are also important to consider. This, for example, uptake of Se was found to increase with increasing pH in sandy and loamy soils but not in a clay soil (Gissel-Nielsen, 1971; Winkel et al., 2015). Generally, organic matter increases Se retention in soil and hence reduces its bioavailability; a number of studies have shown that soil organic matter content is negatively correlated with plant uptake of Se (Winkel et al., 2015). The uptake of Se largely takes place through active membrane transport while the role of passive diffusion is limited; Se^{VI} is believed to transport through sulphate transporters while phosphate transporters seems to play an important role in Se^{IV} transport (Winkel et al., 2015). Therefore, the uptake of Se^{VI} and Se^{IV} is greater in sulphur and phosphorus starved plants respectively (Li et al., 2008). The presence of ions such as SO_4^{2-} and PO_4^{3-} have been found to be negatively correlated with Se accumulation in plants (Mikkelsen et al., 1989).

The intake rate of organic Se is believed to be several times (20-100) greater than those of inorganic Se. There is no specific mechanism known for organic Se uptake so far but it is reported that probably amino acid transporters play an important role (Winkel et al., 2015).

1.9.1 Agronomic biofortification

According to WHO (2019):

"Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology".

However, none of the above-mentioned approaches appears to be enough to act alone in completely eradicating Se deficiency in relevant populations. Globally, researchers are working on the approaches listed above but the current project is focussed on agronomic biofortification of staple crops. This is perhaps the most promising method to reach malnourished people in the developing world who have limited access to diverse diets (de Valença et al., 2017). Agronomic biofortification with Se is achieved through foliar application to crop plants or direct application to soil (Lyons et al., 2004; Broadley et al., 2007; Broadley et al., 2010). Foliar application has been reported to be more efficient in increasing plant Se concentrations (Gupta and Gupta, 2017; Lyons, 2018) as it increases absorption of Se by plant tissues and reduces the loss of Se to fixation reactions in soil (Winkel et al., 2015). In the majority of studies it is reported that application of Se^{VI} is most suitable for biofortification because it is more soluble in soil than Se^{IV}, easily taken up by plants and readily distributed from roots to aerial parts of the plant (Broadley et al., 2007; Woch and Hawrylak-Nowak, 2019); Se^{IV} is reported to be a more 'long standing' source of Se for plants but with a much lower initial uptake efficiency (Broadley et al., 2007). Both Se^{IV} and Se^{VI} have been found to increase plant Se content when applied as aqueous solution to plant leaves (Ducsay and Ložek, 2006).

1.10 Selenium and health

Selenium is an important micronutrient for human and animal health though it is not known to be essential for plants growth (Rayman, 2000; Ellis and Salt, 2003; Winkel et al., 2015). It is an essential component of seleno-proteins and mostly involved in redox reactions (Brown and Arthur, 2001; Di Bella et al., 2010). Seleno-proteins such as glutathione peroxidase plays a major role in preventing cell degeneration from oxidative stress (Di Bella et al., 2010). Selenium is essential for normal functioning of the thyroid gland and thyroid hormone synthesis (Köhrle, 2005; Ventura et al., 2017). It protects the thyroid gland from damage by excessive exposure to I (Köhrle, 1999). It is required in very small amount and has a very narrow range between dietary deficiency (<55 μg day^{-1}) and toxicity (>400 μg day^{-1}) (MacFarquhar et al., 2010; Fordyce, 2013).

1.11 Consequence of selenium deficiency and toxicity

Selenium deficiency is dietary in origin and occurs mostly in areas where soils are low in bioavailable Se (Hartikainen, 2005). It causes a number of health risks to humans and animals (Tinggi, 2003; Hartikainen, 2005). In humans, deficiency has been associated with two endemic diseases including Keshan (cardiomyopathy) disease and Kashin-Beck (a deforming arthritis) disease in Se-deficient regions of China (Rayman, 2000; Risher, 2003). Zimmermann and Köhrle (2002) reported that collective deficiency of Se and I results in myxoedematous cretinism. It has also been reported that Se deficiency causes a number of other health complications including heart disease, hypothyroidism, a weakened immune system, skeletal muscle

disorders, progression of HIV infection and mortality, increased incidence of cancer and complications in reproductive growth, depressed mood, anxiety, confusion and hostility (Rayman, 2000; Ellis and Salt, 2003; Di Bella et al., 2010; Fordyce, 2013).

Excessive intake of Se has toxic effects both on human and animals (MacFarquhar et al., 2010). Selenium toxicity was largely recognised in the 1930's in animals that had fed on plants capable of accumulating Se (Spallholz, 1994; Tinggi, 2003). The symptoms of Se toxicity were probably first described when Marco Polo visited remote areas of China in the middle of the 13th century, before the element was discovered (Hartikainen, 2005). The symptoms of Se toxicity in human include vomiting, nausea, tiredness, irritability, nail and hair loss, nail discoloration, bad breath (garlic breath), joint pain and headache (Reid et al., 2004; MacFarquhar et al., 2010). Selenium toxicity causes alkali diseases, blind staggers, abnormal foetal development and congenital malformation in animals, and reduced hatchability and deformed embryos in birds (Spallholz and Hoffman, 2002; Fordyce, 2013).

1.12 Selenium biomarkers

There are multiple biomarkers for assessing Se status of an individual. These include Se concentration in urine, hair, toenails and blood (whole blood, serum or plasma) (Hunter et al., 1990; Sheehan and Halls, 1999; Johnson et al., 2010; Fairweather-Tait et al., 2011). Hair and toenails are usually used for determining the long-term status of Se while plasma or urine mostly represent the short-term Se status (Hunter et al., 1990; Thomson, 2004; Fairweather-Tait et al., 2011). The concentration of Se in plasma or serum is considered to be the most widely accepted biomarker for

determining Se status (Fairweather-Tait et al., 2011; Phiri et al., 2019). It is also reported that urinary Se is closely associated with plasma Se and dietary intake (Thomson, 2004). Thus urinary Se can be used as a useful biomarker in field studies to determine the Se status of a population as Se content changes with dietary variation (Fordyce, 2013) and is far less intrusive to collect compared to blood sampling. Selenium concentration in different biomarkers (whole blood, serum, toenails and urine) correlate well with dietary intake (Longnecker et al., 1996; Satia et al., 2006; Yoneyama et al., 2007). Due to the large variation of Se status in different countries, there is no accepted normal global range (Thomson, 2004), however, a reference value for various regions or countries can be given. The reference value for Se in blood plasma is 50-120 µg L^{-1} in Germany (Wilhelm et al., 2004) and 63-158 µg L^{-1} in the UK (Sheehan and Halls, 1999).

Section 3. Study area

1.13 Description of the study area: Gilgit-Baltistan

Gilgit-Baltistan (GB), previously known as 'The Northern Areas', is a high altitude mountainous province situated in the extreme north east of Pakistan, latitude 35° to 37° North and longitude 72° to 75° East (Hashmi and Shafiullah, 2003; Malik and Azam, 2009; Karrar and Iqba, 2011). The province covers an area of about 72,500 km^2 (Khalil, 2003; Razza et al., 2014) and is situated at the confluence of three of the world's most famous mountain ranges: the Himalayas, Karakuram and Hindukush (Din, 2010; Hussain et al., 2011; Karrar and Iqba, 2011). Gilgit-Baltistan is divided into 8 districts: Granche, Skardu, Gilgit, Diamir, Ghizar, Astore, Hunza and Nagar (Hussain,

2017) with Gilgit city as the provincial capital (Karrar and Iqba, 2011). Based on the 1998 census the total population of the area was recorded as 870,347, comprising 453,221 males and 417,126 females (Harlech-Jones et al., 2005) with a population density of approximately 12 persons km^2 (Khalil, 2003; Hashmi and Shafiullah, 2003; Dryland and Jawad, 2011). The majority of the population live in rural areas (Iftikhar, 2003).

The landscape of the area is very mountainous and is home to some of the highest peaks in the world including K-2 (the second highest mountain in the world). More than half of the area is located 4,500 m above sea level (Khan, 2003; Government of Pakistan and IUCN, 2003). Some parts of GB are heavily glaciated and contain the largest glaciers outside the polar region, such as Siachin and Baltoro which are 72 and 62 km long and occupy an area of 685 and 529 km^2 (Din, 2010). The glaciers cover about 10-20% of the Northern Areas and a further 30-40% of the area is covered by seasonal snowfall. The glaciers of GB are an important factor in controlling water availability in the upper Indus basin (Ahmed and Joyia, 2003). Gilgit-Baltistan form the watershed of the Indus River (Ahmed, 2013) which is one of the longest river systems in the world and the most important river of Pakistan (Inam et al., 2007; Mohiuddin, 2007).

Gilgit-Baltistan has a fairly diverse and variable climate, ranging from arid and semi-arid cold desert in the northern Karakoram and Hindu Kush to the monsoon-influenced humid areas in the western Himalayas (Ismail, 2018). The wide topography of the region significantly affects the temperature and precipitation. The temperature ranges from 40°C in summer to -10°C in winter in the bottom of valleys

(Nawaz et al., 2019). The precipitation is low at low altitude (below 3000 m) and hardly ever exceeds 200 mm annually but at high altitude above 6000 m there is heavy snow equivalent to about 2000 mm annually (Government of Pakistan and IUCN, 2003; Malik and Azam, 2009). The rainfall ranges from 125 mm in the north to 375 mm in the south (Malik and Azam, 2009). The overall average annual rainfall in the region is under 200 mm (Iftikhar, 2003). However, there is occasional intense and heavy rainfall in the area which has substantial hydrological and geomorphological significance, particularly in terms of erosion and the production of debris flows in rivers (Ahmed and Joyia, 2003).

Geologically GB is characterized by the presence of various types of igneous and different grades of metamorphic rocks (Khan et al., 2010). The pattern of rock distribution is not regular and differs regionally and with elevation (Malik and Azam, 2009). The area is mainly composed of three tectonic plates which are known, from north to south, as the Karakorum plate, the Kohistan island arc and the Indo-Pakistan plate (Tahikheli and Jan, 1979; Kazmi and Qasimjan, 1997). The region has two major tectonic thrusts: the main Karakoram thrust in the north and the Indus suture zone in the south (Tahirkheli and Jan, 1979; Lisa and Khwaja, 2005).

1.13.1 Water resources and irrigation

Water supply is largely derived from three sources: precipitation, spring water and streams flow. Precipitation in the form of rainfall is low and scarcely contributes to water consumption. The availability of spring water is also inadequate and limited to certain areas. The major source of water for consumption is therefore local streams.

The water level in streams varies seasonally as they largely fed by glaciers (Ahmed and Joyia, 2003). Snowmelt begins in March at lower altitudes with glacial inputs from May substantially raising the flow (Ahmed and Joyia, 2003). In July and August, the runoff is 15 to 40 times higher than in March with 40 to 70% of the total runoff of the Indus and its tributaries occurring (Ahmed and Joyia, 2003; Khan, 2014). The total annual runoff in streams is variable and affected both by winter snowfall and summer weather condition which affects the rate of snow melting (Ahmed and Joyia, 2003; Bashir and Rasul, 2010; Ashraf et al., 2012).

Apart from a small number of settlements on riverside in district Skardu and a few villages in Gilgit, where people draw water from shallow wells, the water supply in GB is largely relied on the utilisation of surface water (Ahmed and Joyia, 2003). The mountains have only limited groundwater supplies as the rocks of the area generally have low porosity and permeability which limits groundwater availability to local fracture zones or pockets of alluvium (Ahmed and Joyia, 2003; Hashmi and Shafiullah, 2003; Mukhopadhyay and Dutta, 2010).

There is a general shortage of irrigation water in GB (Hashmi and Shafiullah, 2003) as the irrigation is largely dependent on surface water. To a lesser extent, and at a local scale, spring water is also used for irrigation. The irrigation water is distributed through 'Khuls', small, lengthy surface channels maintained and constructed by farmers and villagers. The snow fed irrigation channels show annual flow variability and are less reliable than small perennial rivers and springs (Velde, 1989; Ahmed and Joyia, 2003; Hashmi and Shafiullah, 2003).

1.13.2 Soil

The arable lands are largely established on alluvial fans and, to a smaller extent, arise from the reclamation of old river terraces in valleys (Hashmi and Shafiullah, 2003). The hill slopes are generally bare without any soil cover (Malik and Azam, 2009). The soils are largely calcareous with pH values above 7.0 (Government of Pakistan and IUCN, 2003; Hashmi and Shafiullah, 2003). The soil is generally low in clay content and organic matter (less than 1%) but high in silt and sand. Due to the small clay content the water retention capacity of the soil is low and thus the water availability to crops is limited. Free-draining and irrigation lead to leaching of major nutrients (Hashmi and Shafiullah, 2003).

1.13.3 Agriculture land-use

The utilised land is mainly used for arable farming, fruit growing, livestock grazing and forestry. Only 2% of the total area is cultivable and, of this, roughly half is already in use for agriculture (Government of Pakistan and IUCN, 2003; Hashmi and Shafiullah, 2003). The agricultural system varies with altitude. At low altitudes (between 1200 and 1900 m) both summer and winter crops are grown whilst above 2000 m and below 3000 m only summer crops (either maize or wheat) are grown (Malik and Azam, 2009). Cultivation ends at 3000 m and above which high forest and alpine pastures begin (Government of Pakistan and IUCN, 2003; Hashmi and Shafiullah, 2003).

The staple cereal crops are wheat, maize and barley which are grown on an area of 52,837 ha; fruit trees are usually grown on boundaries of fields and cover about

12,056 ha of land (Ahmed, 2003). The main fruits grown are apple, apricot, cherry, mulberry, grapes, peache, almond and walnuts (Ahmed, 2003). Approximately 10,638 ha of land is used to grow vegetables such as onion, peas, tomato, potato, cauliflower, cabbage, spinach, carrot, radish, turnip, lettuce, beans, garlic and aubergine (Hashmi and Shafiullah, 2003).

The area is generally devoid of good quality animal fodder which becomes an acute problem in winter during extreme weather condition. Bushes and grasses, growing on shallow soils developed in cracks and crevices of rocks on mountainsides, provide green fodder for animals. Such animal grazing involves an area of 47,558 ha but this does not meet the requirements of livestock in the area (Government of Pakistan and IUCN, 2003; Malik and Azam, 2009).

1.14 Prevalence of IDDs in Gilgit-Baltistan

The population of GB is largely considered I deficient (Government of Pakistan, 2011). Stewart (1990) studied the occurrence of endemic goitre in northern Pakistan between 1981 and 1986 and reported a significant difference between northern and southern regions of GB with regards to the prevalence of IDDs. The northern valleys were more I deficient compared to southern valleys and had a higher frequency of IDDs (Stewart, 1990). Historically, a goitre prevalence of approximately 70% has been reported in the population of GB (Khattak et al., 2017).

Section 4

1.15 Importance of the study

Different approaches can be used to tackle I deficiency although each one has recognised shortcomings. It is therefore vital to have a better understanding of I bio-geochemistry to examine how dietary I can be enhanced via the food chain. This may include making the existing soil I more bioavailable or addition of I to soil via crop irrigation water or fertilizers. Although studies have been conducted previously in this area they were limited only to examining the availability of I in drinking water and surveying urinary I concentration in local populations. This study extends the scope of the investigation to soil, water, vegetables, fruits and other edible crops and examines the Se status of the area, including the population of the area.

1.15.1 Hypothesis, Aim and Objectives

Hypothesis

The I and Se status of the population of GB is mainly controlled by geochemical factors.

Aim

To assess the factors controlling I and Se status in the population of GB.

Objectives by chapter

Chapter 3. To undertake a survey investigating (i) the speciation and fractionation of I and Se in soil and water in GB; (ii) transfer of I and Se to food crops; (iii) the regional variation in I and Se status in GB; (iv) the sources of dietary I and Se in GB.

Chapter 4. Using ^{77}Se isotope labelling to study (i) the viability of Se biofortification in cereal crops grown on an alkaline GB soil in a biofortification trial and (ii) the longer term dynamics of Se applications to arable soils in the region.

Chapter 5. A survey of the population in selected areas, including (i) analysis of I and Se in urine samples and (ii) use and composition of (iodised) salt, to assess the I and Se status of people living in GB.

2 MATERIALS AND METHODS

2.1 Introduction

The Mountain Agriculture Research Centre (MARC) Gilgit carried out sampling and all other fieldwork for this study. This involved: (i) undertaking water, soil and plant sampling in different districts of Gilgit-Baltistan (GB); (ii) conducting a field trial on cereal crops with application of isotopically enriched Se (^{77}Se) to wheat (Dec2017-July2018) followed by two further crops (wheat and maize) in consecutive growing seasons; (iii) collection of human biomarkers (urine samples) from local populations; (iv) undertaking a salt consumption survey in the local population. This chapter describes the general methods and approaches, which have been used throughout this study; methods which are specific to individual experiments are described in relevant chapters.

2.2 Sampling

The specific sampling procedures for water, soil, plant, culinary salt and urine are discussed in the relevant sections of forthcoming chapters.

2.3 Water characterisation

2.3.1 Water pH, electric conductivity (EC) and temperature

Water pH, EC and temperature of water samples were measured by a portable pH and EC meter (HANNA HI 98129) at the source. Buffer solutions 4.01 and 7.00 were used to calibrate the pH meter before every batch of measurements.

2.3.2 Measurement of carbon in water samples

Dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and total carbon (TC) in water samples were measured on a Shimadzu TOC-VCPH coupled with an ASI-V unit (Shimadzu UK Ltd) after diluting the water sample (10 ml sample + 10 ml Milli-Q water (18.2 MΩ cm)). A range of concentrations (10 to 50 mg L^{-1}) of potassium hydrogen phthalate ($C_8H_5KO_4$) were used to calibrate the instrument for TC analysis while Na_2CO_3 (10 to 50 mg L^{-1}) was used for DIC calibration. Dissolved organic carbon was determined by difference (TC – DIC).

2.3.3 Measurement of anions in water samples

Approximately 0.5 ml of water was used to determine the concentration of anions (fluoride, chloride, nitrate, phosphate and sulphate) by ion chromatography (Dionex IC-1100). The eluent was prepared by combining solutions of 3 mmol L^{-1} Na_2CO_3 and 1 mmol L^{-1} $NaHCO_3$ in Milli-Q water. Several concentrations, in the range 1 to 20 mg L^{-1}, of calibration standards were prepared with the eluent as background matrix.

2.4 Soil characterisation

2.4.1 Soil pH

Soil pH was measured using a combined glass electrode and pH meter (Hanna, model pH 209). The measurement of soil pH was undertaken on a soil-water suspension with a ratio (w/v) of 1:2.5 – typically suspending 5 g of soil in 12.5 mL of Milli-Q water in a 50 mL centrifuge tube (Rowell, 1994). The soil suspensions were shaken end-over-end for 30 minutes to attain equilibrium and then placed in an upright position in a rack. The normal calibration procedure was carried out using pH buffer 7.00 then 4.01 and again 7.00. After washing the electrode the pH of all the samples was measured by immersing the electrode in the soil suspension just off the bottom of the tube. Five minutes was allowed for the pH reading to stabilize before recording the reading.

2.4.2 Soil carbon concentration

The total carbon concentration (SC) of soil samples was determined using two different CN analysers. Using a Flash EA 1112 (CE Instruments) approximately 20 mg of agate ball-milled soil sample was weighed into a tin capsule and combusted at 900°C with copper oxide using an electrolytic copper column; calibration employed a reference soil standard (B2176; peat soil) with a known carbon content of 15.95%. On a LECO corporation instrument (Model no: 630-300-300, serial no: 4250) a much larger amount of ball-milled soil (500 mg) was combusted in a ceramic boat at high temperature (1350 °C); calibration used ethylene diamine tetra acetic acid (EDTA) standards.

The inorganic carbon in soil was measured using a Shimadzu TOC-VCPH coupled with an SSM-5000A solids module (Shimadzu UK Ltd). Approximately 100 mg of ball-milled soil was acidified with 0.7 ml of 25% phosphoric acid (H_3PO_4) in ceramic boats and heated to 200°C. A chalk-based soil (standard reference B2188) and sodium carbonate (Na_2CO_3), with inorganic carbon concentrations of 2.09% and 11.30% respectively, were used as calibration standards. A range of weights (50, 75 and 100 mg) of Na_2CO_3 and the chalk-based soil were used for calibrating the instrument. Before using the ceramic boats they were acid-washed in 2% nitric acid (HNO_3) overnight and then heated in muffle furnace at 450°C for 8 hours.

2.4.3 Soil texture (mechanical analysis)

Samples for mechanical analysis were prepared by suspending 0.5 g of air-dried sieved (< 2 mm) soil in 30 ml hydrogen peroxide (H_2O_2) in a 50 ml centrifuge tube. The centrifuge tubes were heated in a water bath at 80^{o}C for 2 hr to oxidise the organic matter. Twenty ml of Milli-Q water was added to each tube and, after shaking, the soil suspensions were centrifuged at 3500 rpm for 5 minutes and the supernatant was discarded. The remaining plug of sediment was re-suspended in 25 ml of Milli-Q water, hand-shaken, centrifuged and supernatant again discarded. Then 25 ml of Calgon (35 g of sodium hexametaphosphate, 7 g sodium carbonate in 1 L Milli-Q water) was added to each tube and the suspension was agitated in an ultrasonic bath for 30 minutes before analysis by laser granulometry. Clay (<4 μm), silt (≥4 μm and ≤63 μm) and sand (>63 μm) were determined. A grain size < 4 μm has been used to define clay particles (Kerry et al., 2009).

2.4.4 Soil metal oxides (Fe, Al, Mn hydrous oxides)

Hydrous oxides of Fe, Mn and Al were solubilised in a citrate-bicarbonate-dithionite (CBD) solution consisting of 0.3 M Na-citrate, 1 M sodium bicarbonate ($NaHCO_3$) and 0.07 M Na-dithionite. Approximately 0.2 g of ball-milled soil was shaken in 20 ml of CBD solution, in a 50 ml centrifuge tube, in a water bath at 20^{o}C for 24 hours. The centrifuge tubes were then centrifuged at 2400 rpm for 15 minutes and the supernatant filtered to <0.22 μm using Millipore polyethersulfone (PES) syringe filters. A subsample of supernatant was acidified by adding 0.4 ml of 50% HNO_3 to 9.6 ml of sample for Fe (iron), Mn (manganese) and Al (aluminium) oxide analysis by ICP-MS.

2.4.5 Acid digestion of soil samples for elemental analysis

Ball-milled soil samples (c. 0.2 g) were initially digested with 2 ml of 70% HNO_3 and 1 ml of 60% perchloric acid ($HCLO_4$) in PFA vessels using a teflon-coated graphite block digester (Model A3, Analysco Ltd) controlled by a Eurotherm unit. The temperature program started at 80 °C for 8 hr followed by 100°C for 2 hr. The resultant dried material was then digested with 2.5 ml of 70% hydrofluoric acid (HF) at 120°C for 1 hr, followed by 140°C for 3 hr and finally 160°C for 4 hr. The dry residue was then suspended in 2.5 ml HNO_3 and 2.5 ml Milli-Q water at 50°C for 1 hr. The resulting soil digest solutions were diluted to 50 ml with Milli-Q water and stored in 7 ml plastic vials. A 1-in-10 dilution with Milli-Q water was carried out by aliquoting 1 ml of solution and 9 ml of Milli-Q water into ICP sample tubes for total selenium (Se_T) and other elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS).

2.4.6 Extraction of total soil iodine and organically bound selenium

Soil samples (c. 1 g; ball-milled) were suspended in 10 ml of 10% tetra methyl ammonium hydroxide (TMAH) in 50 ml centrifuge tubes, hand-shaken and heated in an oven (Scientific Laboratory Supplies Ltd) for 3 hr at 70°C with lids loosened; the tubes were shaken once, after 1.5 hr. The suspensions were then centrifuged at 3500 rpm for 30 min and the supernatants syringe-filtered to <0.22 µm (Millipore). The filtered samples were transferred to universal tubes and were further diluted to a final matrix of 1% TMAH prior to analysis for total iodine (I_T) and organically bound Se (Se_{TMAH}) by ICP-MS.

2.4.7 Fractionation of soil iodine and selenium

A three stage sequential extraction of soil samples was undertaken with potassium nitrate (0.01 M KNO_3) followed by potassium di hydrogen phosphate (0.016 M KH_2PO_4) and finally with 10% TMAH to determine 'Soluble', 'Adsorbed' and 'Organic' fractions of I and Se respectively.

2.4.7.1 'Soluble' Se and I: extraction with KNO_3

Approximately 4 g of air-dried sieved (< 2 mm) soil was equilibrated in a 50 ml centrifuge tube with 20 ml of 0.01 M KNO_3. The centrifuge tubes were shaken end-over-end for 2 hr then centrifuged at 3500 rpm for 30 minutes and the supernatant syringe-filtered to <0.22 µm (Millipore). Prior to measurement of I concentration (I_{sol}), 4.8 ml of soil extract was mixed with 0.2 ml of 25% TMAH to give a final matrix of 1% TMAH. For Se determination (Se_{sol}) the samples were preserved in 2% HNO_3 by

addition of 0.2 ml of 50% HNO_3 to 4.8 ml of soil extract. Speciation analysis of I (iodide; I^- and iodate; IO_3^-) and Se (selenite; Se^{IV} and selenate; Se^{VI}) was undertaken on an 'unpreserved' 1.5 ml aliquot of KNO_3 extracts using HPLC-coupled ICP-MS.

2.4.7.2 'Adsorbed' I and Se: extraction with KH_2PO_4

After completion of the KNO_3 extraction, the tubes with soil plugs were weighed and re-suspended in 20 ml 0.016 M KH_2PO_4. The soil suspensions were shaken end-over-end for 1 hr and centrifuged at 3500 rpm for 30 minutes. Supernatants were syringe-filtered (<0.22 μm) and solutions prepared for analysis of I_{ads} and Se_{ads} by ICP-MS following the procedure described for the KNO_3 extracts (Section 2.4.7.1). Speciation analysis of I and Se (IO_3^-, I^-, Se^{VI}, Se^{IV}) was undertaken on an 'unpreserved' 1.5 ml aliquot of KH_2PO_4 extracts using HPLC-coupled ICP-MS. Carry-over of Se and I from the previous KNO_3 extraction was compensated for using the weights of the tubes with wet soil plugs measured prior to adding KH_2PO_4.

2.4.7.3 'Organic' I and Se: extraction with TMAH

Following KH_2PO_4 extraction, soil samples were weighed again and re-suspended in 10 ml of 10% TMAH solution. A vortex mixer (~ 1 min) was used to ensure that the soil plug was fully re-suspended in the TMAH extractant. The samples were heated in an oven (Scientific Laboratory Supplies Ltd) at 70°C for 3 hr, with lids slightly loosened, and shaken once, after 1.5 hr. The suspensions were then centrifuged at 3500 rpm for 30 min and the supernatant solutions transferred to universal tubes

without filtration. A 1-in-10 dilution of the supernatants in Milli-Q water was carried out prior to analysis of total I_{TMAH} and Se_{TMAH} concentrations by ICP-MS.

2.5 Analysis of plant samples

2.5.1 Microwave assisted acid digestion of plant samples

Generally, finely ground plant samples (c. 0.2 g) were microwave-digested in 6 ml HNO_3 (68% Primar Plus grade). For grain samples, 0.3 g was digested in 3 ml HNO_3 (70%), 3 ml Milli-Q water and 2 ml H_2O_2 (30%). The microwave vessels were held in a rotor and samples were digested under pressure at high temperature; the temperature profile was 10 min ramp up to 140^{o}C, 20 min holding time at 140^{o}C and 15 minutes cooling time at 55^{o}C. The final volume of digested sample was made to 20 ml (plants) and 15 ml (grain). The solutions were stored in universal tubes pending elemental analysis. A 1:10 dilution with Milli-Q water was done prior to multi-elemental analysis (including Se) via ICP-MS.

2.5.2 Microwave TMAH extraction of plant samples

Microwave-assisted TMAH extraction of plant samples was initiated by weighing 0.2 g of samples and adding 5 ml of 5% TMAH into a microwave vessel. The tubes with lids tightened were placed in microwave rotor. The samples were extracted for 40 min using a microwave programme including a 5 min ramp time to 110^{o}C, 20 min holding time at 110^{o}C and 15 min at 50^{o}C). The extracts were diluted to 25 ml with Milli-Q water, centrifuged and syringe filtered to <0.22 µm. The filtered solutions were stored in universal tubes pending I analysis.

2.6 ICP-MS analysis

Two different models of quadrupole-based ICP-MS, an iCAP Q and an iCAP TQ (Thermo Fisher Scientific; Bremen, Germany) were used for all elemental analysis. Sample introduction was via auto-samplers: a Cetac (Model ASX-520) with the icapQ and an ESI 4DX auto-sampler with the iCAP TQ.

2.6.1 Analysis of selenium by ICP-MS

Analysis of Se in all samples (soils, plants and water) was normally undertaken in an acidic matrix (2% HNO_3) with the ICP-MS (iCAP Q) operating in 'hydrogen cell' mode. The use of H_2 as a cell gas fulfils the role of a collision cell in reducing poly-atomic species but does not impede Se^+ ions as much as He. However, because a more advanced triple-quadrupole instrument became available, analysis of ^{77}Se in field trial samples was undertaken using the iCAP TQ in 'oxygen cell mode'. This enables mass-shifting of the ^{77}Se and ^{80}Se to m/z values of 93 and 96 respectively (+16) and thereby removes the Se signal from interferences. Internal standards of 5 µg L^{-1} Rhodium (Rh) and Rhenium (Re) in 4% methanol and 2% HNO_3 were used.

2.6.2 Analysis of iodine and TMAH-extractable selenium

For I and Se analysis in 1% TMAH matrices, the ICP-MS instruments were run in standard mode (no cell gas) for all samples (water, soil, plant and salt). Rhenium (5.00 µg L^{-1}) prepared in 1% TMAH was used as an internal standard.

2.7 Speciation analysis

Selenium and I speciation analyses of soil extracts and water samples were undertaken on two different HPLC units (Dionex ICS-3000 and ISC-5000) coupled to an ICP-MS during the course of this project. The chromatography eluent consisted of 4.00 g L^{-1} NH_4NO_3, 20 ml L^{-1} methanol, 0.00325 g L^{-1} NH_4-EDTA and 12.1 g L^{-1} Tris buffer. The stationary phase used was a Hamilton PRX-100 anion exchange column (100 x 4.1 mm; 5 µm particle size); the eluent flow rate was 1.4 mL min^{-1}. Working standards of I and Se reduced species (I^- and Se^{IV}) and oxidised species (IO_3^- and Se^{VI}) were prepared by diluting their respective stock solutions (1000 mg L^{-1}) with Milli-Q water. Standards were run between the samples at regular intervals to enable correction for instrumental drift. The peaks obtained for each sample were manually integrated using chromeleon software and then peak areas were converted to concentration in excel 2016 by considering the peak area of standards (Figure 2.1 and Figure 2.2) as reference.

2.8 Statistical analysis

Basic statistical calculations including mean, median, standard error and standard deviation were performed in Microsoft Excel 2016. Minitab (versions 17.2.1 and 18.1) was used for calculating correlation, regression, analysis of variance (ANOVA) and Tukey's HSD test. Graphpad prism (version 7.03) and excel 2016 were used to generate graphs.

2.9 Data quality assurance

All samples were measured with replication for I, Se and all other analyses; generally, replicates were within ±1% for individual samples. Operational blanks were run within each batch of analysis to correct for any contamination associated with the sample preparation and analytical procedures. The analysis of operational blanks normally indicated there was no significant contamination from the reagents used in sample processing and solution preparation. Calibration blanks and certified reference materials (CRM) of soil (Montana soil – NIST 2711a) and plants (wheat – NIST 1567b, rice flour – NIST 1568b and tomato leaves – NIST 1573a) were used for QA purposes. The average recoveries of Se in the CRMs were within ±10 % of the reported values. Calibration solutions of I and Se were always run prior to and during sample analysis by ICP-MS to check for any unforeseen effects on sample analysis which were not corrected by the internal standard correction for drift in instrument sensitivity.

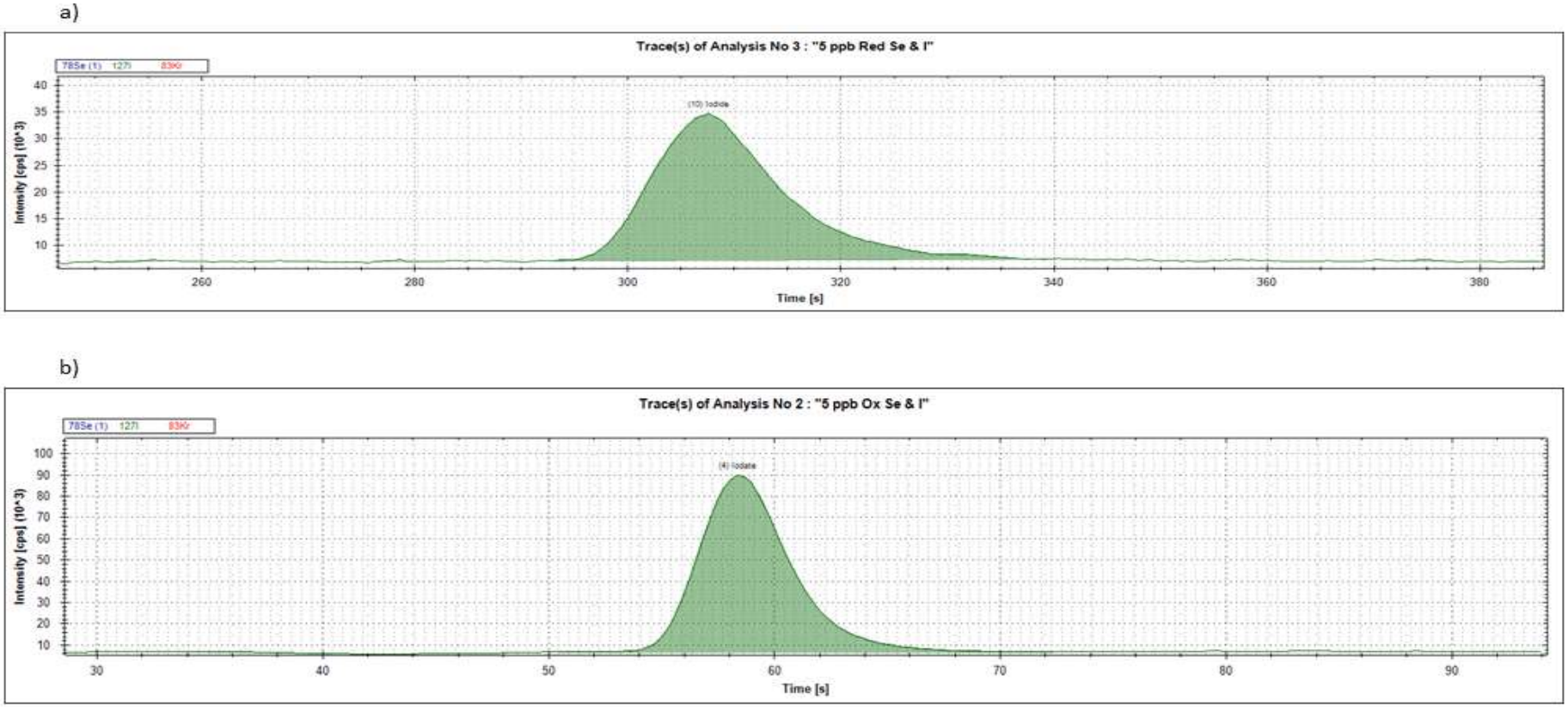


Figure 2.1. Chromatographs of reduced and oxidised standards of iodine - a) iodide (I^-) and b) iodate (IO_3^-)

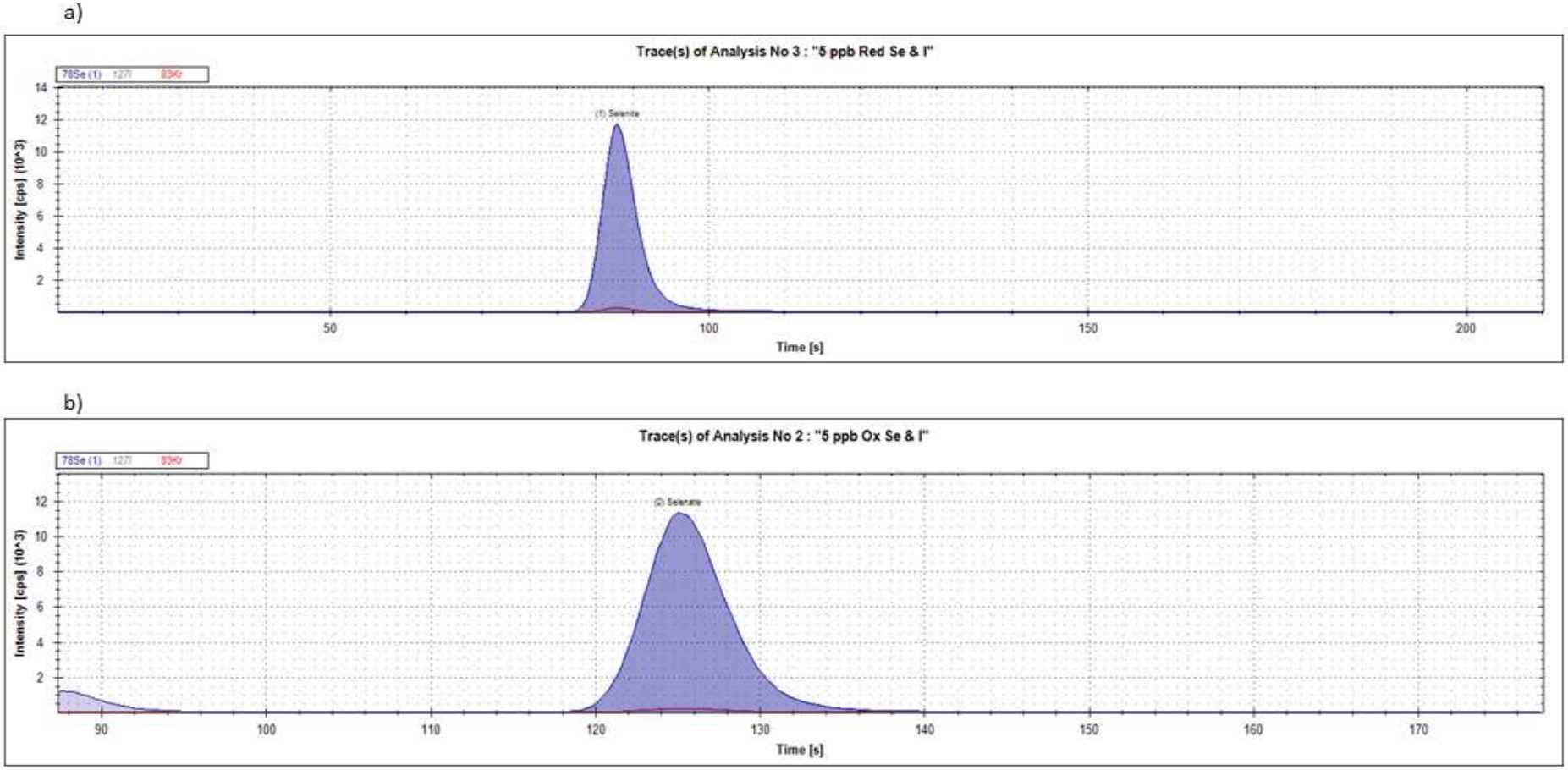


Figure 2.2. Chromatographs of reduced and oxidised standards of selenium - a) selenite (Se^{IV}) and b) selenate (Se^{VI})

3 IODINE AND SELENIUM IN GILGIT-BALTISTAN

3.1 Introduction

Iodine (I) has a wide, but uneven, distribution in the environment (Wu et al., 2013). Rocks are the largest reservoirs of I but, unlike most other elements, weathering of rocks and sediments is not a major source of I for the soil-plant system (Johnson, 2003). Only a small amount of the soil I available to plants is derived directly from rock weathering (Fuge and Johnson, 2015). In contrast, oceans are major reservoirs of I (Fuge, 2013; Fuge and Johnson, 2015) and volatilisation from ocean water and movement through the atmosphere plays an essential role in I cycling through the environment and the biosphere (Johnson, 2003). It is recognised that I concentrations are higher in coastal areas compared to inland and mountainous regions located away from coasts (Fuge and Johnson, 2015). Apart from inputs, the concentration of I in soil is affected by several factors affecting retention capacity of the soil, including parent material, topography and soil characteristics such as organic matter concentration and pH (Johnson, 2003; Fuge and Johnson, 2015). An I rich soil does not necessarily produce I rich plants because available soil I is not directly related to total soil I content (Fuge and Johnson, 1986). Fresh waters generally have low I concentrations (Johnson, 2003) unless rivers run through I-rich sedimentary rocks (Fuge, 1989). Iodine input from atmosphere as dry or wet precipitation also contributes to soil and plant I in many cases (Fuge and Johnson, 1986; Bowley, 2013; Johnson, 2003).

Selenium (Se) is among the most widely distributed elements in the earth crust and mainly associated with sulphide minerals (Shamberger, 1981; Johnson et al., 2010; Fordyce, 2013). Weathering of rocks is one of the primary sources of Se in soil (Shamberger, 1981). The majority of rocks contain low concentrations of Se therefore Se-deficient environments are more common than seleniferous ones (Fordyce, 2013). Selenium concentration in most soils ranges from 0.01 – 2 mg kg^{-1} while some seleniferous soils have Se concentration up to 1200 mg kg^{-1} (Fordyce et al., 2010; Winkel et al., 2012; Fordyce, 2013). Surface waters generally have smaller concentrations of Se (0.06-0.12 $\mu g\ L^{-1}$) while in groundwater it reaches up to 6000 $\mu g\ L^{-1}$ in some areas (Fordyce, 2013; Alexander, 2015). Selenium is not considered essential for higher plants but it is taken up by plants via sulphate (selenate) and phosphate (selenite) transporters (Alexander, 2015; White, 2016). Different plants vary in their ability to accumulate Se (Alexander, 2015; Barillas et al., 2011).

Both I and Se are important micronutrients for human and animal health and their deficiency or toxicity can result in serious health complications. Deficiencies can be resolved by supplying sufficient I and Se in the diets of affected populations (Hetzel, 1983; Fordyce, 2013). As mentioned in Chapter 1, I deficiency is generally widespread in remote mountainous areas (Kelly and Snedden, 1958). Thus Gilgit-Baltistan (GB), being located in the Himalayan region, has a history of I deficiency disorders (IDD) (Stewart, 1990). Furthermore, co-existing deficiency of I and Se can results in some extreme forms of IDD (e.g. cretinism) (Vanderpas et al., 1990) and incidence of cretinism has been historically reported in GB (Stewart, 1990). The population of GB

largely consume locally grown agricultural produce and there is limited data available on the status of environmental I and Se in GB.

Aims and objectives

This chapter is an investigation of the status of I and Se in soil, plants and water samples collected from different districts of GB. The overall aim of the study was to assess the factors controlling I and Se concentrations in soils and water in GB, the availability of I and Se to plants and ultimately the status of the local population. The study objectives were to investigate:

(i) the speciation and fractionation of I and Se in soil and water in GB and transfer to food crops;

(ii) regional variation in I and Se status in GB;

(iii) the principal sources of dietary I and Se in GB;

3.2 Materials and Methods

3.2.1 Sites selection and soil sampling

Figure 3.1 presents the sampling locations of all the samples. At the time of sampling GB had seven districts, five of which were chosen for sampling based on their accessibility and the availability of fertile agriculture land. The sampling districts included Gilgit, Diamer, Hunza-N, Astor and Skardu. Overall, twenty-six villages were surveyed with five villages from each district apart from Hunza-N where six villages were sampled. The aim of sampling from the range of locations chosen was to

investigate regional variation in I and Se status over a range of soil types and crop plants.

Soil samples were collected from arable land only. In every village, one agricultural field was chosen based on accessibility due to the remoteness of the area. A central 10 m x 10 m square area in the middle of the field was selected as a sample support to recover a representative soil sample. Topsoil (0-20 cm) was collected with an auger from the four corners and middle of the sample support square. Soil sampled from these 5 points was mixed to obtain a composite sample for that field.

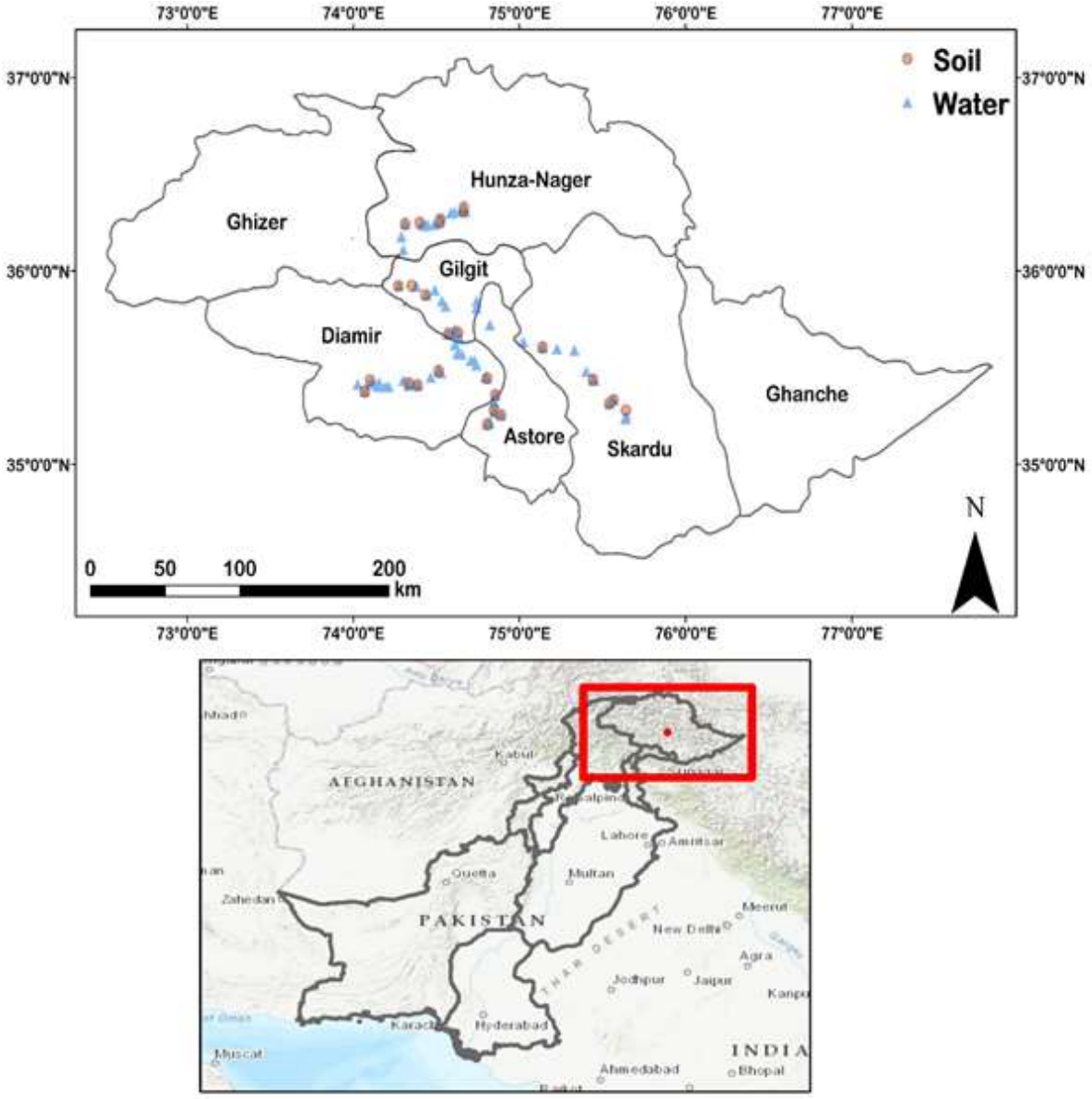


Figure 3.1. Soil and water sampling locations across Gilgit-Baltistan. Brown circle represent soil and blue triangle represent water

Samples were air dried, sieved to <2 mm and thoroughly mixed; 1 kg of the sieved soil was shipped to the University of Nottingham, UK. After arrival at the University of Nottingham, approximately 10 g of <2 mm sieved soil was finely ground using an agate ball mill (Retsch; Model PM400) at 300 rpm for 10 minutes to be used for acid

digestion, organic and inorganic carbon (SOC and SIC) and metal (Fe, Al, Mn) oxides (Chapter 2; Sections 2.4.2, 2.4.4 and 2.4.5).

Other soil characteristics including soil pH and texture were determined on <2 mm sieved soil (Chapter 2; Sections 2.4.1 and 2.4.3). Soils were extracted with tetra methyl ammonium hydroxide (TMAH) for extractable Se and total I (Chapter 2; Section 2.4.6). Soils were sequentially extracted with potassium nitrate (0.01 M KNO_3), potassium di hydrogen phosphate (0.016 M KH_2PO_4) and 10% TMAH for 'Soluble', 'Adsorbed' and 'Organic' Se and I (Chapter 2; Section 2.4.7). The KNO_3 and KH_2PO_4 extracts were also analysed for I and Se speciation by HPLC-ICP-MS (Chapter 2; Section 2.7). The acid digests and TMAH extracts of soil samples were used for elemental analysis by ICP-MS (Chapter 2; Section 2.6).

3.2.2 Water sampling

Water was sampled in the villages used for soil sampling and also between soil sampling locations (Figure 3.1). In total, 107 water samples were collected from a mix of rivers, streams, lakes and groundwaters (springs and wells), of which 37 were drinking water sources, 25 were used for irrigation and the remaining 45 sources were used both for drinking water and irrigation. Water samples were filtered at the point of sampling, using syringe filters (<0.22 µm), into a universal tube and kept in the dark during transportation to the Mountain Agriculture Research Centre (MARC) laboratory in Gilgit where they were stored at 4^{o}C pending shipping to the University of Nottingham for analysis by ICP-MS (elemental composition) and ion chromatography (anions) (Chapter 2; Section 2.3).

3.2.3 Plant sampling and sample preparation

Plants were sampled from all 26 villages where the soil sampling was undertaken. A wide range of locally grown edible plant samples were collected from each village. Plant samples included cereal crops, vegetables, fruits, tubers and nuts. In total 281 samples were collected representing 34 different species. Plants were air dried in the MARC laboratory and packed in paper bags for shipping to the UK. Upon arrival at the University of Nottingham, the samples were further oven-dried at 40^{o}C. The fully dried samples were then milled in an ultra-centrifugal mill to obtain finely ground powder for analysis by ICP-MS following microwave-assisted acid digestion and TMAH extraction (Chapter 2; Section 2.5).

3.3 Results and discussion

3.3.1 Basic characteristics and multi-element analysis of water samples

The basic characteristics of all water samples along with concentrations of major and minor elements are provided in Appendix 1. The median pH of all samples was 7.93 and ranged from 7.0 to 9.2. Water electrical conductivity (EC) was less than 1 dS m^{-1} in all samples with mean and median values of 0.21 and 0.173 dS m^{-1} respectively. The usual range of EC for non-saline irrigation water is 0 – 3 dS m^{-1} (Ayers and Westcot, 1986; Jeong et al., 2016). Dissolved inorganic carbon (DIC) accounted for a large proportion (>85%) of total carbon compared to dissolved organic carbon (DOC). Median DIC and DOC concentrations were 10.5 and 1.38 mg L^{-1} respectively in all samples. Groundwater had higher concentrations of both DIC and DOC compared to surface water. The sodium adsorption ratio (SAR) in all samples of irrigation water

was <1 which means there is unlikely to be a problem with soil salinity. Water with values of SAR <3 are suitable for a wide range of crops and unlikely to cause any soil health problems (Bortolini et al., 2018). The hardness of water, calculated as the apparent concentration of $CaCO_3$, ranged from < 60 mg L^{-1} to > 180 mg L^{-1} in both surface and groundwater (categories from 'soft' to 'very hard'). Concentrations of $CaCO_3$ less than of 60 mg L^{-1}, 60 – 120 mg L^{-1}, 120 – 180 mg L^{-1} and more than 180 mg L^{-1} are categorised as soft, moderately hard, hard and very hard respectively (McGowan, 2000; WHO, 2011b).

For the majority of samples Ca was the dominant cation. A Piper diagram (Fig. 3.2) indicated that a range of water types were available including 'calcium chloride', 'magnesium bicarbonate' and mixed types across the range of surface and groundwater sources.

Concentrations of certain trace elements, including arsenic (As) and uranium (U), were in excess of the widely accepted drinking standards of 10 μg L^{-1} for arsenic and 30 μg L^{-1} for uranium (USEPA, 2001; WHO, 2011a; WHO, 2017), in some water sources (Appendix 1).

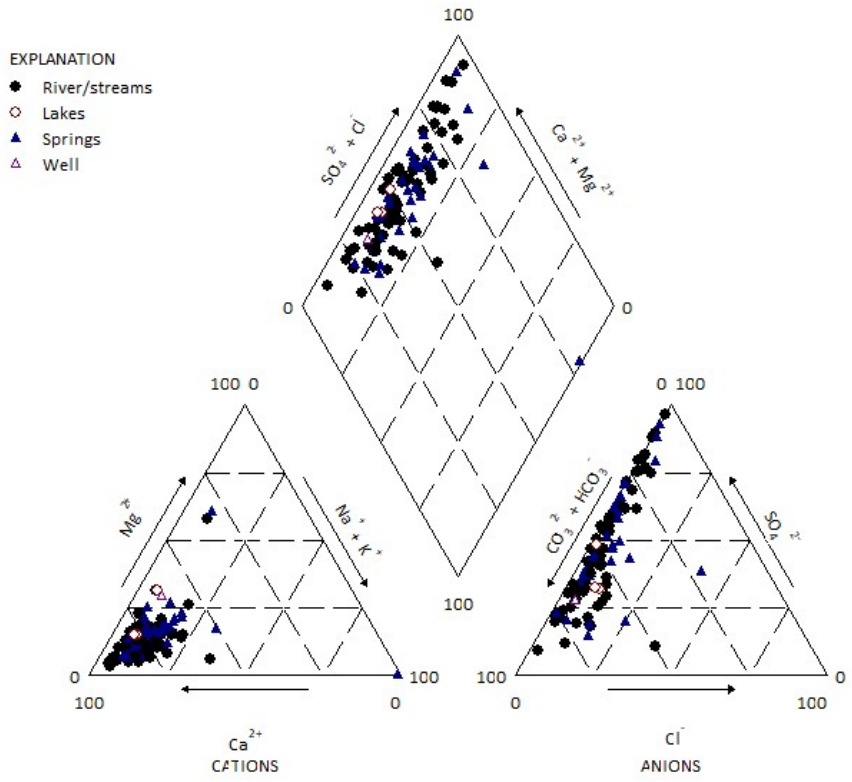


Figure 3.2. Piper diagram illustrating water types in surface sources (rivers/streams and lakes) and groundwater sources (springs and wells)

3.3.2 Iodine in water (I_w)

Iodine concentration in all water sources (surface and ground; n = 107) used for irrigation, drinking or both, ranged from 0 – 10.2 μg L^{-1} with an overall median of 0.227 μg L^{-1}. Approximately 85% (91 out of 107) of the sources had I_w < 1 μg L^{-1} (Appendix 2). There was no significant difference between I_w in drinking water and irrigation water ($p > 0.05$). However, groundwater typically had greater I_w values than surface waters (ANOVA, $p < 0.05$; Figure 3.3).

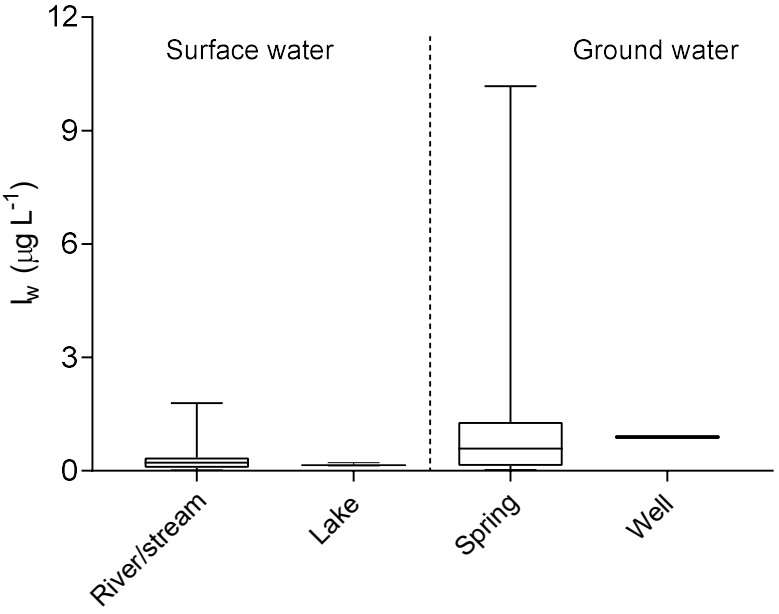


Figure 3.3. Box and whisker plot of I_w in surface water and groundwater

Figure 3.4 presents the spatial distribution of I from all sources. Values of I_w in both surface water and groundwater varied between districts (Table 3.1 and Figure 3.4). The Diamer district had the highest values of I_w in both surface water and groundwater while the Astor district had the lowest I concentrations (ANOVA, $p < 0.05$) (Figure 3.5). The other three districts (Gilgit, Hunza-N and Skardu) did not differ significantly from either Diamer or Astor. The range seen may reflect the diverse geology of the area. Water draining through sedimentary rocks generally has higher I concentrations compared to water in regions of igneous rocks (Moran et al., 2002; Johnson, 2003; Fuge and Johnson, 2015).

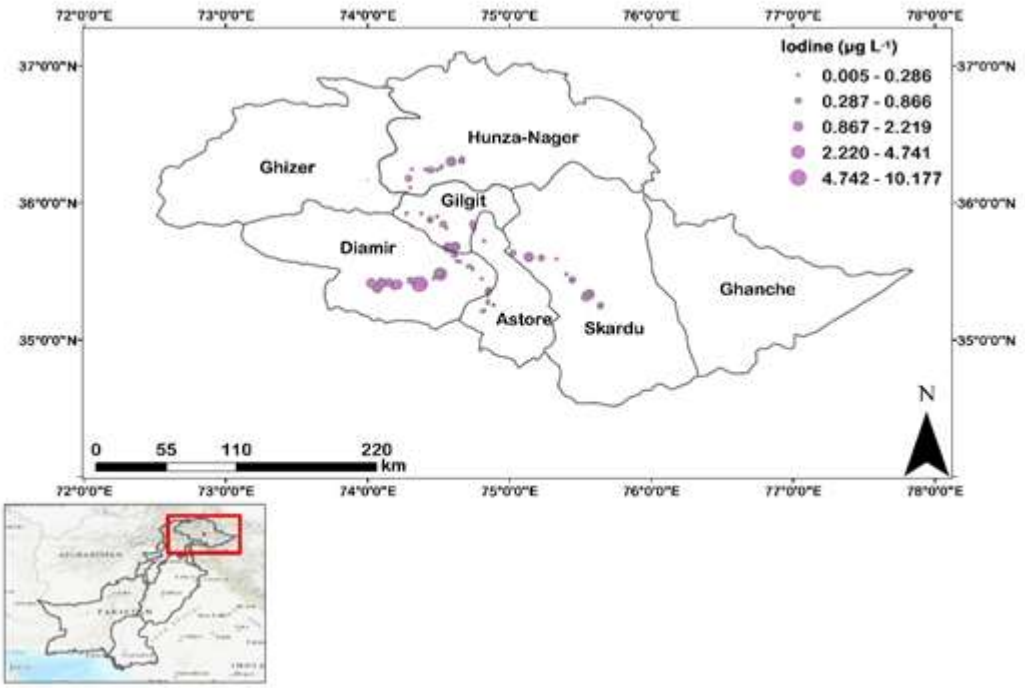


Figure 3.4. Spatial distribution of I_w (ground and surface water) concentration in different districts of Gilgit-Baltistan

Table 3.1. Iodine concentrations (I_w) in surface water and groundwater sources

Sample source and location	n	Total iodine concentration (µg L^{-1})		
		Mean	Median	Range
Surface water				
Stream and river				
Gilgit	19	0.241	0.230	0 - 0.463
Diamer	14	0.452	0.300	0.012 - 1.79
Hunza-N	14	0.141	0.113	0 - 0.400
Astor	12	0.099	0.124	0 - 0.262
Skardu	10	0.422	0.296	0.018 - 1.19
Lake				
Hunza-N	1	0.218	0.218	-
Skardu	2	0.123	0.143	0.094 - 0.152
Groundwater				
Spring				
Gilgit	5	1.17	1.14	0.192 - 2.18
Diamer	8	2.64	1.17	0.430 - 10.2
Hunza-N	5	0.481	0.167	0.027 - 1.72
Astor	8	0.275	0.121	0.017 - 1.27
Skardu	8	0.800	0.591	0.025 - 2.22
Well				
Skardu	1	0.893	0.893	-

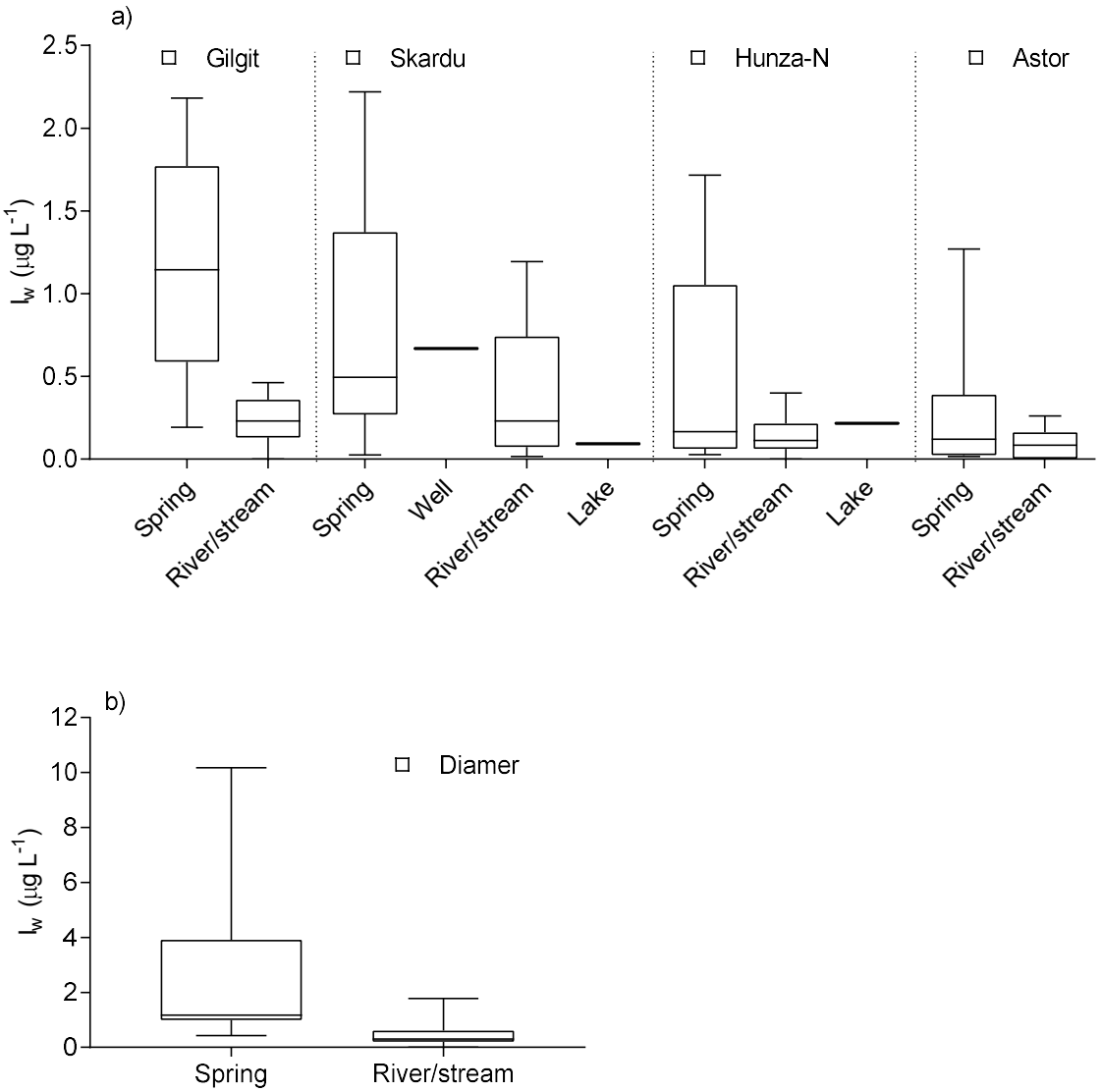


Figure 3.5. Box plots of iodine concentration in surface water (rivers, streams and lakes) and groundwater (springs and wells) sources from different districts: (a) Gilgit, Skardu, Hunza-N and Astor; (b) Diamer. Note the different scales in graphs a and b.

3.3.2.1 Surface water

Concentration of I in surface water (n = 72) ranged from 0.01 – 1.79 μg L^{-1} with a median value of 0.207 μg L^{-1}. These values are at the lower end of the global surface water concentration range (0.01 – 212 μg L^{-1}) reported by Fuge and Johnson (1986) and Fuge and Johnson (2015). Values of I_w were also less than I concentration in water from other regions with similar mountainous topography, such as Kabul and Nangarhar in Afghanistan at 5 - 23 μg L^{-1} (Watts and Mitchell, 2009) or San Juan province in Argentina at 16 - 95 μg L^{-1} (Watts et al., 2010). However, the results for GB are comparable with I concentrations (< 0.1 μg L^{-1}) reported by Day and Powell-Jackson (1972) in the Himalayan region of Nepal. Multiple factors, such as distance from the sea, rainfall and underlying geology, affect I concentration in fresh water (Fuge and Johnson, 2015). The nearest sea to the study area is at a distance of about 1400 km (Figure 3.6), which reduces the chances of any marine influence in water I recharge. Furthermore, the area is located in a rain shadow area and hence receives little rainfall with an annual average of 254 mm per year. The majority of the rocks are igneous or metamorphic in nature and highly variable (Appendix 3) (Malik and Azam, 2009). The low precipitation rates and presence of igneous/metamorphic rocks are contributing factors to the low I concentrations in surface waters of the study area.

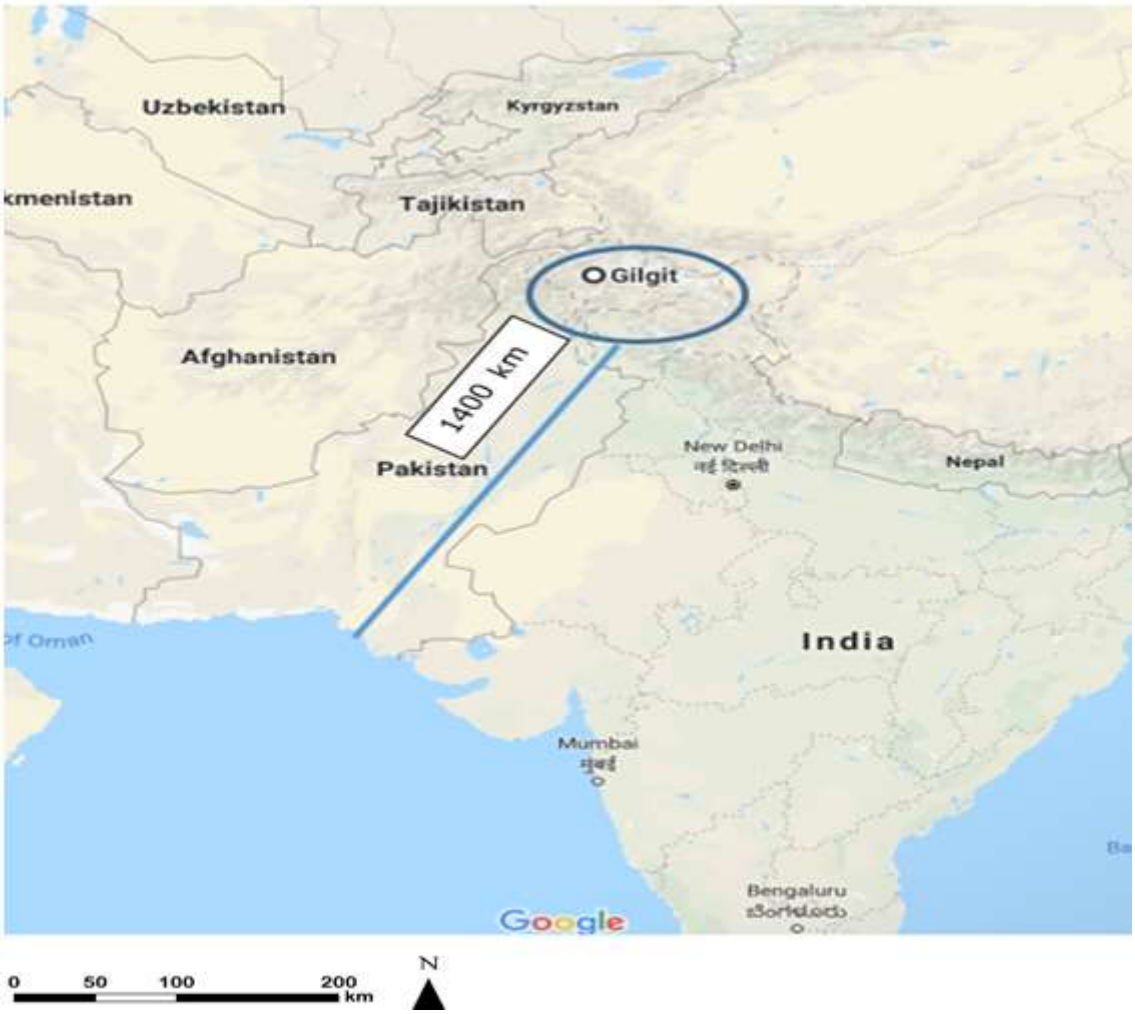


Figure 3.6. Distance between study area (Gilgit-Baltistan) and the nearest marine water (Arabian Sea). Image obtained from Google Earth.

3.3.2.2 Groundwater

In groundwater samples the median value of I_w was 0.643 µg L^{-1}, ranging from 0.017 – 10.2 µg L^{-1}. Iodine concentrations were higher than in surface water but, despite this, groundwater had lower I concentrations than in other parts of the country. Zia et al. (2014) reported an average concentration of 8.5 µg L^{-1} I in groundwater from

other provinces of Pakistan. The relatively higher concentration in groundwater could be due to both leaching from sandy sediments and evapo-concentration in the arid and semi-arid environment of the area. Iodine concentration increased with EC ($r = 0.473$, $p < 0.05$) (Figure 3.7), which may suggest that evapo-concentration has influenced the I concentrations in groundwater. Li et al. (2013) also reported a relationship between EC and evapo-concentration of I in groundwater in China.

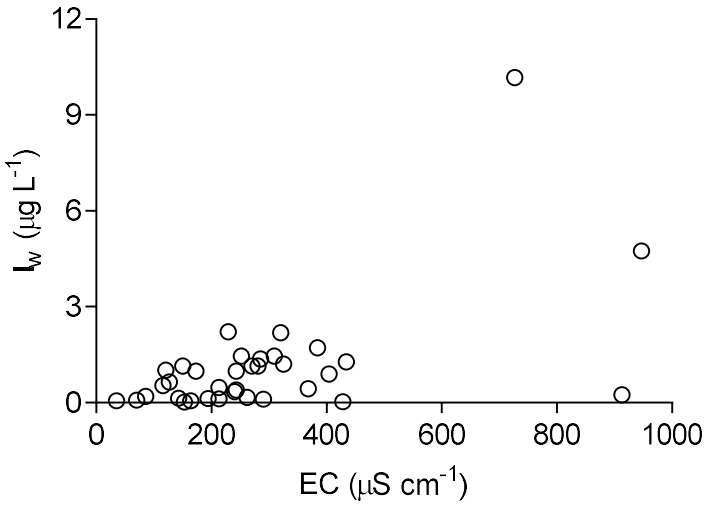


Figure 3.7. Relationship between EC and iodine concentration in ground water

3.3.2.3 Speciation of iodine in water

Iodine was present both in inorganic and organic forms in almost all surface and groundwater samples. The ratio of inorganic to organic species varied between samples and did not follow any obvious trend with location. However, inorganic species typically accounted for a larger percentage (median = 70.5%) of total I in the majority of samples. This is comparable with data reported by Karim (2018) who measured I speciation in irrigation waters from Sulaimani province of Iraqi Kurdistan and found a higher proportion of inorganic species. A correlation ($r = 0.414$, $p < 0.001$)

between DIC and inorganic I was observed which may reflect local geology. Dissolved inorganic carbon accounted for $>$ 85% of total carbon and showed a positive correlation with inorganic I (Figure 3.8). The smaller proportion of organic I (median = 29.5%) may reflect the low concentration of DOC; organic I in surface water and groundwater had a weak positive correlation with DOC ($r = 0.478$, $p < 0.001$) (Figure 3.9). The ratio of DOC to organic I (DOC/I_{org}) was variable across samples and did not show any significant trend. There was no statistically significant difference in DOC/I_{org} ratio between surface water and groundwater sources ($p > 0.05$).

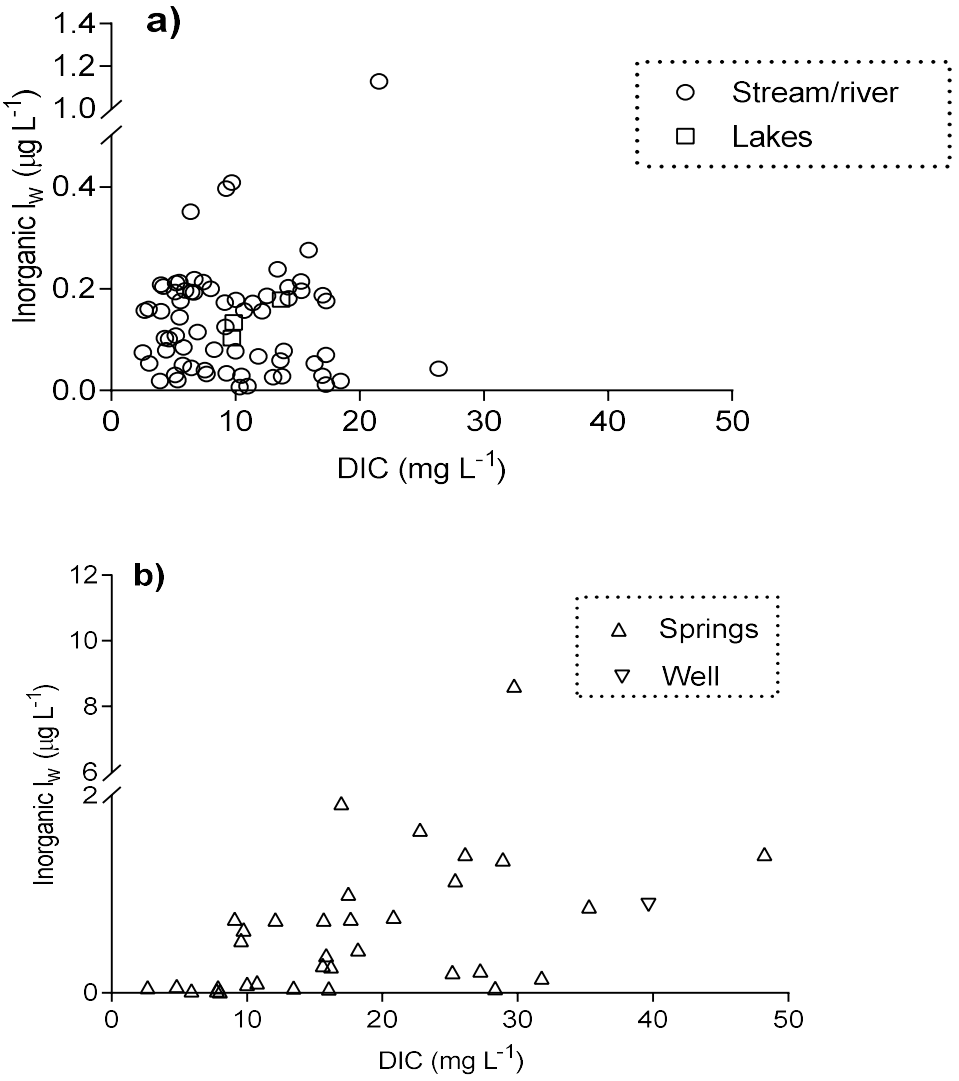


Figure 3.8. Relationship between DIC and inorganic iodine concentration in (a) surface water and (b) groundwater

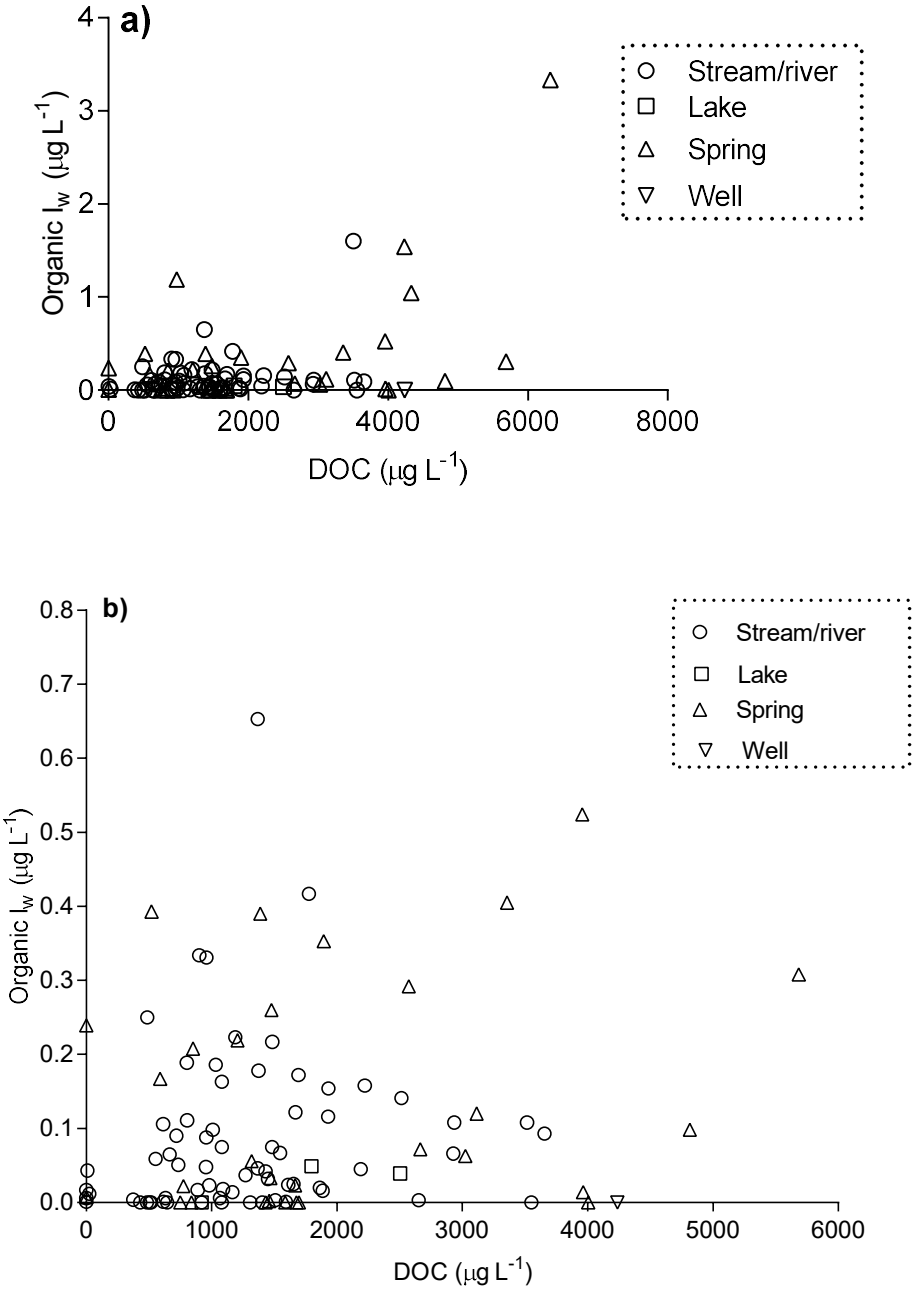


Figure 3.9. Relationship between DOC and organic iodine concentration in surface water (streams, rivers, lakes) and groundwater (springs, wells). Note the different scale in graphs a and b.

Inorganic I was predominantly present as iodate (IO_3^-), possibly reflecting the alkaline conditions as nearly all samples had pH values above 7. Rucklidge et al. (1994), cited

in Moran et al. (2002), suggested that IO_3^- is typically the predominant inorganic I species under alkaline conditions in water and soil. Our data is comparable with the findings reported by Takaku et al. (1995) and Karim (2018) that inorganic I is largely present as IO_3^- in natural water. However, iodide (I^-) dominated in a subset of samples (surface water and groundwater) potentially driven by its presence in this form in rocks and soil of the watershed. Smith and Butler (1979) reported that high concentrations of I^- in Yarra river in Australia were probably because of the dominant prescence of I^- in soils and rocks in the river Yarra drainage basin and assumed that no conversion of inorganic I species occurred. However, an alternative reason for I^- enrichment in water is the reduction of IO_3^- to I^- due to microbial activity under reducing conditions (Li et al., 2017a).

3.3.3 Soil characteristics

Details of basic soil characteristics including soil pH, texture, concentrations of inorganic and organic carbon, and metal oxides are given in Table 3.2 and Figure 3.10 for all sites. Soils fell in the pH range (6.70 to 8.33) (neutral to moderately alkaline). There was significant variation in pH across sampling districts ($P < 0.05$): the district Hunza-N had the highest pH value (8.07) while Astor had the lowest pH at 6.99. Particle size analysis demonstrated that soils in all districts were largely sandy loams apart from one site each in Hunza-N (N13) and Skardu (S24) where the soils were silty loams (sand = 36.7% , silt = 51.3%, clay = 11.9%) and medium loams (sand = 39.6 , silt = 51.3 and clay = 9.10) respectively (Table 3.2).

All the sampling districts had average soil inorganic carbon (SIC) contents of < 1%. Districts Astor and Hunza-N accounted for the lowest and highest mean SIC contents of 0.001% and 0.726% respectively. Some individual sites in Gilgit, Diamer and Astor did not have measureable SIC (Table 3.2) reflecting the highly diverse geology of the parent material. Soil organic carbon (SOC) across all sites was relatively low and ranged from 0.410 to 2.77%; Hunza-N had the highest SOC content of 2.34%. The other four districts, i.e. Gilgit, Diamer, Astor and Skardu, had mean values of 1.20%, 1.14%, 1.57% and 1.31% SOC respectively. The sites with relatively higher SOC had more numerous perennial fruit orchards possibly reflecting inputs of plants residues and lack of ploughing which would contribute towards higher organic carbon.

Table 3.2. Key characteristics of soil samples from all sampling districts

District	Village	Sample code	pH	SIC	SOC	Sand	Silt	Clay	Fe-ox	Mn-ox	Al-ox	Comb-ox
				%					(g/kg)			
Gilgit	Juglote-MARC	G01	7.19	0.000	0.983	79.1	18.7	2.25	3.33	0.119	0.322	3.77
	Basin	G02	7.77	0.008	1.52	74.2	22.7	3.12	3.29	0.097	0.349	3.73
	Daynor	G03	8.06	0.525	1.48	55.4	39.8	4.87	2.94	0.162	0.250	3.35
	Minawar	G04	7.22	0.006	0.661	54.3	42.8	2.89	1.92	0.055	0.250	2.23
	Bargin	G05	7.43	0.001	1.37	67.5	29.8	2.68	4.08	0.078	0.268	4.42
Diamer	Gorabad	D06	7.90	0.040	0.976	81.0	16.4	2.62	2.04	0.073	0.197	2.31
	Gonar Farm	D07	7.70	0.000	0.841	74.4	23.3	2.31	2.38	0.055	0.247	2.68
	Chillas-MARC	D08	7.93	0.024	0.989	72.5	23.8	3.71	3.91	0.137	0.302	4.35
	Khai Batuga	D09	7.21	0.007	1.90	74.4	21.3	4.26	3.59	0.130	0.266	3.99
	Gas Bala	D10	7.89	0.002	1.04	70.9	25.6	3.55	2.38	0.071	0.223	2.67
Hunza-N	Chalt Nagar	N11	8.26	1.41	1.45	59.4	34.9	5.68	6.68	0.160	0.276	7.11
	Hussainabad	H12	7.87	0.686	2.75	61.7	33.8	4.54	7.27	0.184	0.294	7.75
	Pissan Nagar	N13	8.19	0.691	2.65	36.7	51.3	11.9	3.89	0.192	0.261	4.35
	Nasirabad	H14	8.28	0.469	1.94	63.9	33.0	3.06	4.82	0.084	0.232	5.14
	Karimabad	H15	7.87	0.137	2.46	76.6	21.8	1.59	4.20	0.077	0.257	4.54
	Smar Khay	N16	7.92	0.963	2.77	60.5	35.9	3.51	3.24	0.090	0.186	3.52
Astor	Harachu	A17	7.34	0.005	2.03	72.2	25.2	2.56	2.04	0.053	0.167	2.26
	Chungra	A18	6.83	0.000	1.57	74.1	23.0	2.91	1.92	0.060	0.242	2.22
	Gorikot-MARC	A19	6.90	0.000	0.410	82.2	16.3	1.48	3.23	0.054	0.220	3.50
	Rahman Pur	A20	7.16	0.000	2.21	74.2	22.2	3.67	4.48	0.179	0.453	5.12
	Mankiyal	A21	6.70	0.000	1.62	74.8	21.9	3.35	2.79	0.096	0.370	3.26
Skardu	Torki	S22	8.18	0.038	1.11	58.6	38.1	3.33	1.97	0.081	0.192	2.24
	Kachura	S23	7.51	0.004	1.99	69.2	27.8	2.98	2.51	0.116	0.307	2.93
	Gamba	S24	8.04	1.30	1.42	39.6	51.3	9.10	4.42	0.146	0.258	4.83
	Ranga	S25	8.33	1.54	1.06	57.2	36.7	6.11	4.12	0.110	0.216	4.45
	Skardu	S26	8.23	0.448	0.984	64.5	31.0	4.53	2.97	0.137	0.240	3.34

Combined metal oxides of Fe, Mn and Al fell in the range of 2.22 to 7.75 g kg^{-1}. Oxides of Mn and Al were less than 1.00 g kg^{-1} in all samples and concentration of Fe oxides was 5.02 g kg^{-1} in Hunza-N samples; the remaining four districts i.e. Gilgit, Diamer, Astor and Skardu, had average Fe oxide concentrations of 3.11, 2.86, 2.89 and 3.20 g kg^{-1} respectively.

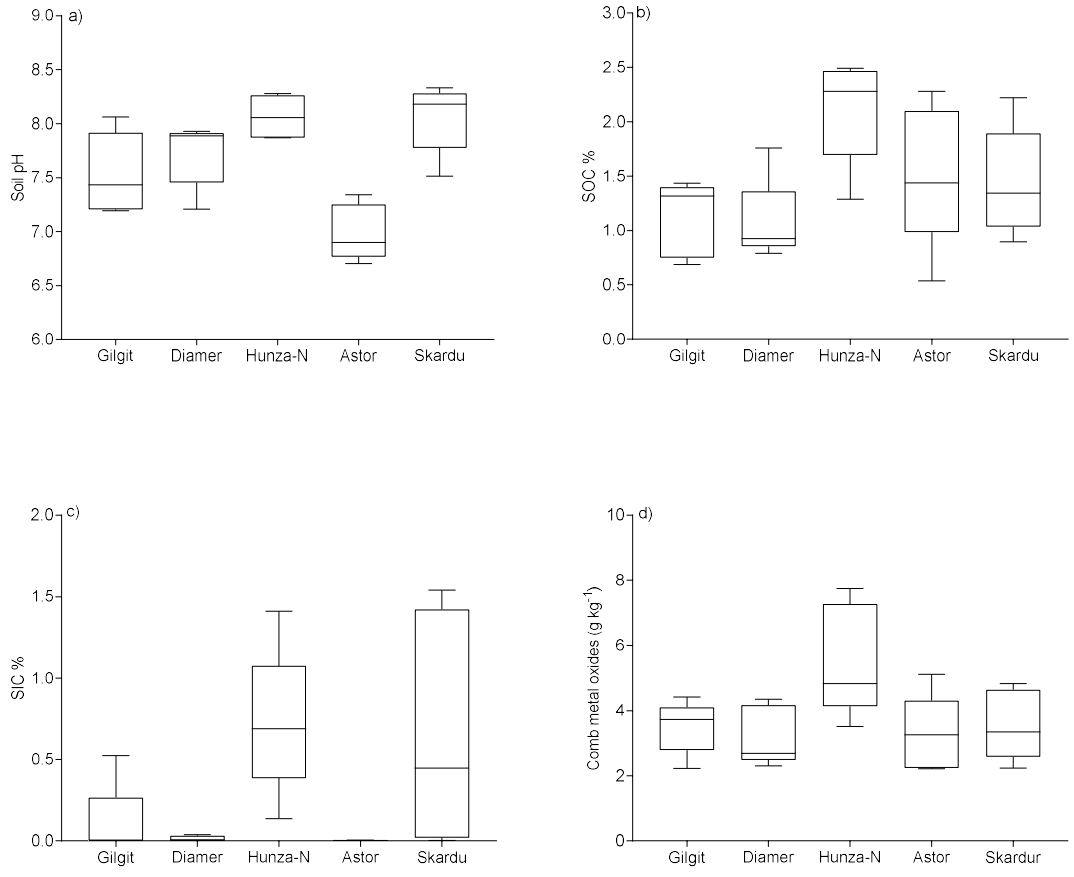


Figure 3.10. Box and whisker plots of basic soil characteristics in different districts: a) soil pH, b) soil organic carbon, c) soil inorganic carbon, d) soil extractable metal (Fe, Mn and Al) oxides

3.3.4 Soil major and trace element analyses

Total concentrations of major and trace elements in soils were determined following hydrofluoric acid digestion (Section 2.4.5) (Appendix 4). The mean total concentrations of the majority of the elements measured were not significantly different across all five districts of the study area. Sodium was the dominant cation and ranged from 7.84 to 22.6 g kg^{-1} (mean = 13.1 g kg^{-1}, median = 14.8 g kg^{-1}) for all samples together in all districts. Concentrations of other elements such as Ca, Mg, Al, K and Zn (Appendix 4) were less than values reported in soil from other parts of Pakistan (Zia et al., 2014, Nazif et al., 2015). Arsenic concentrations in some districts, including Hunza-N, Astor and Skardu were 21.7, 7.33 and 18.2 mg kg^{-1} respectively, which are higher than global average arsenic concentration suggested by Mandal and Suzuki (2002) of 6 mg kg^{-1}.

3.3.5 Iodine in soil

3.3.5.1 Soil total iodine (I_T)

Total I in soil samples (I_T) was extracted with 10% TMAH by the method described in chapter 2 (Section 2.4.6). Figure 3.11 shows the spatial distribution of I_T in the study area. Concentrations of I_T fell in the range of 273 to 1180 µg kg^{-1} with an average value of 685 µg kg^{-1} across all sites. Average I_T concentrations in each district i.e. Gilgit, Diamer, Hunza-N, Astor and Skardu were 674, 895, 650, 489 and 725 µg kg^{-1} respectively (Figure 3.12) and showed no significant regional differences (ANOVA, $P > 0.05$). Values of I_T were low compared to (i) the reported global mean value of 2600 µg kg^{-1} (Figure 3.12) (ii) the average value (920 µg kg^{-1}) reported for soils from other

parts of Pakistan (Zia et al., 2014) (iii) values reported by Karim (2018) for the Kurdistan region of Iraq (4140 μg kg^{-1}) and (iv) alluvium-derived soils worldwide (3560 μg kg^{-1}) (Johnson, 2003); soils in Gilgit-Baltistan are largely alluvial in nature (Malik and Azam, 2009).

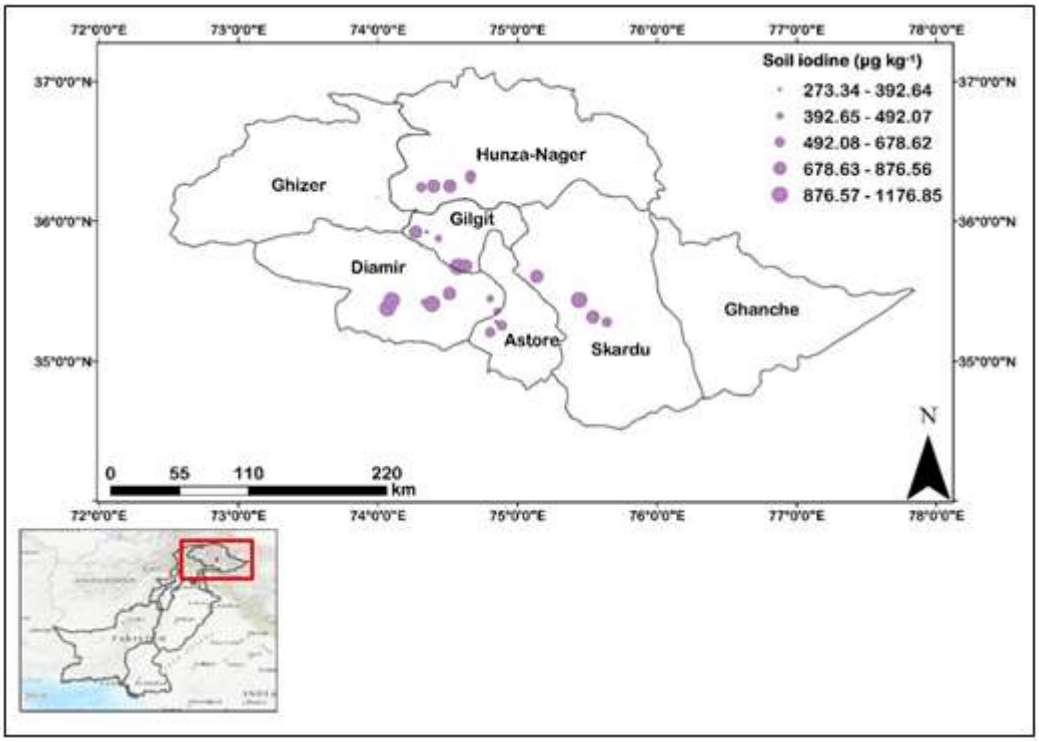


Figure 3.11. Spatial distribution of total iodine concentrations in soils of Gilgit-Baltistan

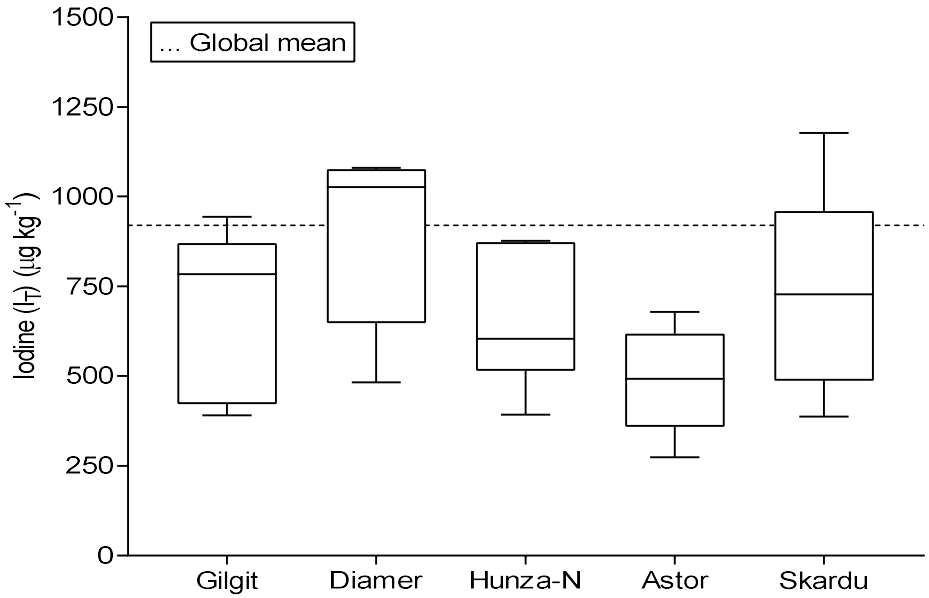


Figure 3.12. Box and whisker plot of total soil iodine concentration (I_T) in the districts of Gilgit-Baltistan

The majority of I in soils is generally derived from oceanic sources through atmospheric dry deposition and rainfall (Fuge and Johnson, 2015). Soil characteristics such as pH, texture and organic matter control retention and, over time, the concentration of total soil I (Zia et al., 2014). Gilgit-Baltistan is far from the sea and located in the rain shadow area of the Himalayan mountains. Thus, the majority of soil I is believed to be geogenic, derived from the soil parent material. The geology of the area is complex but is dominated by igneous and metamorphic rocks (Malik and Azam, 2009) which have less I than sedimentary rocks (Johnson, 2003; Fuge and Johnson, 2015). Furthermore, the soils are predominantly sandy with low organic carbon concentrations, which limits the ability of the soils to retain I (Johnson, 2003; Johnson et al., 2003). The alkaline nature of the soils would also limit I retention;

adsorption of I onto Fe and Al oxyhydroxides and rates of conversion to humus-bound I, are both lower under alkaline conditions (Johnson et al., 2003; Watts and Mitchell, 2009; Shetaya et al., 2012).

No relationship was observed between the concentrations of metal oxides and I in all samples; metal oxides are considered to be an important adsorption site for I in soil but adsorption is most effective at low pH values (< 5) (Whitehead, 1973; Schmitz and Aumann, 1994; Bowley, 2013). Soil pH did not show a correlation with I_T in the majority of sampling districts except Skardu which showed a negative correlation ($r = -0.961$, $p = 0.009$) between soil pH and I_T (Figure 3.12). The absence of correlation between soil pH and I_T may simply be due to the narrow range of pH of the soils, as suggested by Karim (2018) for soils in the Iraqi region of Kurdistan with a pH range similar to that of this study. However, it is contrary to the findings of Bowley (2013), Zia et al. (2014) and Watts et al. (2015) who reported a negative correlation in soils of Northern Ireland, Pakistan and Malawi respectively.

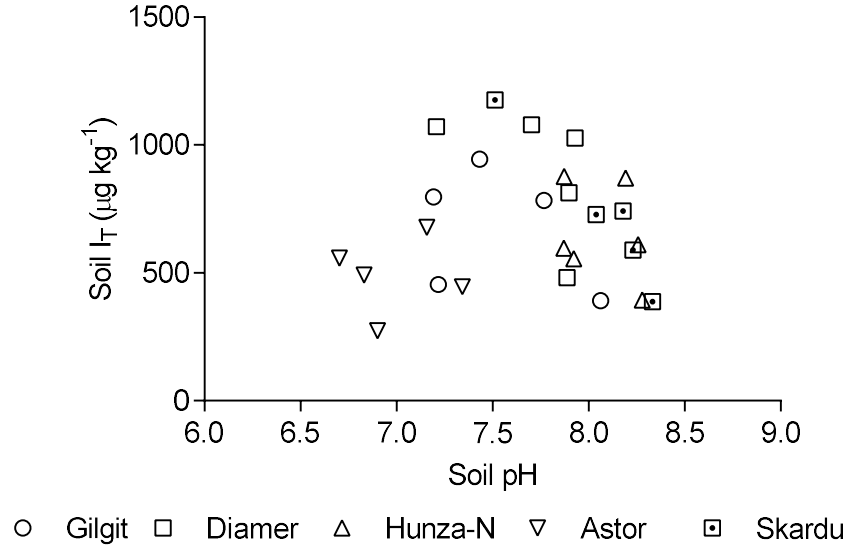


Figure 3.13. Relationship between soil pH and total soil iodine concentration in districts of Gilgit-Baltistan

A positive correlation (r = 0.900, p = 0.037) between SOC% and I_T was only observed in Skardu (Figure 3.14). Soil humus is important for retaining soil I, however the narrow range of SOC (<3%) in all samples may have masked any underlying causal relationship. Fuge and Johnson (1986), Johnson et al. (2002), Bowley (2013), Zia et al. (2014) and Watts et al. (2015) all reported a positive correlation between the concentrations of soil organic matter and I in samples from the UK, Morocco, Northern Ireland, Pakistan and Malawi respectively. However, Karim (2018) reported no correlation with SOC% in soils from Iraqi Kurdistan.

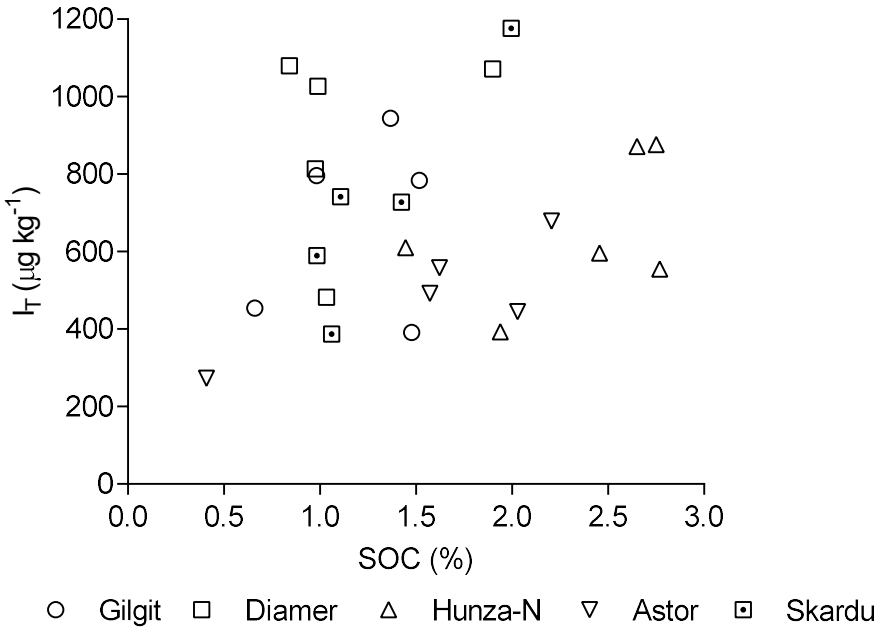


Figure 3.14. Relationship between SOC (%) and total soil iodine concentration in the districts Gilgit-Baltistan

3.3.5.2 Fractionation of soil iodine

Soluble iodine (I_{sol}) in soil

The soluble fraction of I (extracted with 0.01 M KNO_3) accounted for ≤ 3 % of I_T in all samples (Table 3.3). It ranged from 6.66 to 24.7 µg kg^{-1} with an overall median of 10.7 µg kg^{-1}. While the concentration of I_{sol} varied between samples, the proportion of I_T that was extractable in 0.01 M KNO_3 was almost the same in each district and did not reveal significant differences between districts (ANOVA, $p > 0.05$). Figure 3.15 presents the concentrations of I_{sol} in each district.

The small percentage of I_T available as I_{sol} (Table 3.3) is comparable with other studies. Magomedova et al. (1970) found that only 1-12% of I was water-soluble in soil samples from Dagestan, USSR. Fuge and Johnson (1986) reported that less than 10%

of I_T was extractable with water in approximately 80% of soils mainly from Wales (n = 183). Soils from other parts of Pakistan (Zia et al., 2014) and other countries such as Malawi (Watts et al., 2015) and Kurdistan (Karim, 2018) have revealed similar average proportions of water-soluble I concentrations; 2.36%, 1.38% and 1.59% of total soil I respectively. Small proportions of water soluble I have also been measured for German soils (2.5-9.7% of I_T) (Schmitz and Aumann, 1994).

Soil characteristics including pH and SOC did not show a relationship with I_{sol}. It is possible that variation in I_{sol} concentration across different districts may be due to the variation in I concentration in irrigation water. The concentration of I in irrigation water showed a positive correlation with I_{sol} ($r = 0.599$, $P = 0.001$) when samples of all districts were considered as one data set (Figure 3.16).

Adsorbed iodine (I_{ads}) in soil

The adsorbed fraction of soil iodine (I_{ads}) (extracted with 0.016 M KH_2PO_4) ranged from 8.87 to 29.0 $\mu g\ kg^{-1}$ and had a median concentration of 15.0 $\mu g\ kg^{-1}$. It comprised < 4.0% of I_T on average for all samples. Karim (2018) reported a slightly higher ratio of 10.7% present as I_{ads} in his fractionation experiments on soils from the Kurdistan region of Iraq. As a percentage of I_T, I_{ads} varied across the sampling districts (Figure 3.15) but showed no significant correlation with soil properties (ANOVA, $p > 0.05$), probably because of similar soil properties that might affect I adsorption (SOC, oxide content, clay content and pH) (Medrano-Macías et al., 2016).

Table 3.3. Concentration of iodine in soil fractions and its extractability as a proportion of total soil iodine (I_T)

District	Village	Sample code	I_T	I_{sol}	I_{ads}	I_{sol} extractability	I_{ads} extractability
			($\mu g\ kg^{-1}$)			(%)	
Gilgit	Juglote-MARC	G01	796	17.5	20.3	2.20	2.54
	Basin	G02	784	14.3	19.5	1.83	2.48
	Daynor	G03	391	9.93	8.87	2.54	2.27
	Minawar	G04	454	9.44	12.6	2.08	2.78
	Bargin	G05	944	14.3	18.8	1.52	2.00
Diamer	Gorabad	D06	813	21.9	19.5	2.70	2.40
	Gonar Farm	D07	1080	23.1	28.6	2.13	2.65
	Chillas-MARC	D08	1027	24.7	29.0	2.40	2.83
	Khai Batuga	D09	1072	15.4	18.8	1.44	1.75
	Gas Bala	D10	482	11.3	10.2	2.33	2.11
Hunza-N	Chalt Nagar	N11	611	13.9	17.8	2.28	2.92
	Hussainabad	H12	877	15.1	15.4	1.73	1.76
	Pissan Nagar	N13	871	12.3	16.9	1.42	1.94
	Nasirabad	H14	393	8.63	9.62	2.20	2.45
	Karimabad	H15	596	8.15	10.2	1.37	1.70
	Smar Khay	N16	555	8.89	10.6	1.60	1.91
Astor	Harachu	A17	445	7.32	10.3	1.64	2.32
	Chungra	A18	492	8.38	12.2	1.70	2.48
	Gorikot-MARC	A19	273	7.39	10.5	2.71	3.83
	Rahman Pur	A20	679	10.1	14.6	1.49	2.15
	Mankiyal	A21	558	6.66	11.8	1.19	2.11
Skardu	Torki	S22	742	15.9	17.9	2.14	2.42
	Kachura	S23	1177	9.48	15.9	0.81	1.35
	Gamba	S24	728	14.6	20.0	2.00	2.74
	Ranga	S25	387	8.30	10.5	2.14	2.71
	Skardu	S26	590	8.38	11.9	1.42	2.01

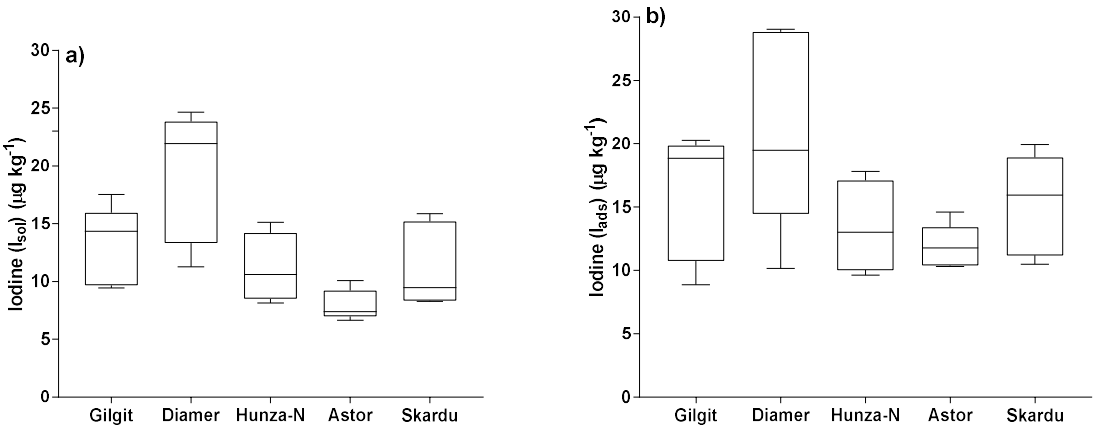


Figure 3.15. Concentrations of (a) soluble and (b) adsorbed soil iodine in districts of the study area

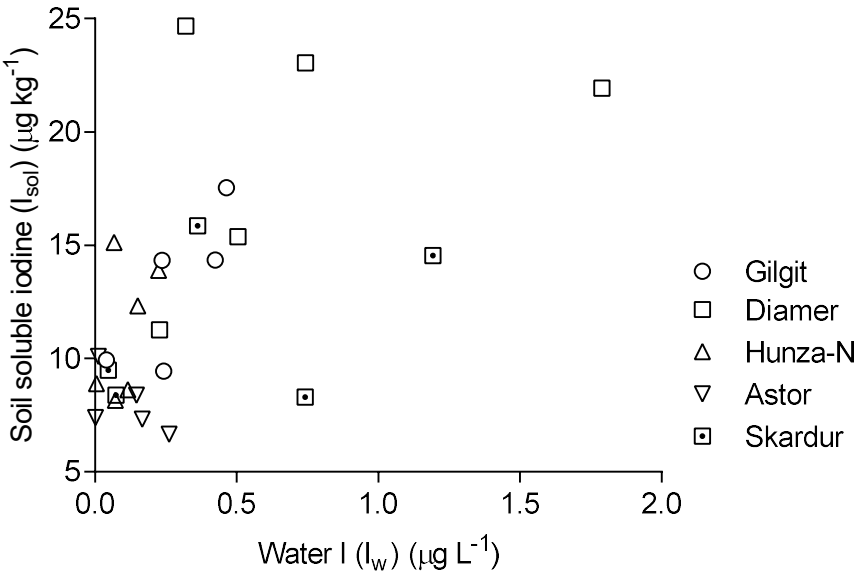


Figure 3.16. Concentration of iodine in irrigation water (I_w) and soluble soil iodine (I_{sol})

3.3.5.3 Speciation of soil iodine

Iodine speciation was carried out on the soluble and adsorbed fractions. In all of the districts most of the I in both I_{sol} and I_{ads} fractions was present as organic I (Table 3.4 and 3.5). The median concentrations of organic I in the soluble and adsorbed fractions were 10.4 and 13.8 μg kg^{-1} which accounted for 98% and 90% of the I_{sol} and I_{ads} respectively across all samples. The large proportion of organic I is comparable with findings from other studies. Hu et al. (2007) reported that a large proportion of I (>90%) in soils is present bound to humic and fulvic acids. It is recognised that there are various enzymes in soils which can convert inorganic I to organic forms (Johanson, 2000).

The ratio of inorganic species, I^- and IO_3^-, was variable across the samples and inconsistent within different districts (Table 3.4 and 3.5). However, on average, I^- generally accounted for a larger proportion of inorganic I in both I_{sol} (63%) and I_{ads} (84%) fractions across all districts; this is comparable to other studies such as Yamada et al. (1999), Hu et al. (2005) and Hu et al. (2007). Iodate is sorbed more strongly in most soils than I^- and is therefore less easily extracted (Hu et al., 2005, Hu et al., 2007, Fuge and Johnson, 2015). The other reason for a large I^- presence is probably its stability in the soil solution. Iodide is the dominant species in most soil solutions because of its stability over a wide range of Eh and pH values (Figure 3.17) (Söderlund et al., 2011).

Table 3.4. Concentration (µg kg^{-1}) of iodine species in I_{sol}; the proportion of organic (Org I_{sol}) and inorganic (Inorg I_{sol}) iodine as a percentage of total I_{sol} and proportion of I^- (iodide) and IO_3^- (iodate) as a percentage of In-org I_{sol}

District	Village	Sample code	Org I_{sol}	Inorg I_{sol}	%Org I_{sol}	%Inorg I_{sol}	$\%I^-_{sol}$	$\%IO_3^-{}_{sol}$
			(µg kg^{-1})		(%)			
Gilgit	Juglote-MARC	G01	16.4	1.18	93.3	6.73	97.6	2.42
	Basin	G02	14.2	0.134	99.1	0.94	89.4	10.6
	Daynor	G03	9.82	0.107	98.9	1.08	57.3	42.7
	Minawar	G04	9.27	0.166	98.2	1.76	60.7	39.3
	Bargin	G05	14.3	0.046	99.7	0.32	54.9	45.1
Diamer	Gorabad	D06	21.5	0.440	98.0	2.01	100	0.00
	Gonar Farm	D07	20.9	2.15	90.7	9.32	92.9	7.07
	Chillas-MARC	D08	22.0	2.66	89.2	10.8	86.3	13.7
	Khai Batuga	D09	14.9	0.450	97.1	2.92	17.5	82.5
	Gas Bala	D10	10.9	0.341	97.0	3.03	38.3	61.7
Hunza-N	Chalt Nagar	N11	13.7	0.155	98.9	1.12	82.9	17.1
	Hussainabad	H12	15.0	0.122	99.2	0.81	47.0	53.0
	Pissan Nagar	N13	12.3	0.072	99.4	0.58	33.6	66.4
	Nasirabad	H14	8.50	0.129	98.5	1.49	46.3	53.7
	Karimabad	H15	8.04	0.113	98.6	1.38	22.1	77.9
	Smar Khay	N16	8.77	0.123	98.6	1.39	55.7	44.3
Astor	Harachu	A17	7.27	0.046	99.4	0.63	51.9	48.1
	Chungra	A18	8.30	0.076	99.1	0.90	65.4	34.6
	Gorikot-MARC	A19	7.22	0.174	97.6	2.35	95.3	4.66
	Rahman Pur	A20	9.93	0.155	98.5	1.54	37.5	62.5
	Mankiyal	A21	6.58	0.072	98.9	1.08	41.9	58.1
Skardu	Torki	S22	15.7	0.142	99.1	0.89	100	0.00
	Kachura	S23	9.29	0.193	98.0	2.04	2.92	97.1
	Gamba	S24	14.4	0.162	98.9	1.11	80.1	19.9
	Ranga	S25	8.19	0.109	98.7	1.32	92.6	7.35
	Skardu	S26	8.24	0.142	98.3	1.69	89.5	10.5

Table 3.5. Concentration (µg kg^{-1}) of iodine species in I_{ads}; the proportion of organic (Org I_{ads}) and inorganic (Inorg I_{ads}) iodine as a percentage of total I_{ads} and proportion of I^- (iodide) and IO_3^- (iodate) as a percentage of In-org I_{ads}

District	Village	Sample code	Org I_{ads}	Inorg I_{ads}	%Org I_{ads}	%Inorg I_{ads}	$\%I^-_{ads}$	$\%IO_3^-{}_{ads}$
			(µg kg^{-1})			(%)		
Gilgit	Juglote-MARC	G01	17.5	2.74	86.5	13.5	96.0	3.98
	Basin	G02	17.3	2.17	88.9	11.1	87.7	12.3
	Daynor	G03	7.70	1.17	86.8	13.2	83.8	16.2
	Minawar	G04	12.1	0.51	96.0	4.01	82.7	17.3
	Bargin	G05	17.8	1.07	94.3	5.70	95.6	4.45
Diamer	Gorabad	D06	16.6	2.84	85.4	14.6	74.0	26.0
	Gonar Farm	D07	22.4	6.20	78.3	21.7	97.7	2.29
	Chillas-MARC	D08	22.4	6.65	77.1	22.9	86.7	13.3
	Khai Batuga	D09	17.1	1.64	91.3	8.73	51.8	48.2
	Gas Bala	D10	9.24	0.92	91.0	9.03	80.2	19.8
Hunza-N	Chalt Nagar	N11	17.1	0.77	95.7	4.33	88.3	11.7
	Hussainabad	H12	14.5	0.96	93.8	6.22	75.1	24.9
	Pissan Nagar	N13	16.2	0.67	96.0	3.99	96.3	3.66
	Nasirabad	H14	8.99	0.63	93.4	6.58	91.2	8.79
	Karimabad	H15	9.93	0.23	97.7	2.31	40.0	60.0
	Smar Khay	N16	10.2	0.45	95.8	4.22	79.2	20.8
Astor	Harachu	A17	9.97	0.34	96.7	3.34	70.8	29.2
	Chungra	A18	10.4	1.77	85.5	14.5	97.9	2.10
	Gorikot-MARC	A19	8.44	2.02	80.7	19.3	97.3	2.71
	Rahman Pur	A20	13.1	1.52	89.6	10.4	95.6	4.44
	Mankiyal	A21	10.5	1.27	89.2	10.8	94.3	5.73
Skardu	Torki	S22	17.6	0.36	98.0	2.01	88.1	11.9
	Kachura	S23	15.3	0.61	96.2	3.82	95.4	4.65
	Gamba	S24	17.6	2.34	88.3	11.7	98.3	1.67
	Ranga	S25	10.1	0.38	96.3	3.66	72.6	27.4
	Skardu	S26	11.1	0.75	93.7	6.28	71.3	28.7

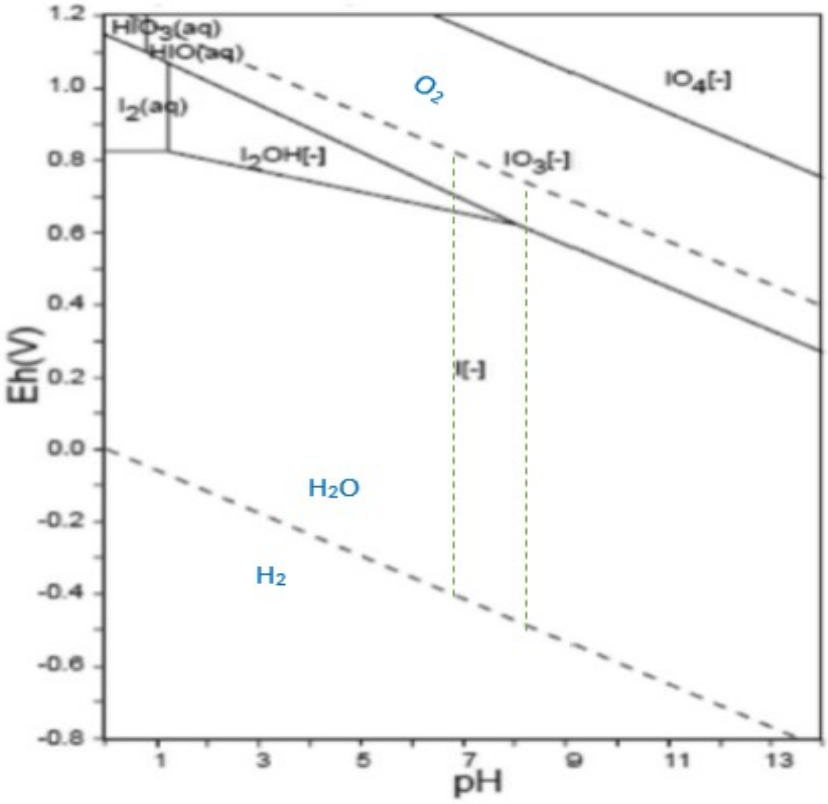


Figure 3.17. Eh-pH diagram of iodine speciation in the soil solution adapted from Soderland (2016). Vertical dotted lines represent the range of pH in soils of this study.

3.3.6 Iodine in crop plants (I_P)

The concentration of I in the dry mass of 281 samples of 34 edible plant species was determined in all sampling districts of the study area (Appendix 5). Plant samples were broadly divided into six groups, i.e. leafy vegetables (n = 56), grains (cereals) (n = 55), crop vegetables (n = 75), tubers (n = 30), fruits (n = 63) and nuts (n = 2). In each district, and also considering all samples together as one data set, I concentrations decreased in the order: leafy vegetables > grains (cereals) > crop vegetables > tubers > nuts > fruits (Figure 3.18). The I concentrations in some plant groups were similar across all districts while others showed variation between districts. Leafy vegetables,

grains and tubers did not show any significant variation between different districts (ANOVA, $p > 0.05$). However, the I concentration of crop vegetables and fruits showed a significant difference between districts (ANOVA, $p < 0.05$). Nuts were collected only from the Gilgit district.

The concentration of I was consistently low in all plants reflecting low I concentrations in water and soil in the area. It varied among plant species in each group (Table 3.6 and Figure 3.19) presumably reflecting differences in the affinity of each plant species for I. Leafy vegetables had the highest average I content. The mean and median values of I_P in leafy vegetables were 36.3 and 27.5 $\mu g\ kg^{-1}$ respectively which are low compared to similar investigations elsewhere (Table 3.7). Within leafy vegetables across the study area (Table 3.6), cabbage (n = 7) and lettuce (n = 1) had the lowest and highest I_P of 5.51 $\mu g\ kg^{-1}$ and 107 $\mu g\ kg^{-1}$ respectively. The mean I_P value in grain was 21.8 $\mu g\ kg^{-1}$ (n = 55) with buckwheat (n = 2, mean = 10.0 $\mu g\ kg^{-1}$) and barley (n = 3, mean = 33.9 $\mu g\ kg^{-1}$) providing the minimum and maximum values of the range. Iodine concentrations in wheat and maize were comparable to values for East Africa reported by Eckhoff and Maage (1997) (Table 3.7). They were lower compared to values reported from Morocco while slightly higher than concentrations published in studies conducted in other parts of the world (Table 3.7). Values of I_P for green pea (n =1) and okra (n = 10) were 0.788 $\mu g\ kg^{-1}$ and 33 $\mu g\ kg^{-1}$ respectively, representing the range for crop vegetables (median = 12.60 $\mu g\ kg^{-1}$; n = 75). The average I_P for all crop vegetables together was less than most literature values (Haldimann et al., 2005).

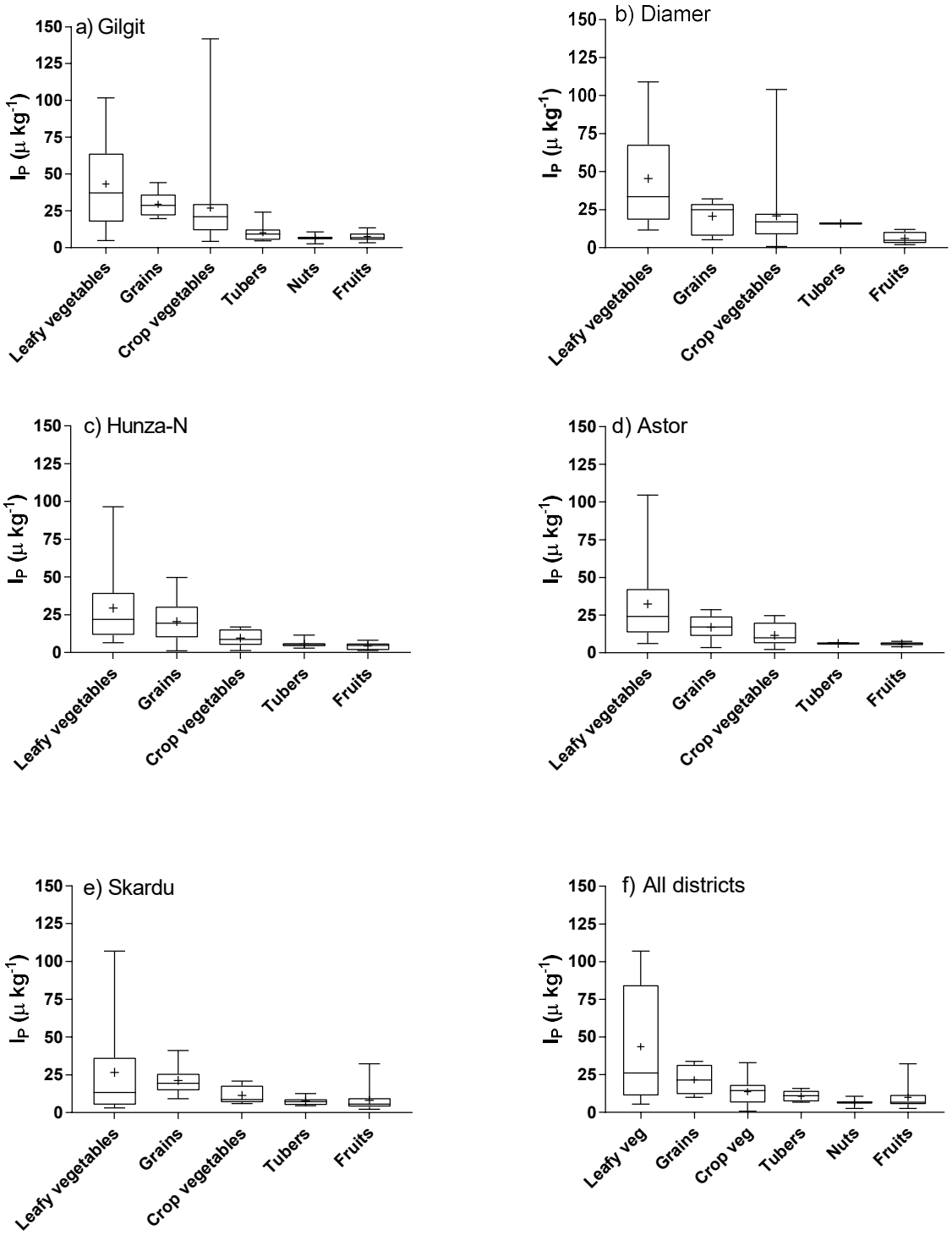


Figure 3.18. Iodine concentration of plant groups in sampling districts of the study area

Table 3.6. Summary statistics for iodine in plant groups and species across all districts

Plant group/species	No of samples	Iodine (μg kg^{-1})			
		Mean	Median	Range	SE(±)
Leafy vegetables	***56***	***36.3***	***27.5***	***3.05 - 109***	***4.04***
Lettuce	1	107	-	-	-
Spinach	21	61.8	53.9	28.3 - 109	5.99
Amaranth leaves	14	26.1	24.0	6.17 - 63.8	4.38
Kholrabi	13	17.2	16.4	12.7 - 27.4	1.23
Cabbage	7	5.51	6.00	3.05 - 8.75	0.743
Grains	***55***	***21.8***	***22.3***	***1.18 - 49.8***	***1.49***
Wheat	25	18.7	14.8	1.18 - 44.2	2.55
Maize	25	24.3	25.0	9.16 - 49.8	1.59
Barley	3	33.9	34.5	30.8 - 36.5	0.583
Buckwheat	2	10.0	-	9.97 - 10.1	-
Crop vegetables	***75***	***16.9***	***12.6***	***0.790 - 142***	***2.29***
Okra	10	33.0	24.9	15.0 - 104	8.29
Tomato	21	21.1	15.6	5.01 - 142	6.16
Pumpkin	6	15.6	16.1	4.07 - 24.7	3.37
Chilli	11	15.4	15.2	7.43 - 30.7	2.37
Cucumber	5	14.5	16.4	5.21 - 20.5	2.82
Aubergine	7	10.6	6.37	4.75 - 29.3	3.29
Capsicum	3	7.57	8.69	4.35 - 9.68	1.64
Beans	11	5.66	5.76	1.42 - 11.4	1.05
Pigeon pea	1	0.79	-	-	-
Tubers	***30***	***9.96***	***6.68***	***2.94 - 66.4***	***2.09***
Radish	1	16.0	-	-	-
Carrot	4	12.6	9.68	6.67 - 24.2	4.11
Onion	12	11.1	6.16	2.94 - 66.4	5.06
Potato	11	7.77	6.51	5.28 - 13.2	0.834
Turnip	2	6.81	-	5.43 - 8.19	-
Nuts	***2***	***6.62***	***-***	2.52 - 10.7	***-***
Almond	1	10.7	-	-	-
Walnut	1	2.52	-	-	-
Fruits	***63***	***6.51***	***5.81***	***1.26 - 32.4***	***0.548***
Grapes	1	32.4	-	-	-
Ber (Chinese date)	1	12.0	-	-	-
Mulberry	3	11.4	10.5	9.80 - 13.7	1.21
Fig	3	8.14	6.00	5.05 - 13.4	2.63
Pear	11	6.64	6.39	4.07 - 9.78	0.575
Apricot	19	5.65	5.64	3.35 - 11.8	0.419
Peach	4	5.45	5.16	3.86 - 7.62	0.939
Apple	18	5.38	4.76	1.26 - 12.4	0.709
Persimmon	3	2.73	2.97	1.74 - 3.47	0.512

Median I_P in tubers was 6.68 μg kg^{-1} (Table 3.6); turnip (n = 2) and radish (n = 1) accounted for the highest (6.81 μg kg^{-1}) and lowest (15.9 μg kg^{-1}) values respectively. Other species of the group such as carrot, onion and potato had average I_P values of 12.6, 11.1 and 7.77 μg kg^{-1} respectively. Again, values of I_P for tubers in this study were mainly less than those from studies conducted in other countries (Table 3.7). However, onion I contents were comparable with data reported from studies in Malawi and Morroco (Table 3.7).

The group of nuts had only two examples (almond and walnut): almond had the highest I concentration of 10.7 μg kg^{-1} compared to walnut with 2.52 μg kg^{-1}. Nuts as a group had an average I_P value (6.62 μg kg^{-1}) less than those reported in the literature from other parts of the world (Fordyce, 2003; Haldimann et al., 2005). Fruits had the smallest I content among all the plant groups. The median I content found in fruits (n = 63) was 5.81 μg kg^{-1} which was less than most values reported from other countries (Table 3.7). Persimmon and grapes accounted for the lowest and highest I contents of 2.73 and 32.4 μg kg^{-1} respectively. The majority of species in fruits group had I_P values <10 μg kg^{-1} (Table 3.7), except mulberry, ber and grapes which had I concentrations of 11.4, 12 and 32.4 μg kg^{-1} respectively.

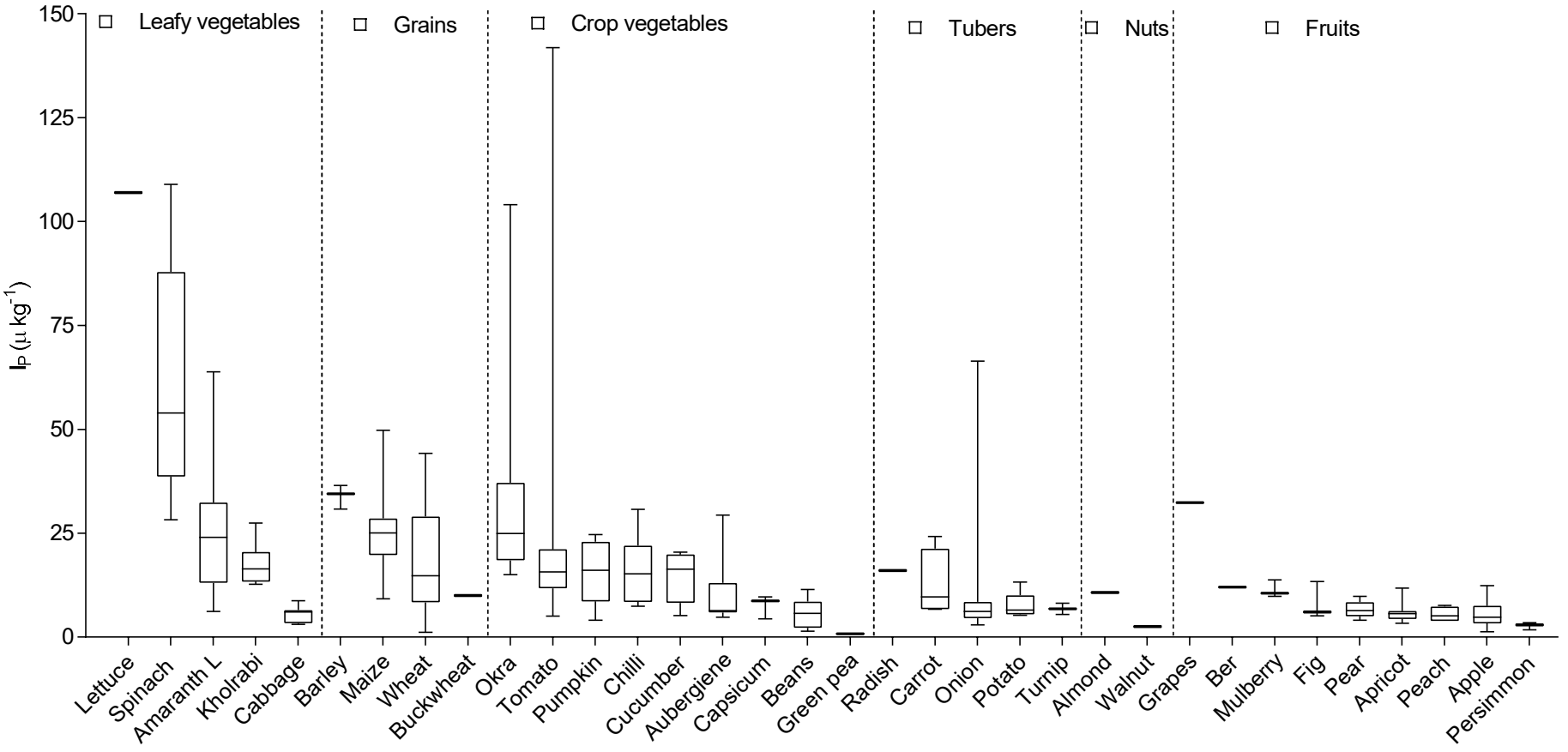


Figure 3.19. Iodine concentration (I_P, µg kg^{-1}) in species of different plant groups in Gilgit-Baltistan

Table 3.7. Comparison of average iodine concentrations in plant groups and species with other investigations

Plant group/species	Number of samples	Current study	Literature data
		Iodine concentration (μg kg^{-1})	
Leafy vegetables	***56***	***36.3***	***236[c], 316[d]***
Lettuce	1	107	455[d]
Spinach	21	60.9	660[d], 462[l], 370[k]
Amaranth leaves	14	26.1	163[f], (70, 160)[n]
Kholrabi	13	17.2	49[d]
Cabbage	7	5.51	(20, 80)[n], 123[d]
Grains	***55***	***21.8***	***6.1[b], 96.5[d], 13[n]***
Barley	3	33.9	90[j], 48.9[d], 9[i]
Maize	25	24.3	91[j], 10[n], (80, 330)[e], 122[d], (19, 30)[k]
Wheat	25	18.7	35[c], 13[a], 15[g], 6.89[b], 227[j], 74.4[d], 12.5[l], 40[i]
Buckwheat	2	9.97	
Crop vegetables	***75***	***16.9***	***130[c], 30[h], 5[m]***
Okra	10	33.0	(110, 210)[n], 255[l], 450[f]
Tomato	21	21.1	(20, 30, 80)[n], 65.1[d], 513[l], 150[f]
Pumpkin	6	15.6	10[n]
Chilli	11	15.4	(110, 210)[n], 201[d]
Cucumber	5	14.5	195[d], 231[l]
Aubergine	7	10.6	306[l]
Capsicum	3	7.57	157[d], 394[l], 351[f]
Beans	11	5.66	(10, 70)[n], 186[d]
Pigeon pea	1	0.788	10[n]
Tubers	***30***	***9.96***	***8[n]***
Radish	1	15.9	224[l]
Carrot	4	12.6	143[d], 25[i]
Onion	12	11.1	10[n], 143[d], 145[l]
Potato	11	7.77	16[c], 82[g], 103[d], <10[i], 190[f], 70[h]
Turnip	2	6.81	333[d]
Nuts	***2***	***6.62***	***218[c], 126[d], 250[h]***
Almond	1	10.7	
Walnut	1	2.52	

Table 3.7. Continued

Plant group/species	No of samples	Current study	Literature data
		Iodine contents (μg kg^{-1})	
Fruits	***63***	***6.51***	***18[c], 71.6[d], 40[h], 3[m]***
Grapes	1	32.4	960[f], 24.3[o]
Ber (Chinese date)	1	12.0	
Mulberry	3	11.4	
Fig	3	8.14	
Pear	11	6.64	62[d]
Apricot	19	5.65	
Peach	4	5.45	
Apple	18	5.38	53[f]
Persimmon	3	2.73	

a-(Zia et al., 2014); b-(Shinonaga et al., 2001); c-(Haldimann et al., 2005); d-(Fordyce, 2003); e-(Longvah and Deosthale, 1998); f-(Mahesh et al., 1992); g)-(Anke et al., 1993); h-(Johnson, 2003); i-(Johnson et al., 2002); j-(Aquaron et al., 1993); k-(Eckhoff and Maage, 1997); l-(Karim, 2018); m-(Fung et al., 2015); n-(Watts et al., 2015); o-(Salau et al., 2011)

3.3.7 Relationship between I_P and iodine concentration in soil and water

There were no correlations between I_P for plant *groups* and soil properties but I_P values for some individual species did show a relationship with soil characteristics (Figure 3.20). Cucumber in the crop vegetables group showed a negative correlation between I_P and SOC% ($r = -0.634$, $p < 0.05$). Values of I_P for kohlrabi plants decreased with increase in soil pH ($r = -0.600$, $p < 0.05$).

For most crop species, no relationship was seen between I_P and (i) the two fractions of soil I (I_{ads}, I_{sol}) or (ii) iodine species (organic, I^-, IO_3^-) in each fraction. Only I_P for potato ($n = 11$) showed a correlation with soluble and adsorbed I fractions ($r = 0.859$ and 0.703 respectively; $p < 0.05$; Figure 3.21). Carrot ($n = 4$) also had a positive correlation with soluble soil I ($r = 0.967$, $p < 0.05$) (Figure 3.21).

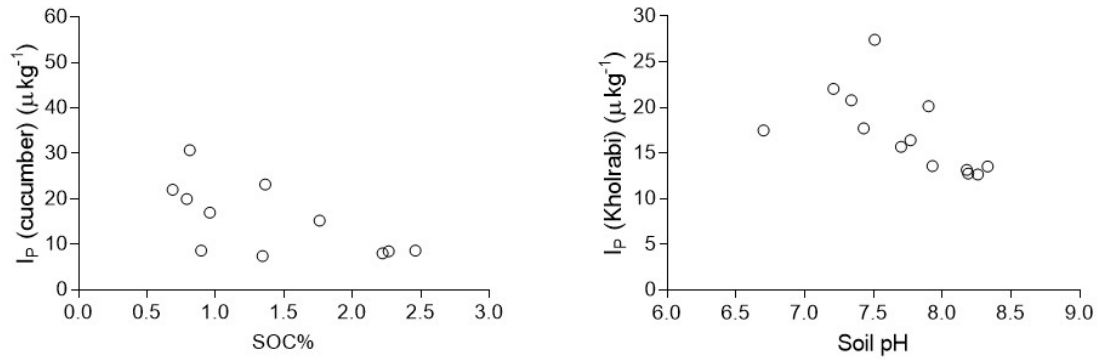


Figure 3.20. Relationship between soil characteristics and values of I_P for selected crop species

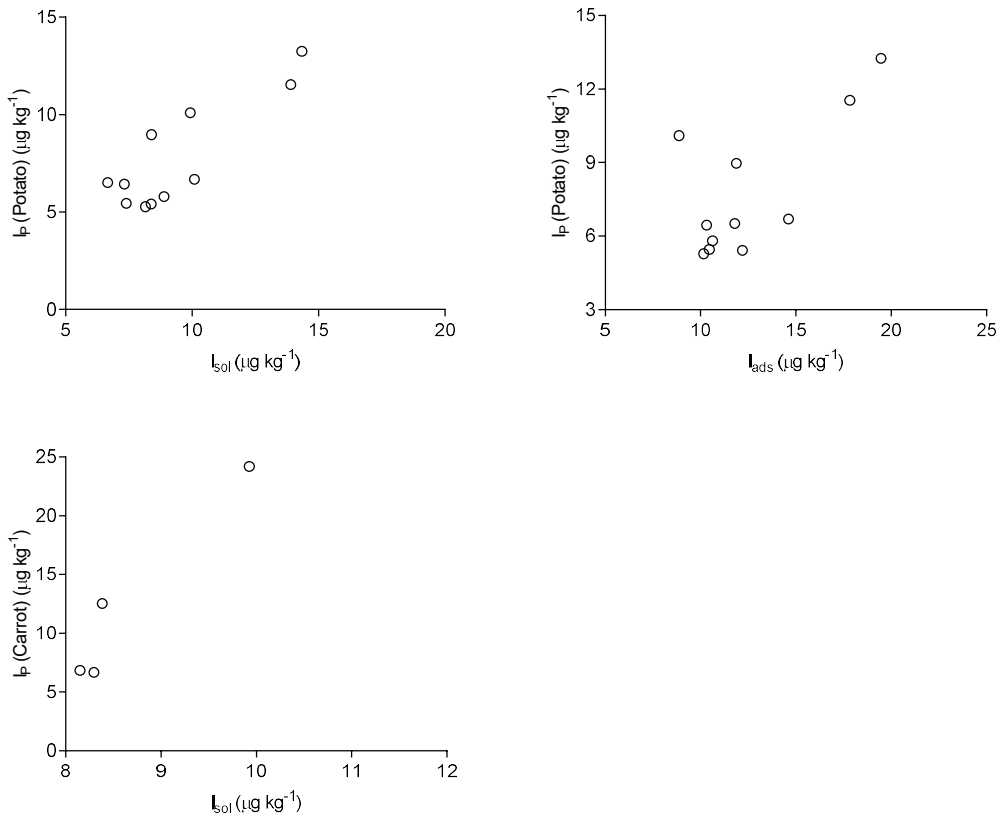


Figure 3.21. Relationship between I_{sol} and I_{ads} with I_P values of selected plant species

Irrigation water I showed no correlation with any of the plant groups or individual plant species within any group.

3.3.8 Soil to plant transfer factor of iodine

Soil to plant transfer factor for I (I-TF) was calculated by dividing I_P by I concentration in the associated soil (I_{soil}) (Equation 3.1).

$$I - TF = \frac{I_P}{I_{soil}} \quad (3.1)$$

Equation 3.1 was used to calculate transfer factors based on total soil I concentration (I_T) as well as soluble (I_{sol}) and adsorbed (I_{ads}) fractions of soil I for plant groups and their species (Table 3.8). The transfer factors decreased in the order of I_{sol}-TF > I_{ads}-TF> I_T-TF in all plant groups, reflecting the relative magnitude of each soil I fraction. On average I-TF of available I (I_{sol} and I_{ads}), was 33-53 times higher than the TF of I_T. The difference between I_{sol}-TF and I_{ads}-TF was smaller for all plant groups, which demonstrate that mobile I (I_{sol} and I_{ads}) is more bioavailable and easily taken up by plants compared to I_T. Transfer factor also varied across the plant groups and was in the order: leafy vegetables > grains> crop vegetables > tubers > fruits > nuts. This variation in I-TF across plant groups broadly follows the trend reported in the literature (Medrano-Macías et al., 2016). Within each plant group there was a diverse range of TF values for each fraction of I, which demonstrates the importance of considering individual crops in terms of their ability to uptake I.

Table 3.8. Soil to plant transfer factor based on total soil iodine (I_T) and its fractions in soil (I_{sol} and I_{ads})

Plant type	No of samples	I_{sol} - TF			I_{ads} - TF			I_T – TF		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Leafy vegetables		***0.363***	***14.2***	***3.37***	***0.257***	***10.0***	***2.59***	***0.005***	***0.383***	***0.067***
Lettuce	1	7.35	7.35	7.35	5.36	5.36	5.36	0.147	0.147	0.147
Spinach	21	1.71	14.2	5.47	1.38	10.03	4.43	0.036	0.383	0.111
Amaranth leaves	14	0.612	4.21	2.16	0.423	3.15	1.730	0.009	0.089	0.043
Kohlrabi	13	0.551	2.89	1.44	0.468	2.01	1.04	0.013	0.047	0.023
Cabbage	7	0.363	0.629	0.445	0.257	0.542	0.382	0.005	0.014	0.009
Grains		***0.078***	***4.69***	***2.13***	***0.076***	***3.80***	***1.72***	***0.001***	***0.097***	***0.040***
Barley	3	2.50	4.24	3.61	1.83	3.80	2.84	0.035	0.093	0.062
Maize	25	0.629	3.74	2.13	0.459	3.350	1.72	0.013	0.095	0.040
Wheat	25	0.078	4.69	1.60	0.076	3.510	1.28	0.001	0.097	0.031
Buckwheat	2	1.16	1.17	1.16	1.04	1.05	1.04	0.026	0.026	0.025
Crop vegetables		***0.032***	***9.89***	***1.11***	***0.032***	***7.29***	***0.900***	***0.001***	***0.181***	***0.022***
Okra	10	6.76	0.993	2.16	5.54	0.933	1.84	0.097	0.017	0.042
Tomato	21	0.217	9.89	1.85	0.175	7.290	1.470	0.005	0.181	0.034
Pumpkin	6	0.472	3.03	1.59	0.423	2.14	1.14	0.010	0.082	0.032
Chilli	11	0.468	2.33	1.19	0.415	2.61	0.996	0.007	0.059	0.023
Cucumber	5	0.640	2.17	1.25	0.513	1.62	0.974	0.009	0.045	0.024
Aubergine	7	0.309	1.67	0.911	0.253	1.47	0.818	0.004	0.037	0.020
Capsicum	3	0.303	0.552	0.417	0.231	0.478	0.385	0.005	0.012	0.009
Beans	11	0.164	1.21	0.591	0.147	0.905	0.448	0.004	0.025	0.010
Green pea	1	0.032	0.032	0.032	0.032	0.032	0.032	0.001	0.001	0.001

Table 3.8. Continued

Plant type	No of samples	I_{sol} - TF			I_{ads} - TF			I_T - TF		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Tubers		***0.259***	***4.318***	***0.887***	***0.636***	***1.71***	***0.551***	***0.005***	***0.062***	***0.017***
Radish	1	0.648	0.648	0.648	0.551	0.551	0.551	0.016	0.016	0.016
Carrot	4	0.804	2.437	1.39	0.636	2.73	1.27	0.011	0.062	0.028
Onion	12	0.259	4.32	0.868	0.224	3.54	0.699	0.005	0.062	0.014
Potato	11	0.646	1.07	0.822	0.444	1.14	0.626	0.009	0.026	0.015
Turnip	2	0.547	0.863	0.705	0.514	0.612	0.563	0.007	0.014	0.010
Fruits		***0.096***	***2.04***	***0.727***	***0.108***	***1.81***	***0.616***	***0.002***	***0.044***	***0.014***
Grapes	1	2.04	2.04	2.04	1.81	1.81	1.81	0.044	0.044	0.044
Ber	1	0.547	0.547	0.547	0.616	0.616	0.616	0.015	0.015	0.015
Mulberry	3	0.735	1.45	1.12	0.559	0.934	0.785	0.011	0.025	0.016
Fig	3	0.397	0.763	0.556	0.389	0.660	0.540	0.007	0.017	0.012
Pear	11	0.346	0.974	0.607	0.270	1.09	0.488	0.007	0.025	0.011
Apricot	19	0.136	0.881	0.493	0.115	0.622	0.387	0.003	0.024	0.009
Peach	4	0.293	0.909	0.512	0.244	0.625	0.391	0.003	0.015	0.008
Apple	18	0.096	1.15	0.484	0.108	0.819	0.381	0.002	0.019	0.008
Persimmon	3	0.115	0.242	0.183	0.113	0.184	0.152	0.002	0.004	0.003
Nuts		-	-	***0.656***	-	-	***0.492***	-	-	***0.013***
Almond	1	1.14	1.14	1.14	0.850	0.850	0.850	0.024	0.024	0.024
Walnut	1	0.176	0.176	0.176	0.134	0.134	0.134	0.003	0.003	0.003

3.3.9 Selenium in water (Se_w)

Figure 3.22 describes the spatial distribution of Se_w in the area. The median concentration of Se_w in all samples (surface and ground) used for various purposes (irrigation/drinking) was 0.246 μg L^{-1} and ranged from 0.016 - 3.04 μg L^{-1}. For the majority of the samples (~93%) Se_w concentration was < 1 μg L^{-1} (Appendix 2). Most groundwaters had larger average Se_w compared to surface waters, considering all samples together or within each district (Table 3.9 and Figure 3.23a and b).

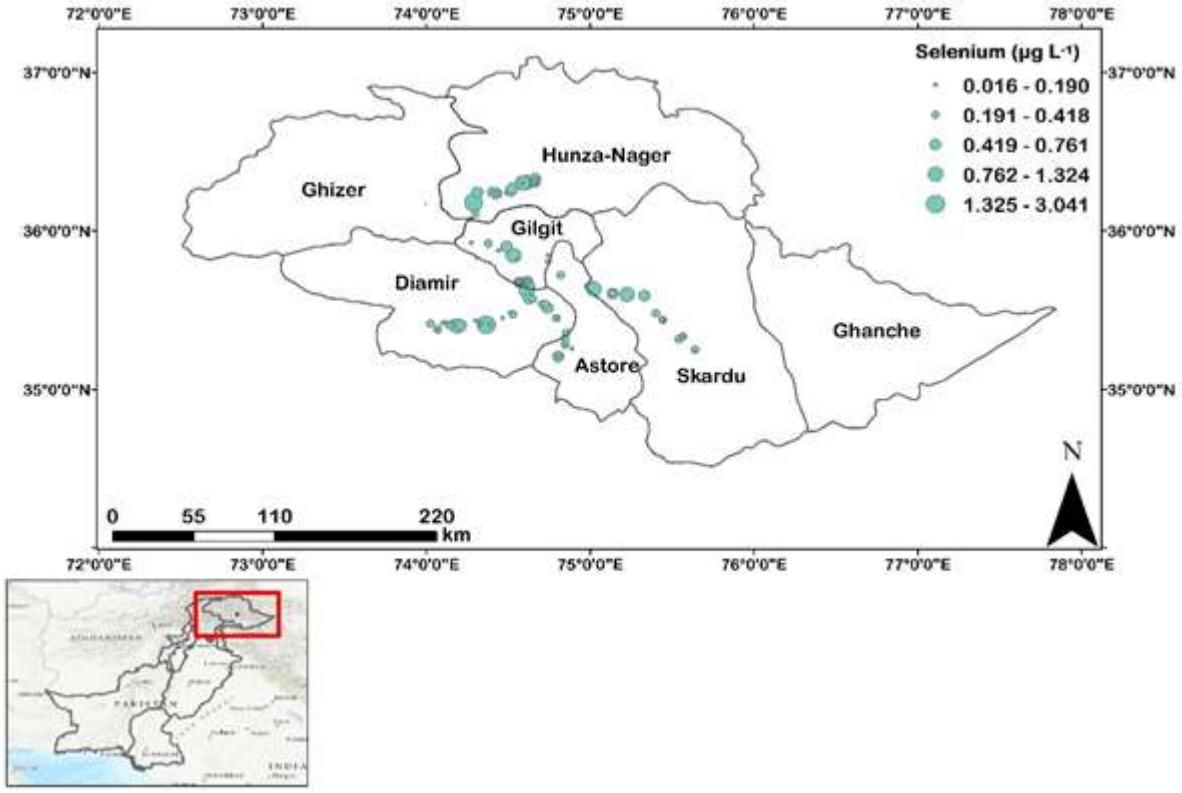


Figure 3.22. Spatial distribution of Se concentration in ground and surface water of Gilgit-Baltistan

Table 3.9. Concentration of Se_w in districts of Gilgit-Baltistan

Sample source and location	n	Se (µg L^{-1})		
		Mean	Median	Range
Surface water				
Stream and river				
Gilgit	19	0.41	0.127	0.041 - 2.17
Diamer	14	0.21	0.103	0.016 - 0.892
Hunza-N	14	0.296	0.254	0.026 - 0.590
Astor	12	0.177	0.161	0.047 - 0.398
Skardu	10	0.319	0.260	0.103 - 0.908
Lake				
Hunza-N	1	0.349	-	-
Skardu	2	0.149	-	0.137 - 0.161
Groundwater				
Spring				
Gilgit	5	0.421	0.418	0.081 - 0.748
Diamer	8	0.755	0.394	0 - 3.04
Hunza-N	5	0.78	0.670	0.528 - 1.19
Astor	8	0.396	0.313	0.047 - 1.073
Skardu	8	0.452	0.332	0.117 - 1.32
Well				
Skardu	1	0.031	-	-

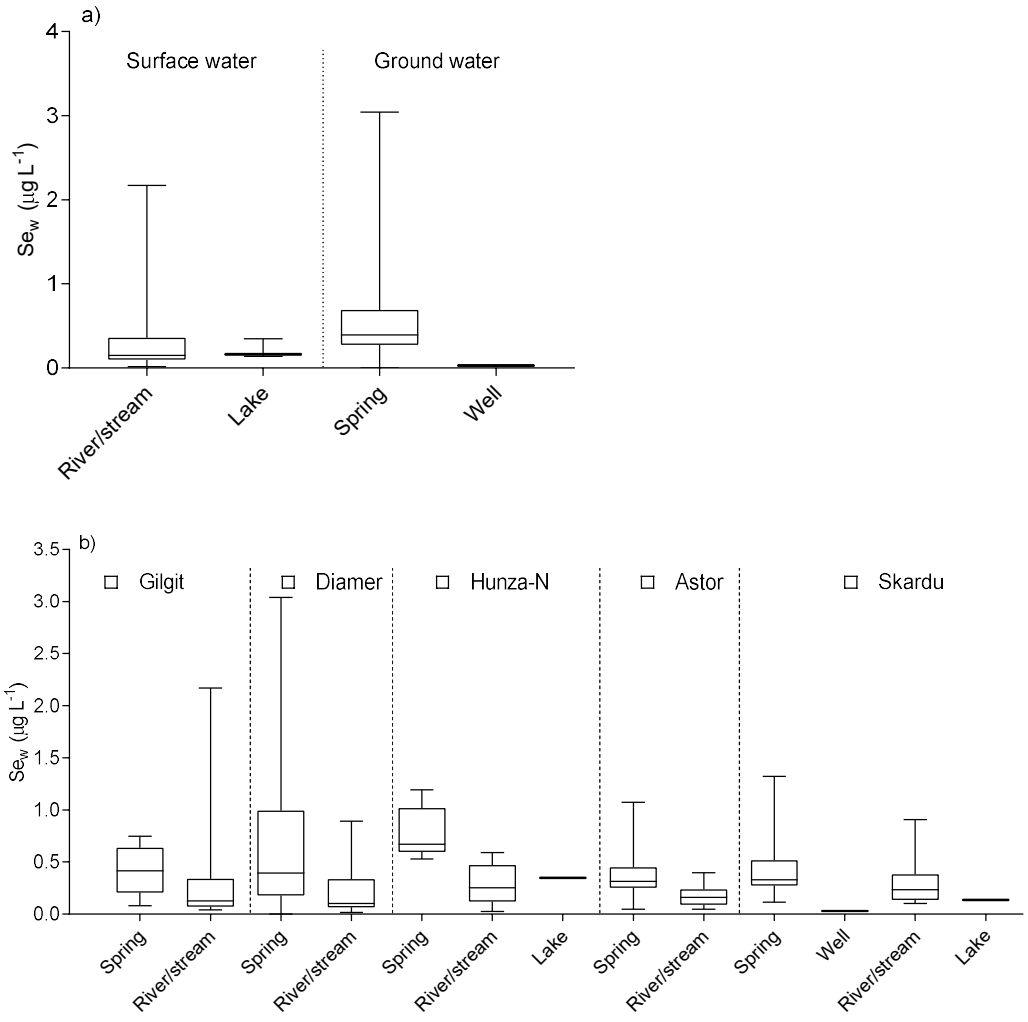


Figure 3.23. a) Box and whisker plot of Se_w in groundwater and surface water considering all samples together, b) Box and whisker plot of Se_w in surface water and groundwater sources in each district

The literature reports that Se in fresh water is generally very low and does not often exceed 10 µg L^{-1} (Fordyce et al., 1998; Fordyce, 2013). It largely falls within the range of 0.1 - 100 µg L^{-1} with most of the values below 3 µg L^{-1} (Fishbein, 1983; Plant et al., 2003; Fordyce, 2007; Fordyce, 2013). The low concentration of Se_w probably reflects

the geology of the area. As mentioned previously, GB has a complex geology with a dominant presence of igneous and metamorphic rocks, which are generally very low in Se. The results of this study correspond well with the observation of Fordyce (2013) that Se-deficient environments are more pervasive than Se-sufficient ones.

3.3.9.1 Surface waters

Selenium in surface waters ranged from 0.016 – 2.17 μg L^{-1} with a median concentration of 0.156 μg L^{-1}. The results of this study are in agreement with other investigations. Wang et al. (1994) reported that the Se concentration in river waters of several European countries, Japan and USA is largely < 1 μg L^{-1}. Lake waters have also shown small Se concentration in different countries such as 0.062 μg L^{-1}, 0.104 μg L^{-1} and 0.135 μg L^{-1} in lake waters from Finland, Sweden and Norway respectively (Wang et al., 1995). Conde and Alaejos (1997) have reported small Se concentrations in lakes, largely less than 1 μg L^{-1}, from various countries across the globe including Australia, Belgium, Germany, Sweden, Finland, Canada, China, Russia and USA.

3.3.9.2 Groundwaters

The median concentration of Se_w in groundwaters was 0.390 μg L^{-1} and ranged up to 3.04 μg L^{-1}. The relatively higher concentration of Se_w in groundwater is comparable with data reported in literature. Watts et al. (2010) reported higher concentrations in groundwater samples than surface water in Argentina. However, some studies have reported concentration of Se as high as 275 μg L^{-1} and 1000 μg L^{-1} in seleniferous aquifers of China and USA (Fordyce et al., 2000a, Fordyce, 2007). The reason for

higher Se_w in ground water is reported by Hem (1985), Watts et al. (2010) and Fordyce, (2013) as being due to a greater contact time of water rock interactions.

3.3.9.3 Speciation of selenium in water

Typically, all surface water and groundwaters had Se_w present both in inorganic and organic forms (Appendix 2). On average, inorganic species accounted for the larger proportion (~65%) of Se_w while the remaining ~35% was present in organic form. In all cases the inorganic Se_w was present as selenate (Se^{VI}); no selenite (Se^{IV}) was detected in any of the water samples (Appendix 2). The dominant presence of inorganic Se^{VI} is comparable with other studies. Wang et al. (1994) reported higher concentrations of inorganic Se, with Se^{VI} as the principal species, in river waters from Finland, Japan and USA. Other workers such as Conde and Alaejos (1997) and Cutter (1985) have also reported that Se^{VI} was the major inorganic species in river waters of various countries across the world. Bujdoš et al. (2005) studied Se speciation in water from Slovakia which indicated Se^{VI} as the major species in water with pH 7 and above. The large concentration of Se^{VI} is partly because its greater solubility and much weaker adsorption on sediments compared to Se^{IV} (Cary and Gissel-Nielsen, 1973; Fishbein, 1983; Wang et al., 1994; Wuilloud and Berton, 2014). The other reason for high concentrations of Se^{VI} may be because Se_w is naturally present in this form at its source in the area. Among groundwater sources, the single well (n = 1) sample had all Se_w present as organic Se. This could be because of microbial activity within the well water; some microbes are capable of converting inorganic Se to organic species (Abd Alla et al., 2017).

3.3.10 Relationship between water characteristics and selenium

There was no correlation observed between concentration of Se_w or Se species and water characteristics such as pH and DOC ($p > 0.05$). However, Se_w had a significant correlation ($r = 0.678$, $p < 0.01$) with DIC in both surface water and groundwaters (Figure 3.24) which may reflect the local geology of the aquifer or basin from which the water has originated.

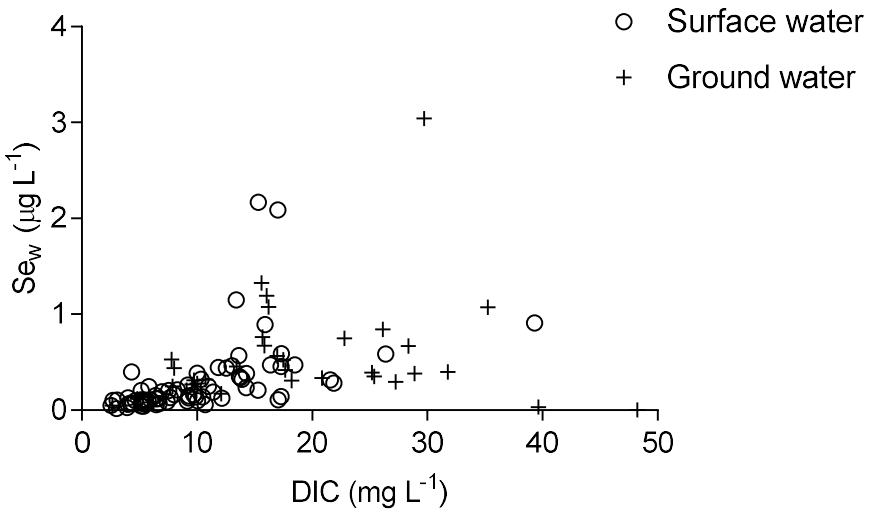


Figure 3.24. Correlation between DIC and Se_w

3.3.11 Soil selenium

3.3.11.1 Total soil selenium (Se_T)

The average Se_T concentration across all districts was 209 µg kg^{-1} and ranged from 92.7 – 453 µg kg^{-1}. Appendix 4 presents soil Se_T concentrations of each site and Figure 3.25 describes its spatial distribution. Hunza-N district had the highest mean Se_T of 346 µg kg^{-1} and was significantly different from the other four districts as presented

in Figure 3.26 (ANOVA, $p < 0.05$). Districts of Gilgit, Diamer, Astor and Skardu had mean Se_T concentrations of 190, 132, 143 and 208 μg kg^{-1} respectively which were not significantly different from each other (ANOVA, $p > 0.05$). All sites had Se_T concentrations less than the global mean of 400 μg kg^{-1} (Fordyce et al., 2000) except two locations in Hunza-N (N11 and N13) (Appendix 4). The generally low Se_T concentrations in the area probably reflect the geology of the area, sandy soil texture and typically low organic carbon. The geology of the study area is dominated by metamorphic and igneous rocks, which usually contain less Se compared to sedimentary rocks (Koljonen, 1973; Fordyce et al., 2010). Underlying rocks type have a major role in Se concentration in most soils (Fordyce, 2007; Fordyce et al., 2009). Sandy soils generally retain less Se compared to clayey soils (Antanaitis et al., 2008) and organic carbon plays an essential role in retaining soil Se (Li et al., 2017b). The slightly higher Se_T in two sites (N11 and N13) may be a localised effect possibly reflecting the effect of prolonged use of irrigation water; the corresponding irrigation waters of N11 and N13 had relatively high Se_w concentrations. Moreover, soils from these sites had relatively high organic carbon contents compared to other sites.

The majority of the soils in this study were marginally deficient in Se based on the threshold values for Se deficiency (< 125 μg kg^{-1}) and marginal deficiency (>125 – 175 μg kg^{-1}) soils reported in Fordyce et al., (2009). The typically low Se_T concentrations in the area are in the range for sandy soils reported in other parts of the world. For example, sandy soils in Poland, Lithuania, Russia, Finland and Canada had 140, 140, 180, 210 and 270 μg kg^{-1} of Se_T respectively (Kabata-Pendias and Mukherjee, 2007). Watts et al. (2010) reported 300 μg kg^{-1} Se in a mountainous area of San Juan in

Argentina which is in a similar range to this study. Similarly Chilimba et al. (2011) reported an average Se concentration of 194 μg kg^{-1} in Malawian soils which is typical of the region due to its geology and advanced weathering of many landscapes.

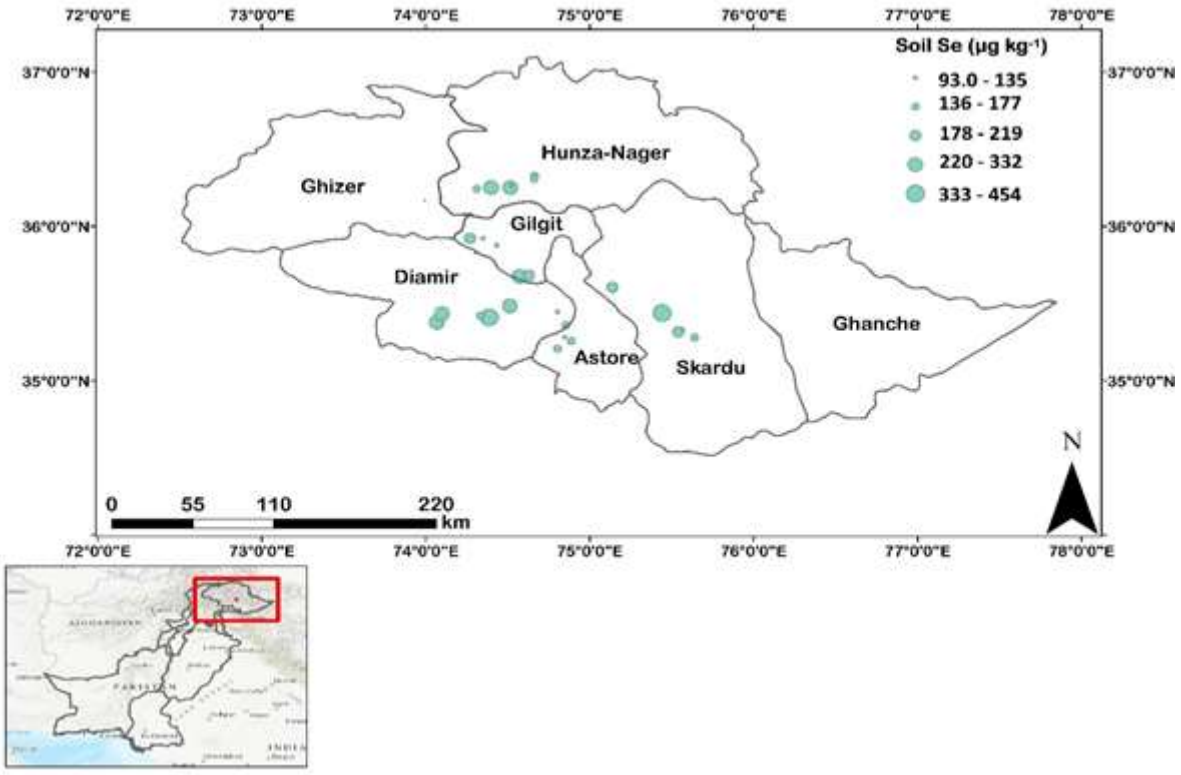


Figure 3.25. Spatial distribution of soil Se_T concentrations in Gilgit-Baltistan

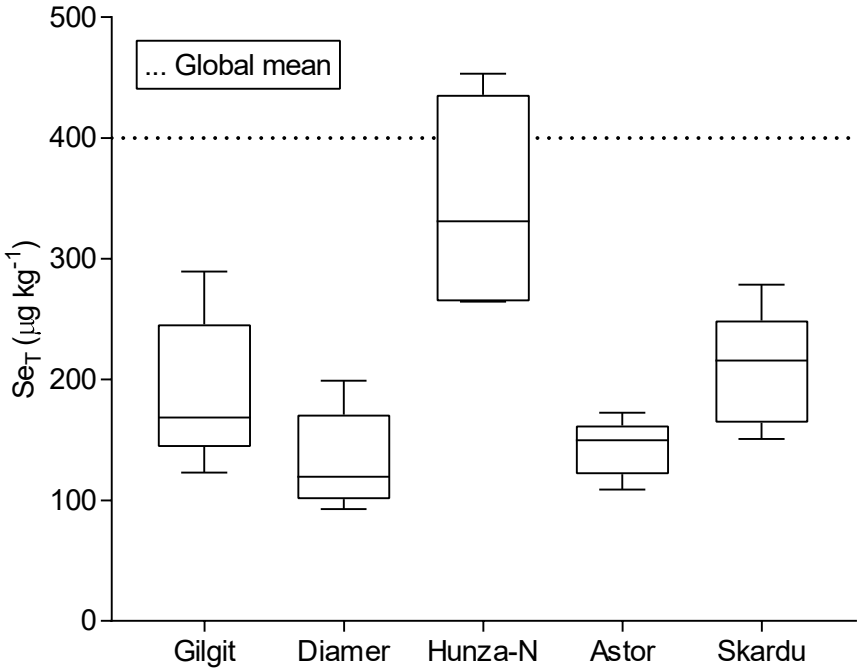


Figure 3.26. Concentration of soil Se_T in different districts of the study area

3.3.11.2 Fractionation of soil selenium

Soluble selenium (Se_{sol})

The soluble Se fraction (extracted with 0.01 M KNO_3) ranged from 0.95 to 6.52 μg kg^{-1} with mean and median values of 2.54 and 2.09 μg kg^{-1}. A significant variation in Se_{sol} was seen between districts (ANOVA, $p < 0.05$). The districts of Hunza-N and Astor had the highest (3.84 μg kg^{-1}) and lowest (1.36 μg kg^{-1}) values of Se_{sol} respectively. Districts Skardu, Gilgit and Diamer had mean Se_{sol} values of 2.71, 2.60 and 1.94 μg kg^{-1} respectively. The range of Se_{sol} concentration in each district is shown in Figure 3.27. The concentration of Se_{sol} as a percentage of soil Se_T (%Se_{sol}) was very low and typically accounted for < 2% of Se_T across all sites (Table 3.10). There was no significant variation between districts (ANOVA, $p > 0.05$) which also suggests %Se_{sol}

was independent of soil Se_T. The low extractability of Se_{sol} in soils is comparable with other studies. Karim (2018) used the same sequential extraction procedure and found that $\%Se_{sol}$ ranged from 0.096 – 2.18% in Kurdistani soils.

Table 3.10. Fractionation of Se as concentration and as a proportion of total soil selenium (Se_T)

District	Village	Sample code	Se_T	Se_{sol}	Se_{ads}	Se_{TMAH}	Se_{sol}	Se_{ads}	Se_{TMAH}
			($\mu g\ kg^{-1}$)				(% Se_T)		
Gilgit	Juglote-MARC	G01	168	2.12	3.09	83.6	1.26	1.83	49.6
	Basin	G02	165	2.00	3.28	92.0	1.21	1.99	55.7
	Daynor	G03	289	4.45	4.26	139	1.54	1.47	48.0
	Minawar	G04	123	1.65	2.25	51.4	1.34	1.83	41.8
	Bargin	G05	202	2.78	4.09	149	1.38	2.03	73.9
Diamer	Gorabad	D06	109	2.11	2.25	54.3	1.93	2.06	49.7
	Gonar Farm	D07	199	3.41	4.61	106	1.72	2.32	53.1
	Chillas-MARC	D08	143	1.79	1.91	59.7	1.25	1.34	41.9
	Khai Batuga	D09	120	1.33	1.26	70.7	1.12	1.05	59.2
	Gas Bala	D10	92.7	1.07	1.00	32.4	1.15	1.08	34.9
Hunza-N	Chalt Nagar	N11	430	5.11	7.41	177	1.19	1.72	41.1
	Hussainabad	H12	329	2.08	1.99	106	0.631	0.604	32.2
	Pissan Nagar	N13	453	6.52	7.82	293	1.44	1.73	64.6
	Nasirabad	H14	333	4.65	4.77	165	1.40	1.44	49.5
	Karimabad	H15	265	2.20	1.62	108	0.830	0.613	40.6
	Smar Khay	N16	265	2.52	1.98	126	0.951	0.748	47.5
Astor	Harachu	A17	151	1.57	1.13	66.8	1.04	0.749	44.2
	Chungra	A18	135	1.64	1.47	65.2	1.22	1.09	48.4
	Gorikot-MARC	A19	150	1.42	1.70	57.0	0.948	1.14	38.1
	Rahman Pur	A20	172	1.22	1.26	61.7	0.708	0.729	35.8
	Mankiyal	A21	109	0.951	0.987	47.8	0.87	0.907	43.9
Skardu	Torki	S22	178	3.67	2.21	91.3	2.06	1.24	51.3
	Kachura	S23	219	1.87	2.01	146	0.852	0.919	66.7
	Gamba	S24	279	3.82	5.21	128	1.37	1.87	46.1
	Ranga	S25	216	2.82	2.51	82.6	1.31	1.17	38.3
	Skardu	S26	151	1.40	0.944	45.5	0.927	0.626	30.2

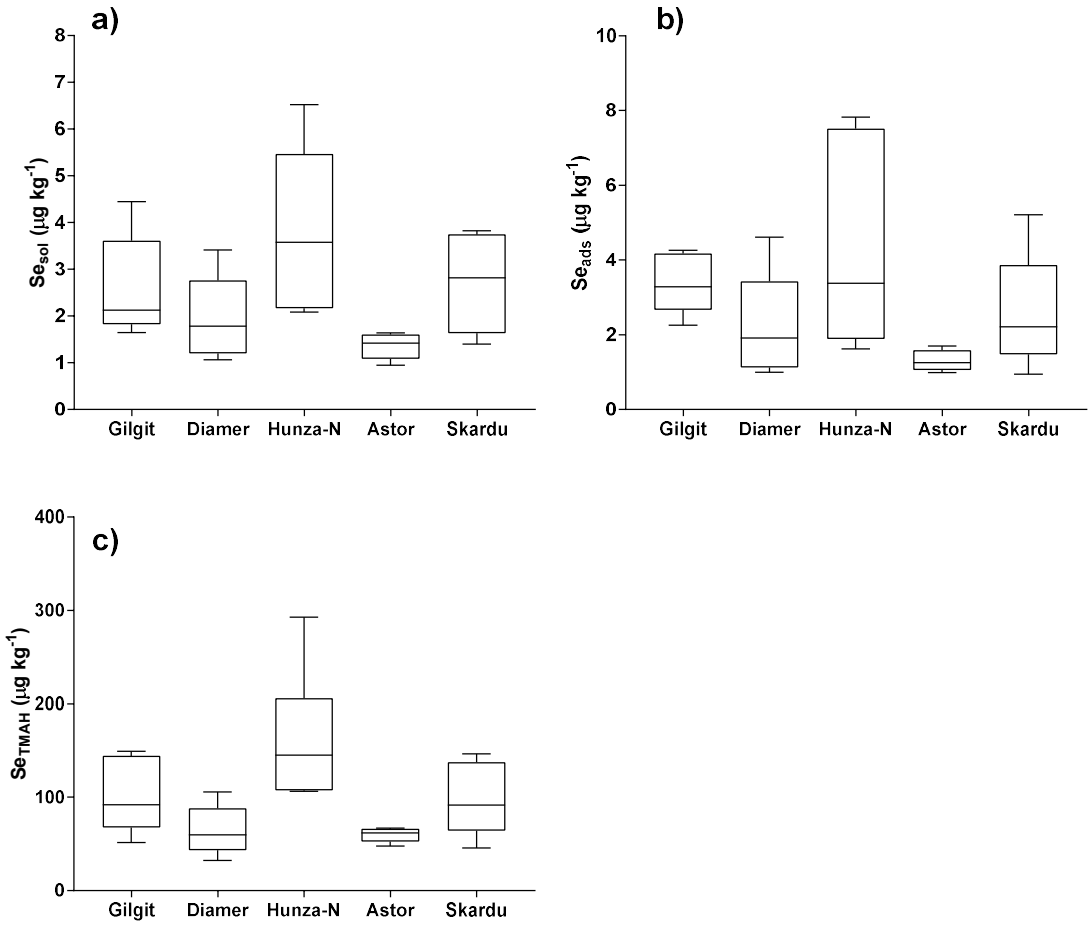


Figure 3.27. Concentrations of a) soluble Se (Se_{sol}), b) adsorbed Se (Se_{ads}) and c) humus-bound Se (Se_{TMAH}) in soil samples from each district of GB

Wang et al. (2012) reported < 1% of soluble Se in agriculture soils of Shaanxi province in China. Tan et al. (2002) studied the concentration of soluble Se in 20 different soil types in China and found it varied from 1.07 – 6.69%. The use of parallel single extraction method on soils from the UK showed that water-soluble Se accounted for 1.4 – 14% of the total soil Se (Tolu et al., 2011). Keskinen et al. (2009) studied the fate of residual Se in Finland soils, amended with Se fertilizers, and observed that soluble Se account for approximately 1% of Se_T.

Adsorbed selenium (Se_{ads})

Adsorbed Se possibly representing the Se fraction associated with metal oxides, was extracted with KH_2PO_4. The range of Se_{ads} concentrations (0.944 – 7.82 μg kg^{-1}) was similar to that of Se_{sol} (Figure 3.27 and Table 3.10); the average Se_{ads} in all samples was 2.81 μg kg^{-1}. A significant variation in Se_{ads} concentration among districts was observed with Hunza-N exhibiting the highest mean value of 4.27 μg kg^{-1}. The average concentrations in other districts; Gilgit, Skardu, Diamer and Astor were 3.39, 2.58, 2.20 and 1.31 μg kg^{-1} respectively.

Adsorbed Se extractability (%Se_{ads}) as a percentage of soil Se_T was not significantly different from that of Se_{sol} ($P > 0.05$, ANOVA). For all samples, Se_{ads} recovery ranged from 0.604 – 2.32 % and had a mean value of 1.31%. The average Se_{ads} extractabilities for Gilgit, Diamer, Skardu, Hunza-N and Astor were 1.83, 1.57, 1.17, 1.14 and 0.923% respectively. Karim (2018) reported a comparable range of adsorbed Se as a % of Se_T with mean value of 1.88% in 97 soil samples from Kurdistan. Mathers (2015) determined %Se_{ads} for 78 Malawi and 236 UK soils and reported values of 3.12% and 2.62% respectively. The results of a Malawi national survey, including 87 soil samples, reported %Se_{ads} values of <1-8% (Chilimba et al., 2011); Stroud et al. (2012) reported %Se_{ads} values of 1.1 – 3.4% for UK soils. Some studies have also reported higher levels of %Se_{ads}: Tolu et al. (2011) measured 20% in a clay loam soil from the UK; Schilling et al. (2011) and Schilling et al. (2014) reported 12 – 35% and 12 – 27% in German and Indian soils respectively; Keskinen et al. (2009) found 15 – 20% in Finnish soils.

Humus-bound selenium

Humus-bound Se (Se_{TMAH}) denoting the organically bound fraction of soil Se_T was extracted with 10% TMAH (pH > 12). Concentrations ranged from 32.4 – 293 μg kg^{-1} with an average value of 100 μg kg^{-1} considering all samples together. There was a significant variation between districts; concentrations in Hunza-N, Gilgit, Skardu, Diamer and Astor were 162, 103, 98.9, 59.7 and 64.5 μg kg^{-1} respectively. The extractability of Se_{TMAH}, as a percentage of Se_T, ranged from 30 to 74% with an overall average of 47%; there was no significant variation between districts (ANOVA, P > 0.05). For the majority of samples (65%) Se_{TMAH} was less than 50% of Se_T. This suggest that a substantial amount of Se is present in a refractory pool, extractable only with the HF-$HClO_4$-HNO_3 digestion procedure. The form of Se which resists solubilization with TMAH is not known but is likely to be present within mineral structures (Mathers, 2015). Comparable (average) recovery of Se_T (41%) with TMAH was observed in 97 soil samples from Kurdistan (Karim, 2018). A wide range of Se recoveries in soils and sediments using alkaline extractions has been reported, including 31.9- 70.1% (Schilling et al., 2014), 31.8 – 52% (Qin et al., 2012), 35-50% (Keskinen et al., 2009), 29- 37% (Ponce de León et al., 2003), 50% (Séby et al., 1997) in soils and sediments from India, China, Finland, Canada and Ireland respectively.

3.3.11.3 Speciation of soil selenium

Speciation analysis was performed on Se_{sol} and Se_{ads} respectively, which demonstrated that Se was present in organic as well as inorganic forms in both fractions. The details of speciation analysis of Se_{sol} and Se_{ads} are provided in Table

3.11 and 3.12 respectively. A large proportion of Se_{sol} was organic Se with an average value of 66.9% and a range from 43.5% to 89.9% considering all samples together. The average proportion of organic Se_{sol} did not show significant variation between districts ($p > 0.05$) and consisted of 60.1, 65.8, 69, 75.5 and 64% of total Se_{sol} in Gilgit, Diamer, Hunza-N, Astor and Skardu respectively. The soluble organic Se was almost certainly linked to soil humus acids but soluble organic Se may also be present in parent materials. Kulp and Pratt (2004) reported that a large proportion of soluble Se was present as organic Se in different rocks from the USA. Similarly, Zhang and Moore (1996) reported a large proportion of the soluble Se fraction in wetland sediments from Montana was organically bound.

Table 3.11. Concentration (µg kg^{-1}) of Se species in Se_{sol}; the proportion of organic (Org Se_{sol}) and inorganic (Inorg Se_{sol}) as a percentage of total Se_{sol} and proportion of Se^{IV} and Se^{VI} as a percentage of Inorg Se_{sol}

District	Village	Sample code	Org Se_{sol}	In-org Se_{sol}	%Org Se_{sol}	%In-org Se_{sol}	$\%Se_{sol}^{IV}$	$\%Se_{sol}^{VI}$
			(µg kg^{-1})		(%)			
Gilgit	Juglote-MARC	G01	1.07	1.05	50.5	49.5	95.8	4.17
	Basin	G02	1.22	0.773	61.3	38.7	97.4	2.55
	Daynor	G03	3.49	0.953	78.6	21.4	96.2	3.85
	Minawar	G04	0.951	0.699	57.6	42.4	100	0.00
	Bargin	G05	1.45	1.32	52.3	47.7	100	0.00
Diamer	Gorabad	D06	1.18	0.923	56.2	43.8	95.7	4.29
	Gonar Farm	D07	1.58	1.84	46.2	53.8	100	0.00
	Chillas-MARC	D08	1.03	0.757	57.6	42.4	97.0	3.00
	Khai Batuga	D09	1.13	0.201	84.9	15.1	96.8	3.15
	Gas Bala	D10	0.895	0.171	83.9	16.1	100	0.00
Hunza-N	Chalt Nagar	N11	2.40	2.71	47.0	53.0	97.0	2.96
	Hussainabad	H12	1.77	0.308	85.2	14.8	95.1	4.86
	Pissan Nagar	N13	4.00	2.52	61.4	38.6	98.8	1.23
	Nasirabad	H14	2.81	1.84	60.4	39.6	100	0.00
	Karimabad	H15	1.71	0.491	77.7	22.3	97.6	2.44
	Smar Khay	N16	2.07	0.444	82.4	17.6	97.2	2.80
Astor	Harachu	A17	1.39	0.183	88.3	11.7	97.7	2.27
	Chungra	A18	1.14	0.499	69.5	30.5	98.7	1.26
	Gorikot-MARC	A19	0.618	0.801	43.6	56.4	98.2	1.83
	Rahman Pur	A20	1.10	0.123	89.9	10.1	83.7	16.3
	Mankiyal	A21	0.816	0.135	85.8	14.2	87.5	12.5
Skardu	Torki	S22	2.21	1.46	60.3	39.7	100	0.00
	Kachura	S23	1.18	0.682	63.5	36.5	96.9	3.12
	Gamba	S24	1.98	1.84	51.8	48.2	99.7	0.31
	Ranga	S25	1.85	0.971	65.5	34.5	98.1	1.94
	Skardu	S26	1.10	0.293	79.0	21.0	99.1	0.92

Table 3.12. Concentration (µg kg^{-1}) of Se species in Se_{ads}; the proportion of organic (Org Se_{ads}) and inorganic (Inorg Se_{ads}) as a percentage of total Se_{ads} and proportion of Se^{IV} and Se^{VI} as a percentage of Inorg Se_{ads}

District	Village	Sample code	Org Se_{ads}	In-org Se_{ads}	%Org Se_{ads}	%In-org Se_{ads}	$\%Se_{ads}^{IV}$	$\%Se_{ads}^{VI}$
			(µg kg^{-1})		(%)			
Gilgit	Juglote-MARC	G01	0.506	2.58	16.4	83.6	99.1	0.887
	Basin	G02	0.813	2.47	24.8	75.2	99.0	0.988
	Daynor	G03	1.85	2.41	43.4	56.6	99.7	0.288
	Minawar	G04	1.07	1.18	47.4	52.6	99.3	0.690
	Bargin	G05	1.29	2.80	31.5	68.5	100	0.268
Diamer	Gorabad	D06	1.23	1.01	55.0	45.0	99.1	0.867
	Gonar Farm	D07	1.25	3.36	27.1	72.9	100	0.438
	Chillas-MARC	D08	0.479	1.44	25.0	75.0	99.0	1.001
	Khai Batuga	D09	0.898	0.358	71.5	28.5	99.2	0.843
	Gas Bala	D10	0.685	0.311	68.8	31.2	100	0.095
Hunza-N	Chalt Nagar	N11	0.00	7.56	0.00	100	99.9	0.107
	Hussainabad	H12	1.27	0.72	64.1	35.9	98.4	1.55
	Pissan Nagar	N13	0.127	7.70	1.63	98.4	99.8	0.226
	Nasirabad	H14	0.673	4.10	14.1	85.9	99.4	0.591
	Karimabad	H15	0.748	0.875	46.1	53.9	99.4	0.648
	Smar Khay	N16	1.14	0.838	57.7	42.3	98.8	1.15
Astor	Harachu	A17	0.876	0.255	77.4	22.6	97.6	2.39
	Chungra	A18	0.877	0.595	59.6	40.4	97.2	2.77
	Gorikot-MARC	A19	0.593	1.11	34.9	65.1	99.1	0.87
	Rahman Pur	A20	1.105	0.151	87.9	12.1	94.4	5.60
	Mankiyal	A21	0.813	0.175	82.3	17.7	94.1	5.88
Skardu	Torki	S22	0.604	1.61	27.3	72.7	99.0	1.04
	Kachura	S23	0.527	1.49	26.2	73.8	99.1	0.90
	Gamba	S24	0.00	5.21	0.00	100	99.8	0.245
	Ranga	S25	0.122	2.39	4.86	95.1	99.4	0.617
	Skardu	S26	0.355	0.589	37.6	62.4	97.2	2.81

In the adsorbed fraction (Se_{ads}), there was a wide variation in the % organic Se, ranging from 0 – 87% with a mean of 39.7% for the whole data set. There was a significant difference between districts ($p < 0.05$) with Astor and Skardu accounting for the highest (68.4%) and lowest (19.2%) respectively. The proportions of organic

Se_{ads} in other districts were: 30.6% (Hunza-N), 32.7% Gilgit and 49.5% Diamer. The variation observed in adsorbed organic species is comparable with the results described in Kulp and Pratt (2004) who reported a range of 13.6 – 85% in phosphate-extractable Se in parent rocks.

The inorganic Se in Se_{sol} and Se_{ads} was present as Se^{IV} and Se^{VI} but the proportion of Se^{IV} in the soluble and adsorbed fractions ranged from 83.7 - 100% and 94.1 - 99.9% respectively. The proportion of Se^{IV} in inorganic Se of both soluble and adsorbed fraction did not show variation among different districts (ANOVA, $p > 0.05$). The large proportion of Se^{IV} in the soluble inorganic fraction contradicts other investigations in the literature but for the adsorbed fraction, the values were comparable to other studies. Karim (2018) reported that inorganic Se_{sol} was largely (65%) present as Se^{VI}; similarly, Kulp and Pratt (2004) also reported a large proportion of Se^{VI} in inorganic Se_{sol}. The large proportion of Se^{IV} in inorganic Se_{sol} in the current study could be due to the native presence of this species in geology of the area. By contrast, the large proportion of Se^{IV} in inorganic Se_{ads} was consistent with other investigations. Karim (2018) found that 96% of inorganic Se_{ads} was present as Se^{IV}. Similarly a study of Se speciation and extractability in Dutch agricultural soils found that Se was largely present as Se^{IV} in the adsorbed fraction extracted with ammonium oxalate (Supriatin et al., 2016). Wang et al. (2012) observed that Se^{IV} was the only inorganic species detected in an adsorbed fraction of soil samples from Shaanxi province in China; Kulp and Pratt (2004) reported the presence of only Se^{IV} in their adsorbed fraction.

3.3.12 Correlations between soil properties and Se contents

Soil organic carbon had a positive correlation with Se_{TMAH} and Se_T respectively for all data considered together (Table 3.13, Figure 3.28) but there was no correlation for intra-district data. This could be the result of small sample sizes and a narrow range of %SOC in each district. The literature reports that organic matter plays an essential role in accumulating Se in soil (Gustafsson et al., 1993; Xing et al., 2015). The %SIC also showed a positive correlation with Se_T and with the soil Se fractions (soluble, adsorbed and humus) for the whole set of data (Table 3.13) but again there were no significant correlations for the intra-district data.

A significant positive correlation of soil pH with all fractions of Se including Se_{sol}, Se_{ads}, Se_{TMAH} and Se_T was observed (Table 3.13, Figure 3.29). Xing et al. (2015) also reported a positive correlation between pH and the different fractions of Se in soils from the Dashan region of China. In contrast to this study, a negative correlation has been reported between pH and both Se_T and Se_{TMAH} (Xing et al., 2015; Mathers, 2015; Karim, 2018).

Table 3.13. Correlation among soil Se and soil characteristics

	Se_T	Se_{sol}	Se_{ads}	Se_{TMAH}	%Humus Se	SOC%	SIC%	pH	Clay	Silt	Sand	Comb_oxides
Se_T	1											
Se_{sol}	0.814*	1										
Se_{ads}	0.672*	0.887*	1									
Se_{TMAH}	0.9*	0.823*	0.745*	1								
%Humus Se	0.12^{NS}	0.374^{NS}	0.461**	0.487**	1							
SOC%	0.486**	0.126^{NS}	-0.04^{NS}	0.491**	0.141^{NS}	1						
SIC%	0.621*	0.62*	0.42**	0.468**	-0.138^{NS}	0.307^{NS}	1					
pH	0.56*	0.667*	0.464**	0.406**	-0.083^{NS}	0.056^{NS}	0.856*	1				
Clay	0.356^{NS}	0.238^{NS}	0.187^{NS}	0.218^{NS}	-0.193^{NS}	0.311^{NS}	0.661*	0.579*	1			
Silt	0.571*	0.576*	0.484**	0.452**	-0.01^{NS}	0.202^{NS}	0.662*	0.646*	0.623*	1		
Sand	-0.56*	-0.544*	-0.464**	-0.442**	0.027^{NS}	-0.225^{NS}	-0.673*	-0.638*	-0.672*	-0.992*	1	
Comb_oxides	0.624*	0.357^{NS}	0.342^{NS}	0.511*	-0.197^{NS}	0.379^{NS}	0.459**	0.335^{NS}	0.423**	0.121^{NS}	-0.158^{NS}	1

* Significant at 0.01, ** Significant at 0.05, ^{NS} Non significant

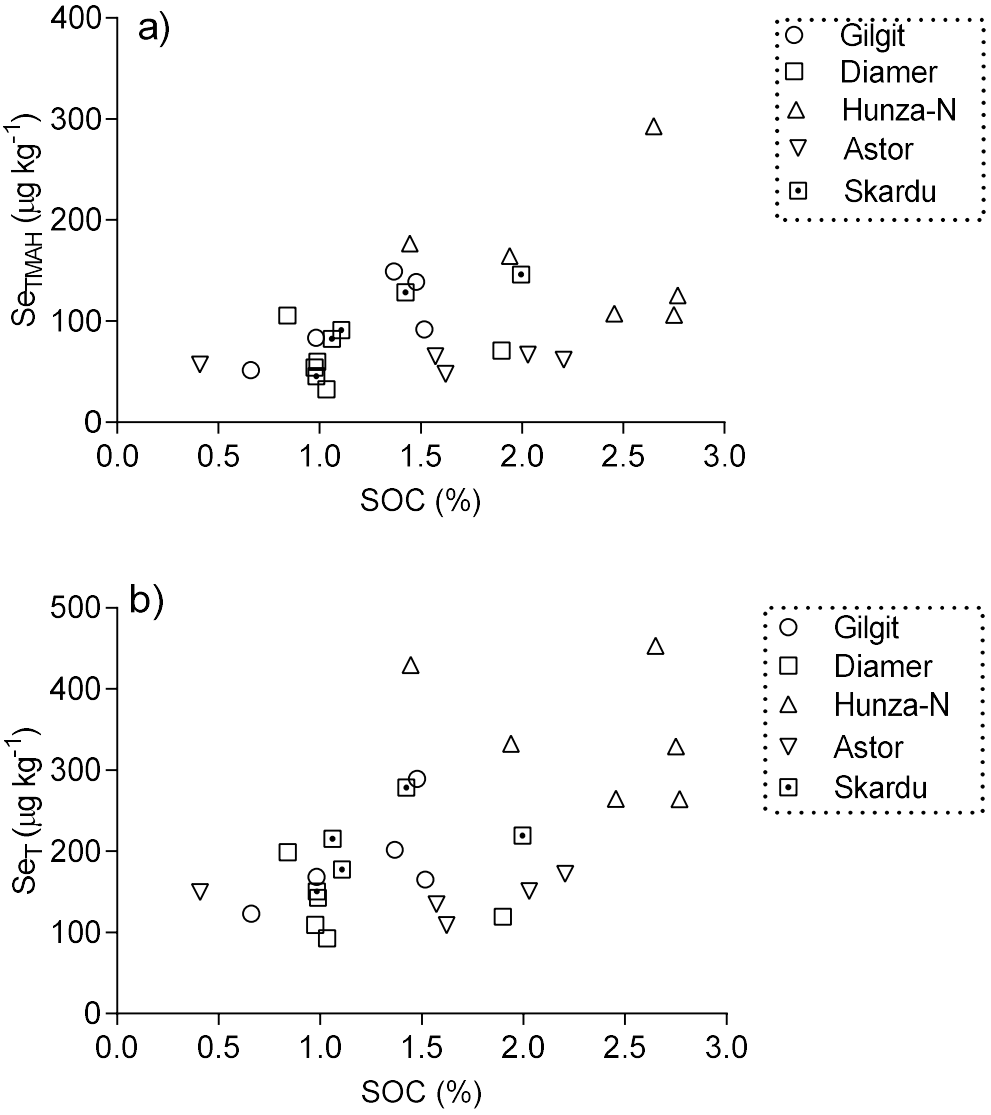


Figure 3.28. Relationship between a) SOC (%) and Se_{TMAH} and b) SOC (%) and Se_T

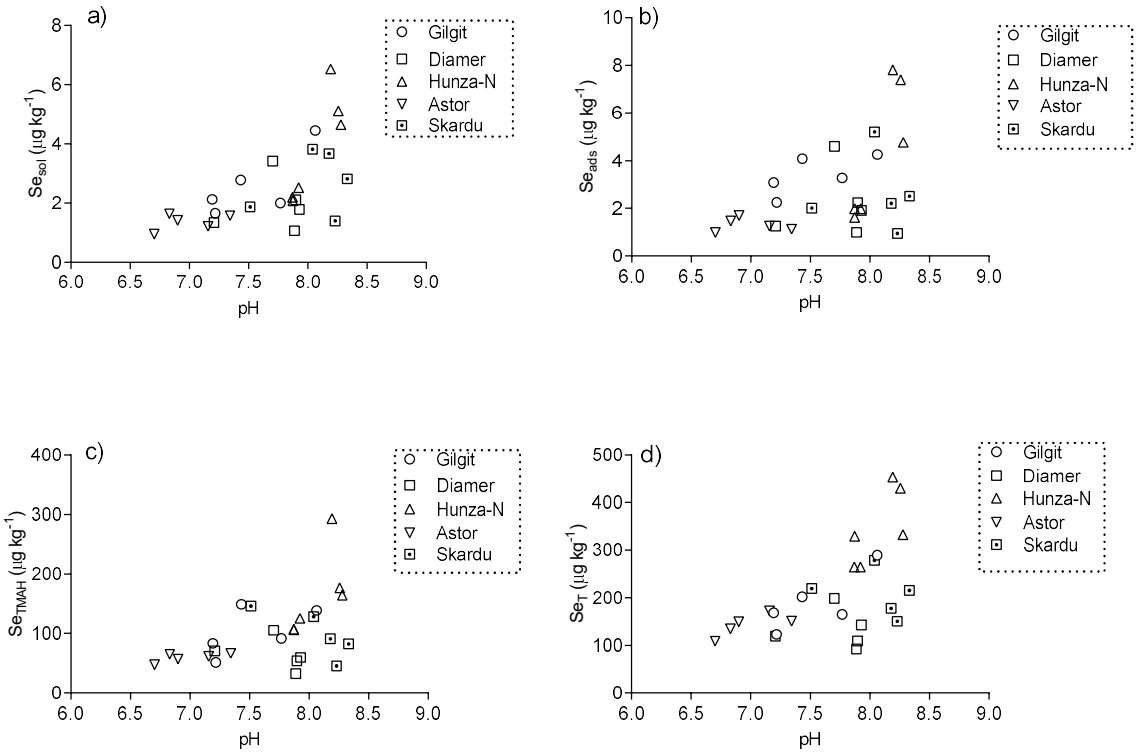


Figure 3.29. Relationship between soil pH and different fractions of soil Se

Xing et al. (2015) reported a positive correlation between clay and Se_T in soils from Hong Kong and mainland China. However, in the current study, the soil clay content did not show a correlation with any form of Se (Table 3.13). This may be due to the narrow range of clay (1.5-11.9%, median = 3.3%) in soils of this study. However, a positive correlation was observed between silt content and different fractions of Se including Se_T (Table 3.13) and there was a corresponding negative correlation between sand content and Se_T, Se_{sol}, Se_{ads} and Se_{TMAH} (Table 3.13).

3.3.13 Selenium in plants (Se_P)

The concentration of Se_P in plant groups and individual plant species from different districts is shown in Appendix 6. It was found that Se_P concentration generally decreased in the order of leafy vegetables > grains > crop vegetables > tubers > nuts > fruits when data of all districts was considered together (Figure 3.30). However, this pattern of change in Se_P concentration was not observed in every district individually (Figure 3.31). Leafy vegetables had the highest average Se_P concentrations in each district (Figure 3.31). The Se_P concentration in plant groups of fruits, tubers and crop vegetables varied significantly across districts (ANOVA; $p < 0.05$). However, the grains and leafy vegetable groups had broadly similar Se_P concentrations across all districts. Selenium concentration was low in the majority of plant species with variation among plant species in each group (Table 3.14 and Figure 3.31). Within leafy vegetables, cabbage (n =7) and lettuce (n = 1) had highest and lowest mean Se_P concentration of 232 $\mu g\ kg^{-1}$ and 31.1 $\mu g\ kg^{-1}$ respectively in all districts together as one data set. Cabbage Se_P concentration ranged from 65.7-515 $\mu g\ kg^{-1}$ with the highest value recorded for Hunza-N and lowest for Skardu (Appendix 6).

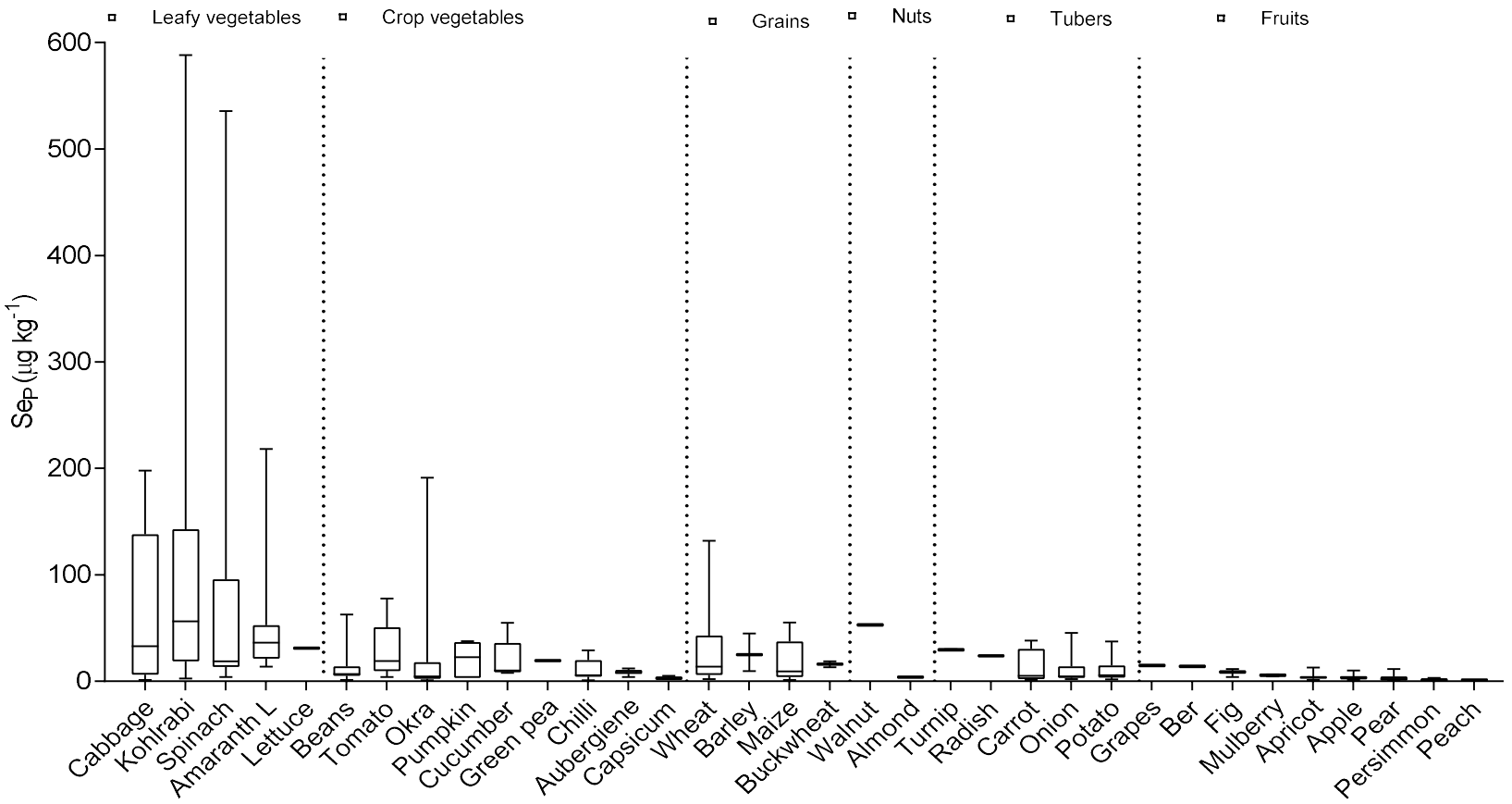


Figure 3.30. Selenium concentration (Se_P, µg kg^{-1}) in plant groups in different districts of Gilgit-Baltistan. Three outliers (one each from cabbage, kohlrabi and beans) were removed, as they did not fall within the 99% confidence interval of all the data

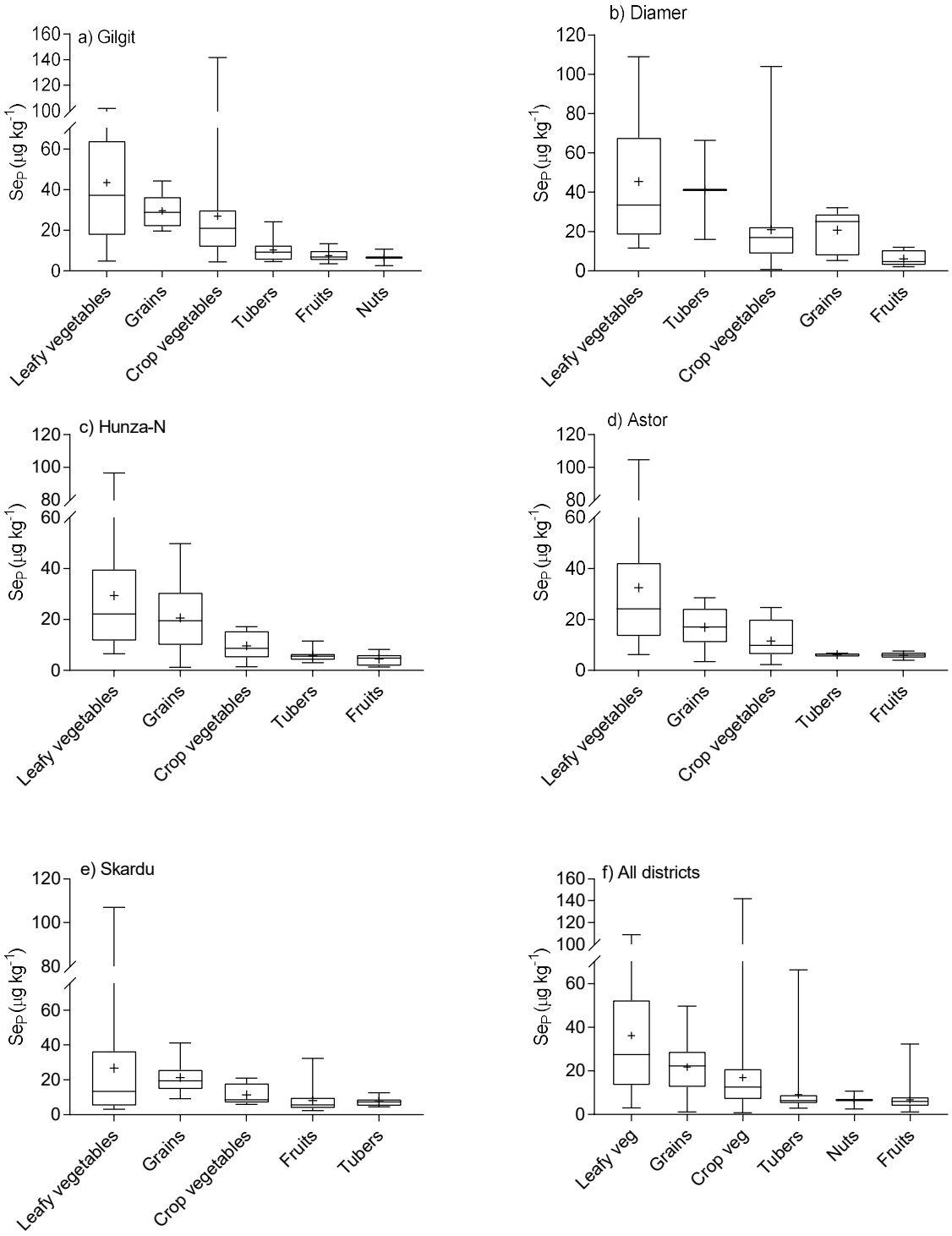


Figure 3.31. Selenium concentration (Se_P; µg kg^{-1}) of plant groups in sampling districts of GB

Table 3.14. Summary statistics for Se_P in plant groups and species in districts of GB

Plant group/species	No of samples	Selenium (μg kg^{-1})			
		Mean	Median	Range	SE(±)
Leafy vegetables	***56***	**105**	**34.8**	***1.17 - 1021***	**26.02**
Lettuce	1	31.1	-	-	-
Spinach	21	77.4	19.0	4.16 - 536	27.7
Amaranth leaves	14	48.6	36.5	13.9 - 218	13.8
Kholrabi	13	164	69.4	2.64 - 807	68.7
Cabbage	7	202	55.5	1.17 - 1021	139
Grains	***55***	**23.8**	**13.4**	***1.38 - 132***	**3.64**
Wheat	3	28.9	13.8	2.04 - 132	20.4
Maize	25	19.0	9.38	1.38 - 55.2	3.56
Barley	25	26.4	25.1	9.40 - 44.8	3.54
Buckwheat	2	16.2	-	13.4 - 18.9	2.72
Crop vegetables	***75***	**34.2**	**10.3**	***0.782 - 1137***	**15.2**
Okra	10	25.9	4.70	0.938 - 191	18.6
Tomato	21	28.5	19.1	4.28 - 77.9	5.03
Pumpkin	6	20.9	22.7	3.11 - 37.6	6.21
Chilli	11	10.9	5.67	0.782 - 29.1	3.02
Cucumber	5	19.8	10.3	8.10 - 54.9	8.92
Aubergine	7	8.62	8.03	4.16 - 12.2	1.00
Capsicum	3	3.46	3.04	2.23 - 5.11	0.858
Beans	11	116	6.87	1.43 - 1137	102
Pigeon pea	1	19.4	-	-	-
Tubers	***30***	**12.9**	**5.25**	***1.49 - 45.7***	**2.56**
Radish	1	24.0	-	-	-
Carrot	4	12.5	5.27	1.49 - 38.2	8.61
Onion	12	12.0	4.82	2.25 - 45.7	4.64
Potato	11	10.0	5.69	1.89 - 37.5	3.26
Turnip	2	29.6	-	29.2 - 30.1	0.41
Nuts	***2***	**28.5**	**-**	**4.01 - 52.9**	**24.4**
Almond	1	4.01	-	-	-
Walnut	1	52.9	-	-	-
Fruits	***63***	**4.39**	**3.45**	***0.741 -14.9***	**0.437**
Grapes	1	14.9	-	-	-
Ber (Chinese date)	1	14.0	-	-	-
Mulberry	3	5.64	5.8	4.66 - 6.50	0.53
Fig	3	8.13	8.65	4.21 - 11.5	2.13
Pear	11	3.63	2.05	0.740 - 11.7	1.13
Apricot	19	4.26	3.33	1.09 - 13.1	0.625
Peach	4	1.49	1.72	0.80 - 1.74	0.233
Apple	18	4.10	3.71	1.15 - 10.3	0.658
Persimmon	3	2.02	1.45	1.43 - 3.17	0.576

Table 3.15 presents a comparison for Se_P (dry weight basis) in different plant species with previous investigations elsewhere.

Leaf vegetables

The average Se_P concentration measured for cabbage was higher than other values reported in the literature for Belgium, India, Kashmir region-Pakistan and Saudi Arabia (De Temmerman et al., 2014; Singh and Garg, 2006; Waheed et al., 2002; Al-Ahmary, 2009). Lettuce samples were collected only from Skardu and no other data for comparison was available for the study area, however it was lower than values reported in Belgium and higher than Saudi Arabia (De Temmerman et al., 2014; Al-Ahmary, 2009). The mean Se_P concentration in spinach (n = 21; 75.4 μg kg^{-1}) was greater than in lettuce but lower than Se_P in amaranth leaves (57.9 μg kg^{-1}, n = 14) and kohlrabi (186 μg kg^{-1}, n = 13). The Se_P concentration in amaranth leaves measured in this study was much lower compared to values reported in the literature (Singh and Garg, 2006) whereas kohlrabi had a much higher Se_P concentration than published values (De Temmerman et al., 2014).

Grains

The average Se_P content of various grains measured in this study, and reported as a group, was less than values reported in Spain (Díaz-Alarcón et al., 1996). Wheat Se_P concentrations measured in this study were much lower compared to values reported from China, Spain, Egypt, India, Belgium, Kashmir region-Pakistan and Saudi Arabia (Mangan et al., 2016; Díaz-Alarcón et al., 1996; Askar and Bielig, 1983; Hussein and Bruggeman, 1999; Singh and Garg, 2006; De Temmerman et al., 2014; Waheed et al., 2002; Al-Ahmary, 2009). By contrast, maize had Se_P concentrations higher than

Table 3.15. Comparison of average selenium content in plant groups and species with other investigations

Sample	n	Current study (dw)	Other investigations (dw)	Other investigations (fw)
			Selenium concentration (μg kg^{-1})	
Leafy vegetables	***56***	***105***		
Lettuce	1	31.1	101[x], 10[z]	9[c], 8[h,j], 0.9[n], 2.4[o], 17.9[l], 7[q], 3[r], 8[s], 94.4[t], (0.3-20)[v]
Spinach	21	92.0	127[m], 117[w], 169[x], 16, 7[z]	7[c], 12[j], 12.4[x]
Amaranth leaves	14	48.6	362[m]	
Kholrabi	13	164	37[x]	2.1[n], 1.8[x]
Cabbage	7	202	38[m], 46[x], 31[y], 9[z]	(13,20)[c], 27[h], 16[l], 2[n], (8.3, 66.1)[p], 20.3[q], (1.1-76.7)[v], 6[x]
Grains	***55***	***23.8***	***(5-720)[a], 27.8[b]***	***380[d], 520[d], 110[d,f], 278[d], 500[e], (30, 80, 70)[f]***
Barley	3	26.4	(88.3-437)[a], 400[e], 625[m], 69[z]	435[c], 660[g], 320[j],
Maize	25	18.99	4.6[b], 149[m], 49.4[w], 110[z]	
Wheat	25	28.9	(70.5,74.1,72.9,73.2)[a], 35.5[b], 500[e], 329[m], 193[t], 54.8[x], 220[y], 165[z]	(43,320)[c], 25.1[n], 70[o], (68, 105)[f], 11.9[v], 48.7[x]
Buckwheat	2	16.2		49[f], 43.3[v]
Crop vegetables	***75***	***34.2***	***118[b]***	***(10, 20, 30, 125)[c]***
Okra	10	25.9	37.6[w], 27[z]	10[c]
Tomato	21	28.5	108[w], 36.2[x], 39[y], 15[z]	10[c], 1[h], 13[j], 0.5[l,n], 2.3[o], 1[t], (7.9, 10.2)[o,p], (1.1-29.1)[v], 1.8[x]
Pumpkin	6	20.9	11.9[x], 12[z]	(0.7,3.9)[v], 1.3[x]
Chilli	11	10.9		
Cucumber	5	19.8	95.1[w], 72.2[x], 21[z]	8[c], 120[h], 6[j], (6.3, 11.5)[p], 14.7[v], 1.8[x]
Aubergine	7	8.03	60.3[w], 28[y], 67[z]	11[c]
Capsicum	3	3.46	59.2[w], 52[z]	11[c], 22[j], 12.1[l], 0.7[n], 4.2[o], (9.1, 11.4)[p], (1.4, 11)[v]
Beans	11	116	210[b], 56[h], 31[f]	143[c], 18[i], 37.5[n], 24.4[o], 7[t], 52.6[v], (33.7, 48.5)[p]
Pigeon pea	1	19.4	60[h], 150[m]	(9, 25)[c], 45[j], 13.5[n]

Table 3.15. Continued

Sample	N	Current study (dw)	Other investigations (dw)	Other investigations (fw)
		Selenium concentration (μg kg^{-1})		
Tubers	***30***	***12.9***		
Radish	1	31.1	95.9[w], 18[x], 13[y], 33[z]	20[c], 40[g], 24[h], 20[j], 0.7[n], 90.3[t], 4.5[v], 0.87[x]
Carrot	4	12.5	43.4[x], 2[z]	15[c], 13[f], 4[h], 29[j], 1.3[n], 6.1[o], (8.1, 19.6)[p], (0.6-11.6)[v], 3.1[x]
Onion	12	12.0	15[c], 20[e], 180[m], 55.3[w], 40.9[x], 137[y], 43[z]	21[c], 13[h], (23, 31)[j], 5.8[n], 7.3[o], (12.4, 15.3)[p], (1.1-10.5)[v], 5.4[x]
Potato	11	10.0	30[e,h], 28[j], 325[m], (30-70)[k], 28.4[x], 48[y]	10[c], 6[f], 23[h], 16[j], 3.5[n], 4.6[o], (7.2, 9.5)[p], 11[u], 1.5[q,v], 5.8[x]
Turnip	2	29.6	29.1[x], 20[y]	26[h], 9[j], 2.2[x]
Fruits	***63***	***4.40***	***1.2[j]***	***(4, 6, 7, 9)[c], (4,6,7,30)[d]***
Grapes	1	14.9	23[z]	17[c], (12, 78)[f], (40-76)[k], 6.7[l], (8.9, 12.9)[p]
Ber	1	14.0		
Mulberry	3	5.64		
Fig	3	8.13	32[z]	16[c], 20[h]
Pear	11	3.63	20[y]	6[c], 4[h], 4.5[l], 6.3[o]
Apricot	19	4.26		13[c]
Peach	4	1.49	25[y]	13[c], 4[h], 5.8[l], 2[t], 11[p]
Apple	18	4.10	3[j], 4.5[k], 38[y], 11[z]	8[c], 4[h], 4.5[l], 1.4[n,o], (7.8, 8.8)[p]
Persimmon	3	2.02		
Nuts	***2***	***28.5***	***295[b]***	
Almond	1	4.1	92[b], 15[u,z]	39[c], 47[j], 50[l], 7[o]
Walnut	1	52.9	406[b], 31[u]	55[c], 74[j], 19.2[o], 46.1[p]

a-(Mangan et al., 2016); b-(Díaz-Alarcón et al., 1996); c-(Bratakos et al., 1987); d-(National-Research-Council, 1983); e-(Askar and Bielig, 1983); f-(Simonoff et al., 1988); g-(Levander and Morris, 1970); h-(Arthur, 1972); i-(Murphy and Cashman, 2001); j-(Olson and Palmer, 1984); k-(Navarro-Alarcon and Cabrera-Vique, 2008); l-(McNaughton and Marks, 2002); m-(Singh and Garg, 2006); n-(Kadrabova et al., 1997); o-(Pappa et al., 2006); p-(Klapec et al., 2004); q-(Smrkolj and Stibilj, 2004); r-(Tinggi et al., 1992); s-(Sirichakwal et al., 2005); t-(Hussein and Bruggeman, 1999); u-(Barclay et al., 1995); v-(Smrkolj et al., 2005); w-(Karim, 2018); x-(De Temmerman et al., 2014); y-(Waheed et al., 2002); z-(Al-Ahmary, 2009)

in samples from Spain (Díaz-Alarcón et al., 1996) but lower than examples from India, Iraqi-Kurdistan and Saudi Arabia (Singh and Garg, 2006; Karim, 2018; Al-Ahmary,

2009). Barley had an average Se_P concentration less than reported in several countries (Mangan et al., 2016; Askar and Bielig, 1983; Singh and Garg, 2006; Al-Ahmary, 2009).

Crop vegetables

Among crop vegetables, various species including okra, cucumber, aubergine, capsicum and pigeon pea had average Se_P concentrations less than values reported in the literature from various parts of the world (Table 3.15). Conversely, pumpkin had higher Se_P concentrations than published values for Belgium and Saudi Arabia (De Temmerman et al., 2014; Al-Ahmary, 2009). Tomato and bean had Se_P concentrations of 28.5 µg kg^{-1} and 116 µg kg^{-1} respectively, which were higher than some countries but lower than others.

Tubers

Within Tubers, radish, onion and turnip had mean Se_P concentrations of 31.1, 12.0 and 29.6 µg kg^{-1} which were comparable with values (33, 15, 29.1 µg kg^{-1}) quoted respectively from Al-Ahmary (2009), Bratakos et al. (1987) and De Temmerman et al. (2014). However, the above-mentioned tuber species, and carrots, also had lower Se_P concentrations compared to published values from some countries but were higher than others (Table 3.15). The average potato Se_P concentrations (10.0 µg kg^{-1}) measured in this study was lower than published values from Egypt, Canada, South Dakota, India, Spain, Belgium and Kashmir region-Pakistan (Askar and Bielig, 1983; Arthur, 1972; Olson and Palmer, 1984; Singh and Garg, 2006; Navarro-Alarcon and Cabrera-Vique, 2008; De Temmerman et al., 2014; Waheed et al., 2002).

Fruits

Among fruits, plant species including grapes, figs, pears and peaches had Se_P concentrations lower than values reported for other countries (Table 3.15). The mean Se_P concentration in apple samples (4.10 µg kg^{-1}) was lower than the majority of the values reported in the literature, except for those of Olson and Palmer (1984) and Navarro-Alarcon and Cabrera-Vique (2008), which were comparable with this study. The Se concentrations of other samples such as ber (Chinese date), grapes, mulberry apricots and persimmons are provided in Table 3.15.

Nuts

Only two types of nut were surveyed: almonds and walnuts. The almond Se_P concentration of 4.1 µg kg^{-1} was lower than values published from Spain, the United Kingdom and Saudi Arabia (Díaz-Alarcón et al., 1996; Barclay et al., 1995; Al-Ahmary, 2009). The concentration of Se_p in walnuts (52.9 µg kg^{-1}) was almost double the value reported for a UK sample by Barclay et al., (1995), however, it was much lower than reported in Spain (Díaz-Alarcón et al., 1996).

3.3.14 Relationship between Se_P and soil properties

The relationships described in this section were obtained when data of all districts was considered together as one data set. There was no significant correlation between Se_P of plant groups or individual species with soil characteristics on a district basis, possibly because of the small number of plant samples from each district. Plant groups including grains, crop vegetables and fruits demonstrated a weak positive correlation with soil pH ($r = 0.321, 0.281, 0.279$ respectively $p < 0.05$) (Figure 3.32).

However, there was no correlation observed between SOC% and Se_P content of plant groups. Sand content had a significant negative correlation with grain crops ($r = -0.484$, $p < 0.05$) (Figure 3.32). Soil clay content and combined metal oxides content had no correlation with Se_P concentration for the majority of plant groups, except for tubers where a positive correlation was observed ($r = 0.685$ and 0.661 respectively, $p < 0.05$) (Figure 3.32).

Karim (2018) reported a positive correlation between soil pH and Se concentration in plant groups including species from grains and crop vegetables. Chilimba et al. (2011) also noted a much higher concentration of Se in maize grown in soils with pH > 6.5 compared to acidic soils in Malawi which he partly attributed to the pH-dependence of Se^{VI} and Se^{IV} adsorption on Fe oxides.

Among individual plant species, the majority did not show any correlation between Se_P and soil characteristics. However, exceptions included maize and amaranth leaves in which Se_P had a positive correlation with soil pH ($r = 0.478$ and 0.545, $p< 0.05$) (Figure 3.33). The concentrations of combined metal oxides and clay also had a positive correlation with Se_P in potatoes ($r = 0.810$ and 0.701, $p < 0.05$). Sand content had a negative correlation with maize and wheat Se_P ($r = -0.447$ and -0.501, $p < 0.05$) (Figure 3.33).

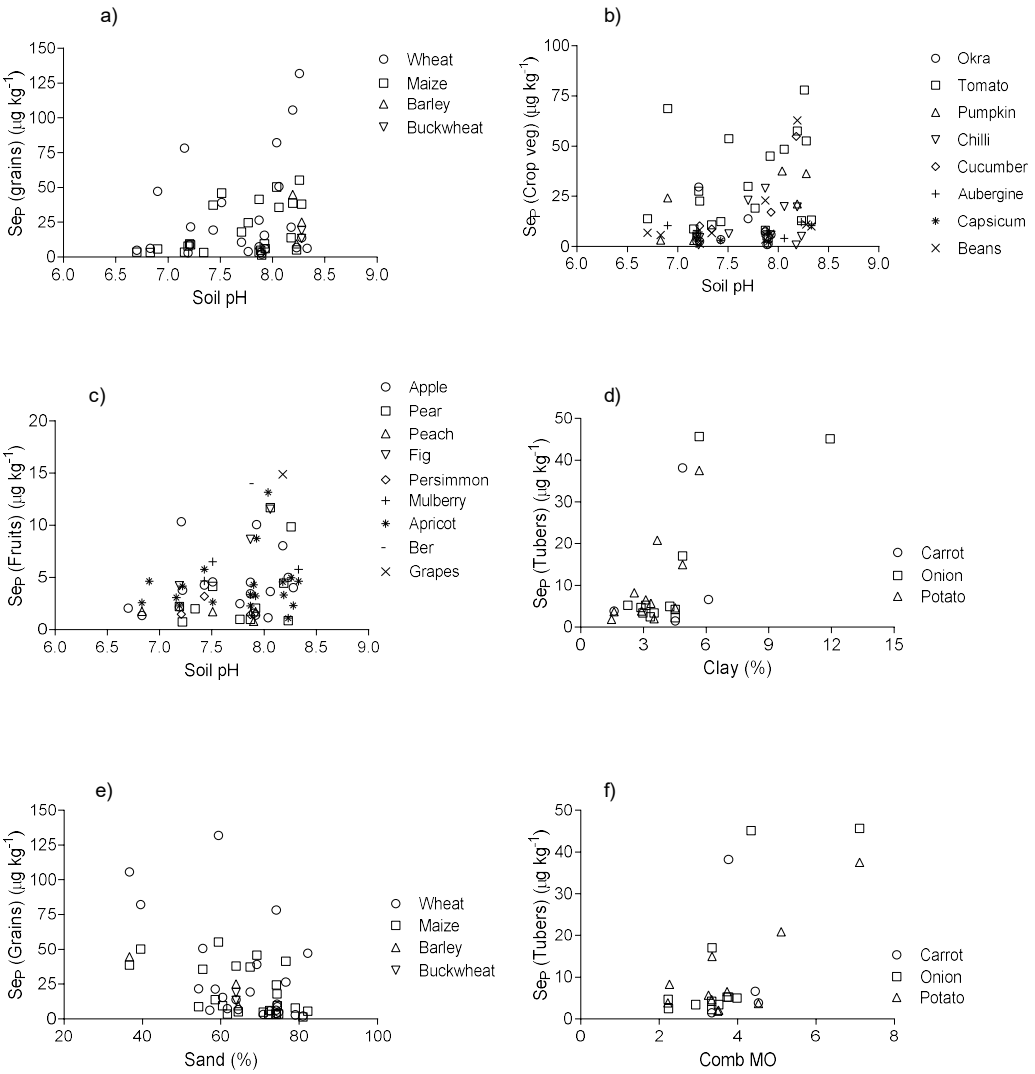


Figure 3.32. Correlation between selected soil characteristics and Se_P in plant groups

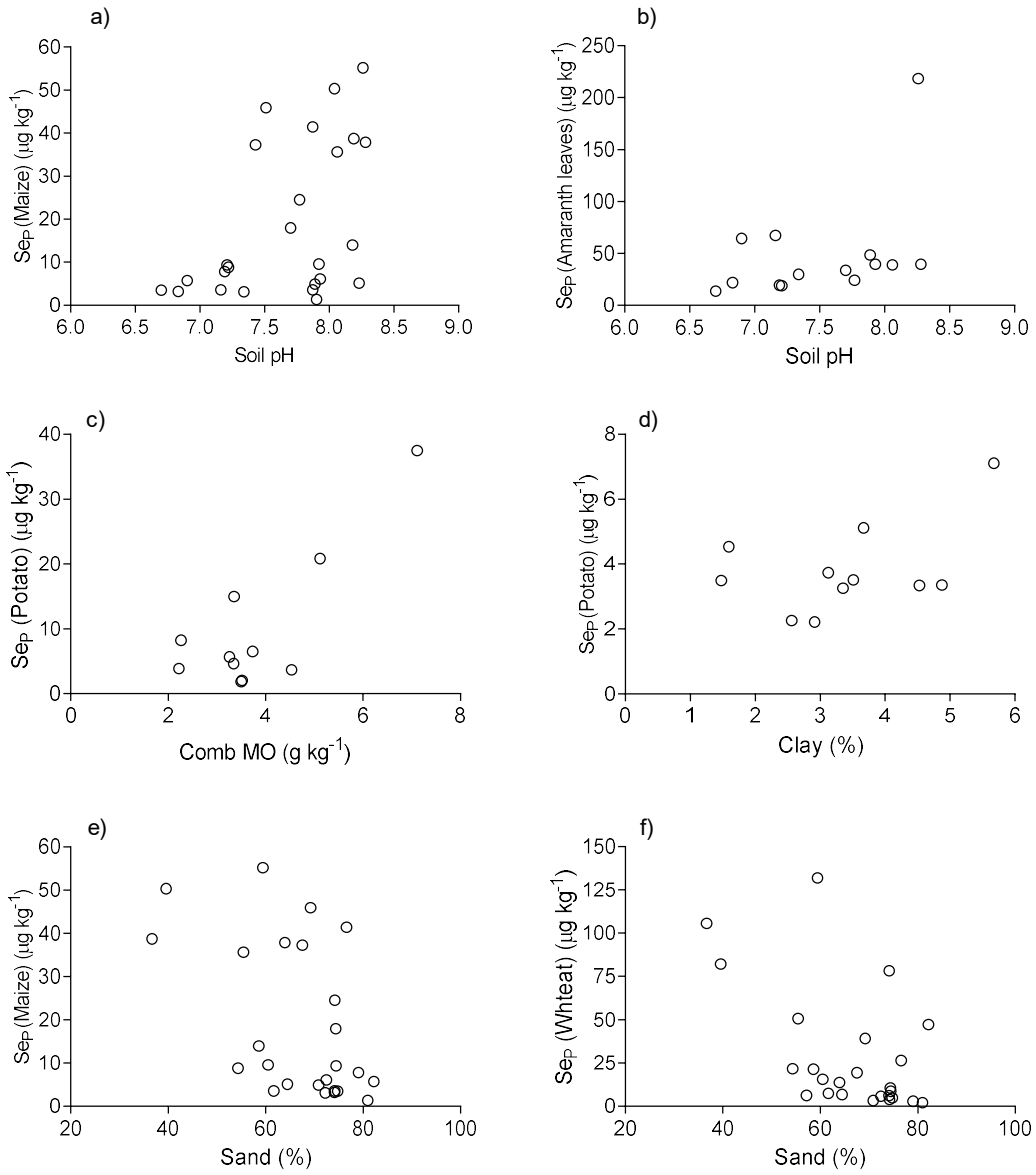


Figure 3.33. Relationship between selected soil characteristics and Se_P in some plant species

The fractions of reactive soil Se, i.e. Se_{sol} and Se_{ads}, had a positive correlation with Se_P in different plant groups (Figure 3.34 and 3.35).

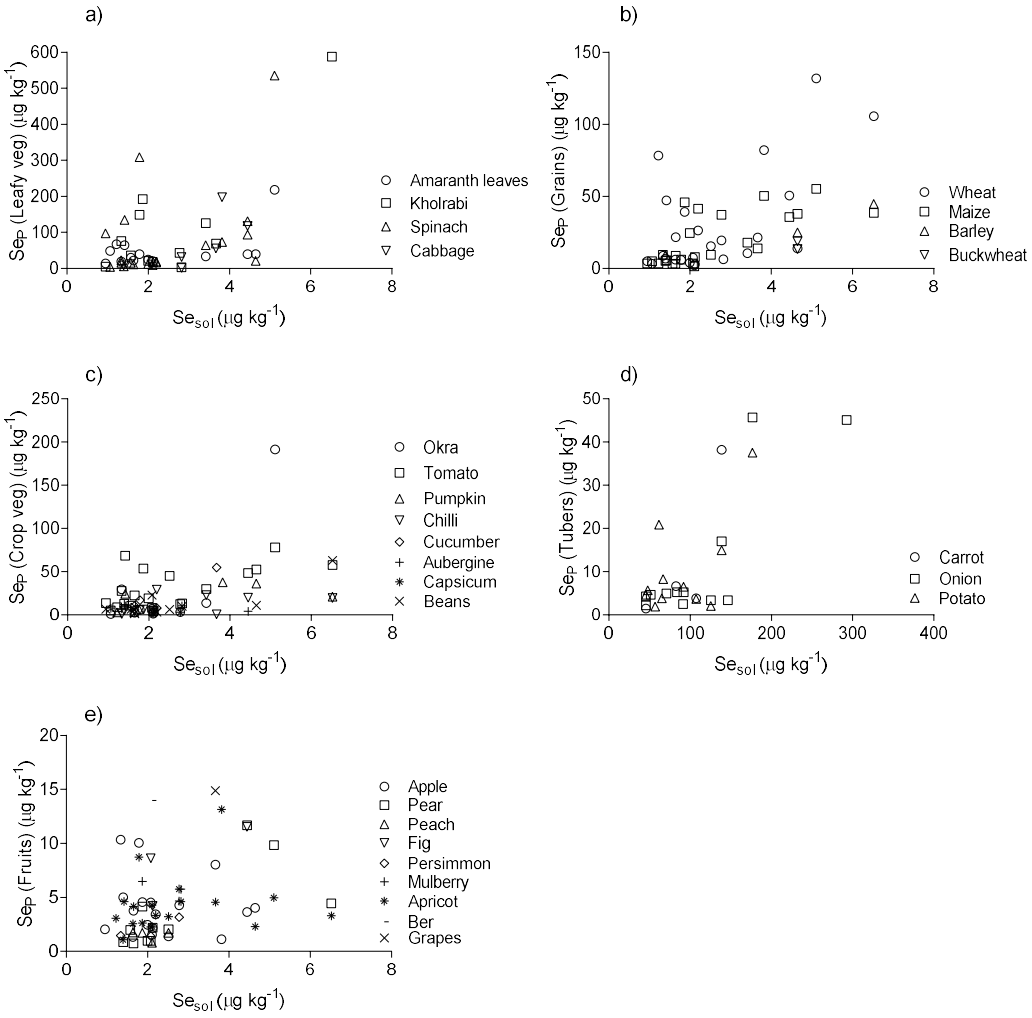


Figure 3.34. Relationship between soil soluble Se (Se_{sol}) and Se_P of different plant groups

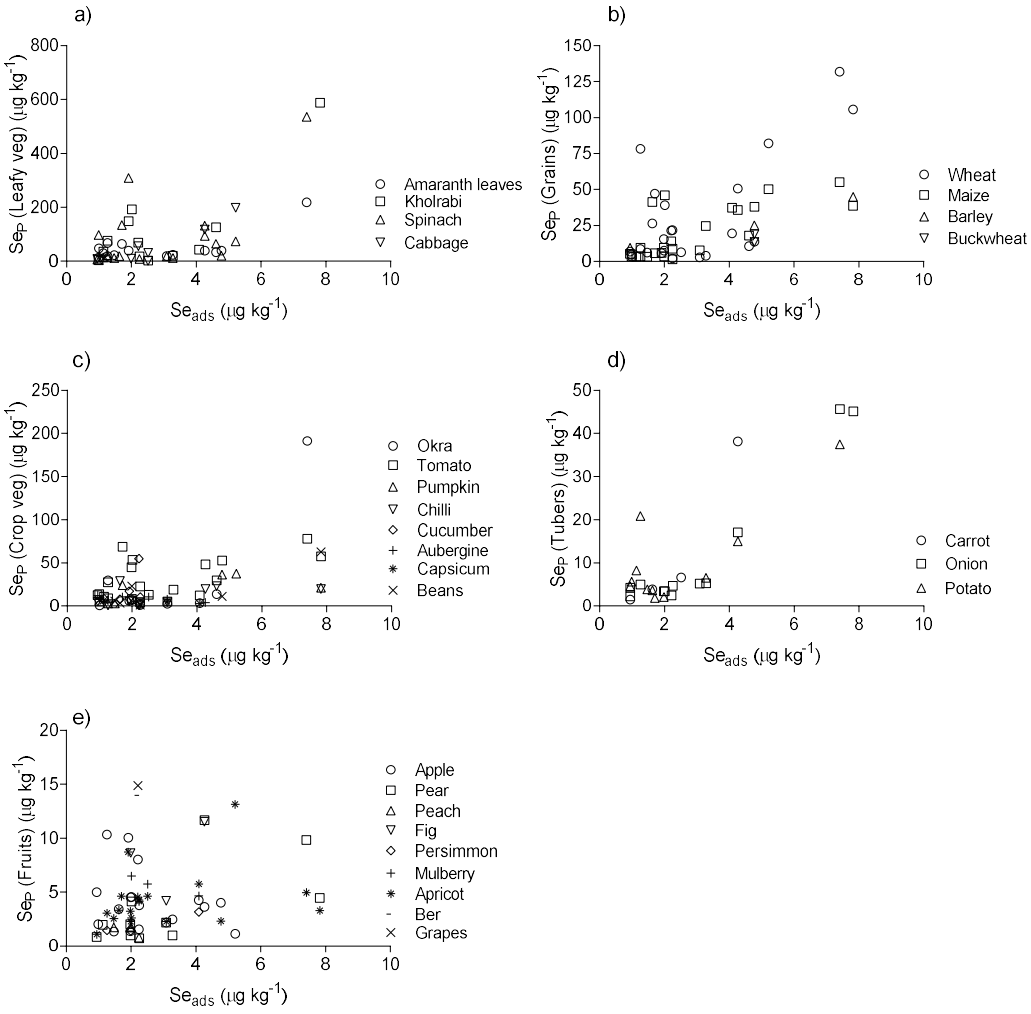


Figure 3.35. Relationship between adsorbed Se (Se_{ads}) and Se_P of different plant groups

Individual plant species did not show a significant correlation with Se_P and Se_{sol} or Se_{ads} in most cases; exceptions included maize, wheat, tomato and onion (Figure 3.36 and 3.37). One reason for relatively weak correlations could be that the soil samples were not collected from the same field where the plants were grown. Soil and plant samples were collected from the same village in most cases rather than the same field.

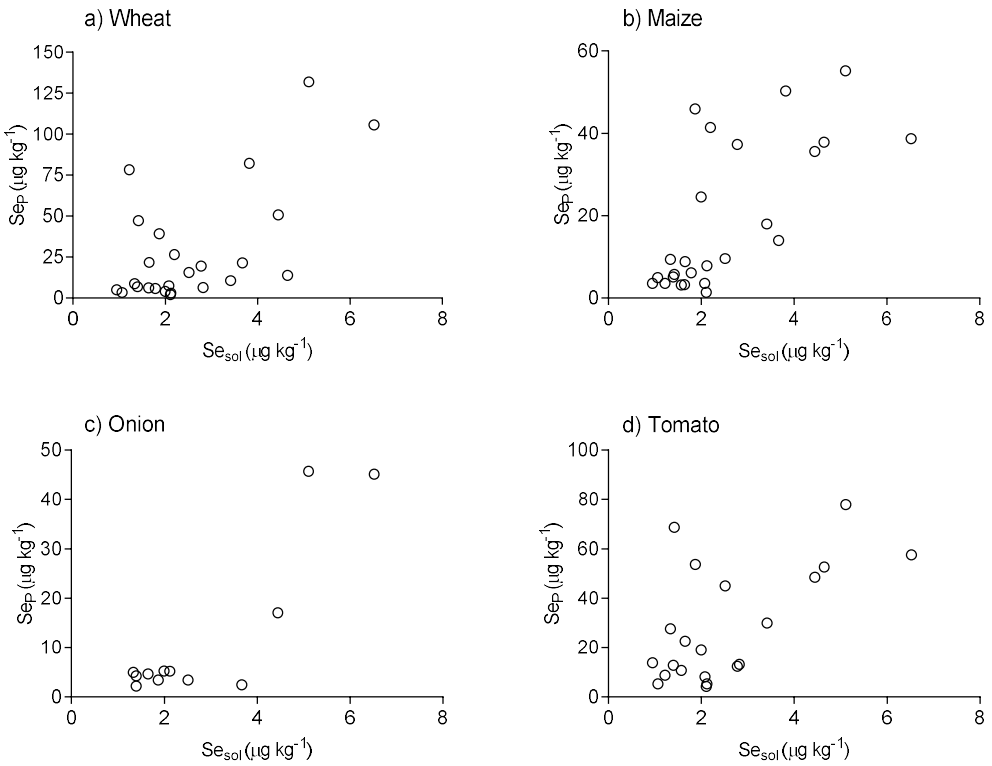


Figure 3.36. Relationship between concentrations of Se_{sol} and Se_P for selected crops: a) wheat, b) maize, c) onion and d) tomato

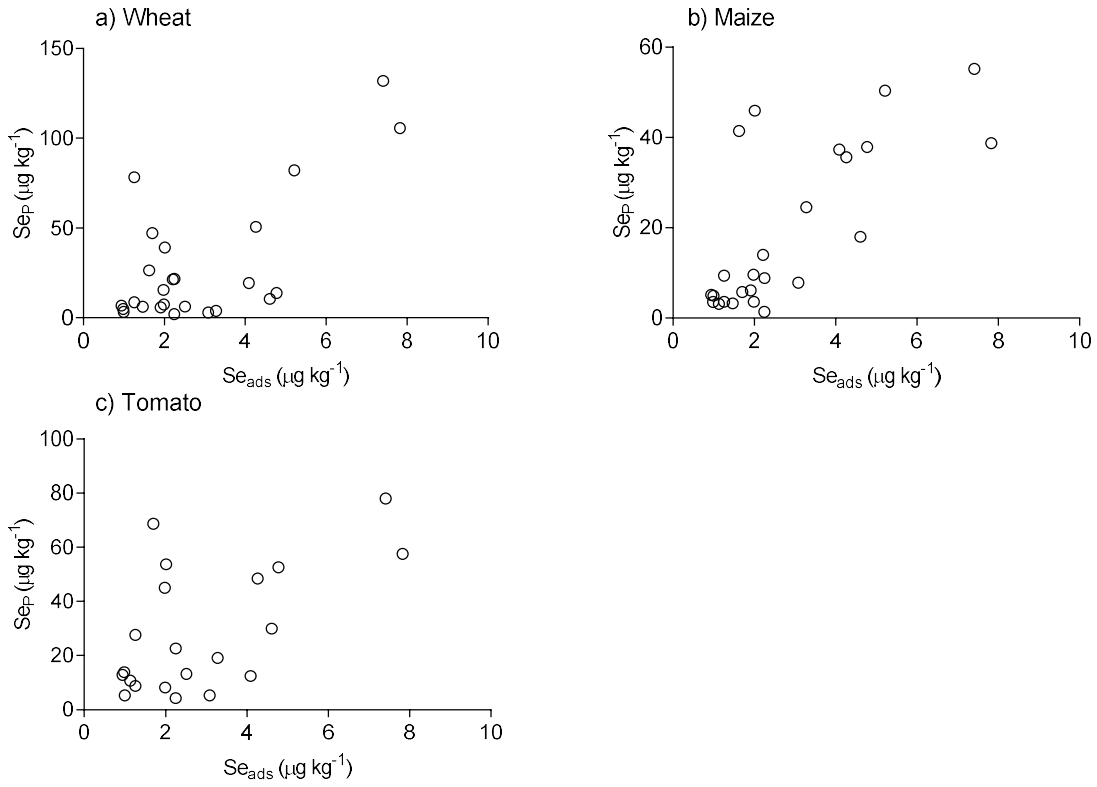


Figure 3.37. Relationship between concentrations of Se_{ads} and Se_P for selected crops: a) wheat, b) maize and c) tomato

The generally low concentration of Se_P in all crops of the study area may be a combined effect of (i) low Se_T in the soil, (ii) a relatively low level of Se bioavailability (<5% of Se_T was present in in the fractions Se_{sol} + Se_{ads}) and the predominance of Se^{IV} in all of soil samples which is less available to plants than Se^{VI} (Gissel-Nielsen et al., 1984, Stroud et al., 2010b).

3.3.15 Irrigation water and plant Se

Concentration of Se_P in crop vegetables had a positive correlation (r = 0.394, p = 0.001) with Se in irrigation water (Se_w; Figure 3.38).

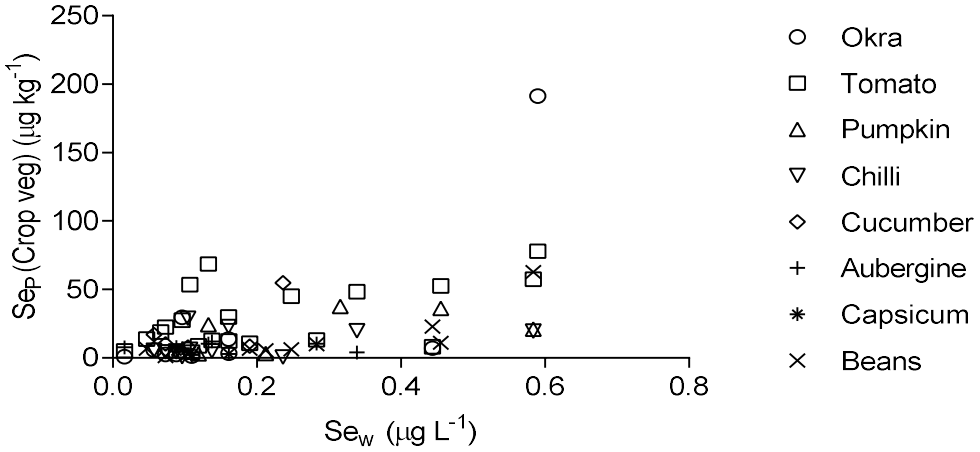


Figure 3.38. Relationship between irrigation water Se (Se_w) and Se_P of vegetable group

A relationship between Se_w in irrigation water and Se_P in individual plant species was largely absent but exceptions included wheat and tomato (r = 0.550 and 0.507 p< 0.05) (Figure 3.39).

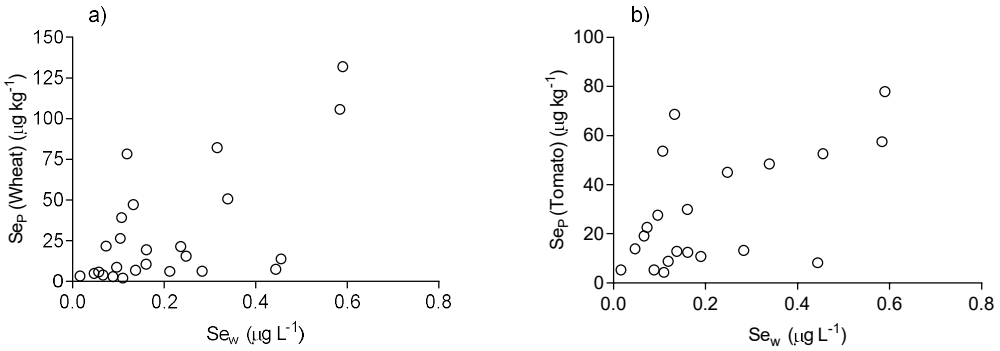


Figure 3.39. Relationship between irrigation water Se (Se_w) and Se_P in a) wheat and b) tomato

3.3.16 Soil to plant transfer factor (TF) of selenium

Table 3.16 describes soil-to-plant transfer factors calculated based on Se_T as well as different fractions of soil Se including Se_{sol}, Se_{ads} and Se_{TMAH} for all plant groups and

individual species in each group. When the data from all districts was considered together, the transfer factors of each plant group typically decreased, as expected, in the order of Se_{sol}-TF > Se_{ads} -TF > Se_{TMAH} -TF > Se_T –TF. However, the fruit group had similar transfer factor values based on Se_{sol} and Se_{ads}. Transfer factors for each plant group were also calculated individually for each district but the results followed the same trend as considering all districts together. Among plant groups, the transfer factor for all fractions of Se (Se_{sol}, Se_{ads}, Se_{TMAH} and Se_T) followed the order leafy vegetables > crop vegetables > nuts > tubers > grains > fruits for data from all districts together. Individually, each district exhibited a different trend of variation in TF with regards to plant groups however, leafy vegetables had higher TF values in all districts except Gilgit where crop vegetables had a higher TF. Fruits group consistently had smaller TF values in each district.

Within leafy vegetables, Se_{sol}-TF ranged from 11 to 47 with lettuce and kohlrabi accounting for lowest and highest values respectively. Amaranth leaves, spinach and cabbage had Se_{sol}-TF of 22.6, 31.6 and 43.5 individually. Among crop vegetables, beans had the highest Se_{sol}-TF value of 41.5; the majority of the other plants in this group had Se_{sol}-TF values <10. The nuts (almond and walnut) had an average Se_{sol}-TF value of 10.7. Among tubers, radish had the highest Ses_{ol}-TF values of 13.5 followed by 11.3 in turnip; the majority of the remaining tubers had Se_{sol}-TF values < 5. Se_{Sol}-TF ranged from 3.48 to 11.2 for grains with buckwheat and wheat representing the extremities. Fruits represented the narrowest range (0.762 – 6.63) with peach and ber (Chinese date) each accounting for lowest and highest values respectively.

Generally, values of Se_{ads}-TF in the majority of plant species were slightly less than Se_{sol}-TF. Values of Se_{TMAH}-TF and Se_T-TF for all species were < 1 except for kohlrabi and cabbage. Transfer factor values calculated based on Se_{sol} and Se_{ads} were much higher than Se_{TMAH} and Se_T, as expected. Different species of the same plant group grown in the same location had variable TF values for both Se_{sol} and Se_{ads} indicating the different intrinsic abilities for crop species to assimilate Se. The same crop species grown in different villages and districts had different soluble and adsorbed TF values, suggesting that soil characteristics or probably the availability of Se from other sources such as irrigation water or rainfall, affected Se_P concentration in plants.

Table 3.16. Soil to plant transfer factor based on total soil selenium (Se_T) and its fractions in soil (Se_{sol}, Se_{ads} and Se_{TMAH})

Plant type	n	Se_{sol} – TF			Se_{ads} – TF			Se_{TMAH} – TF			Se_T - TF		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Leafy vegetables		***0.414***	***200***	***31.2***	***0.465***	***161***	***27.7***	***0.014***	***6.00***	***0.851***	***0.005***	***2.00***	***0.379***
Lettuce	1	11.0	11.0	11.0	12.4	12.4	12.4	0.376	0.376	0.376	0.144	0.144	0.144
Spinach	21	3.91	173	31.6	3.74	161	28.5	0.125	5.18	0.845	0.038	2.17	0.367
Amaranth L	14	8.56	55.3	22.6	6.34	53.7	21.4	0.234	1.50	0.594	0.116	0.524	0.248
Kholrabi	13	0.936	158	46.9	1.05	109	41.5	0.032	4.56	1.15	0.012	1.88	0.581
Cabbage	7	0.414	200	43.5	0.465	138	34.7	0.014	5.77	1.29	0.005	2.38	0.557
Grains		***0.65***	***64.2***	***7.0***	***0.61***	***62.4***	***6.8***	***0.03***	***1.27***	***0.20***	***0.01***	***0.454***	***0.10***
Barley	3	5.40	6.86	6.33	5.26	9.96	6.98	0.152	0.206	0.171	0.062	0.099	0.079
Maize	25	0.654	24.6	6.91	0.613	25.5	6.52	0.025	0.392	0.164	0.011	0.209	0.081
Wheat	25	0.967	64.2	11.2	0.907	62.4	10.4	0.035	1.27	0.270	0.018	0.454	0.120
Buckwheat	2	2.89	4.06	3.48	2.81	3.96	3.38	0.082	0.115	0.098	0.040	0.057	0.049
Crop vegetables		***0.21***	***409***	***10.9***	***0.35***	***278***	***9.47***	***0.01***	***7.62***	***0.26***	***0.00***	***5.63***	***0.140***
Okra	10	0.600	37.4	7.62	0.563	25.8	6.36	0.023	1.08	0.196	0.010	0.445	0.090
Tomato	21	2.03	48.4	12.3	1.73	40.4	11.7	0.064	1.21	0.292	0.025	0.459	0.133
Pumpkin	6	1.94	17.1	7.08	2.16	14.2	6.07	0.049	0.424	0.185	0.018	0.162	0.082
Chilli	11	0.213	13.2	3.95	0.353	17.9	4.19	0.009	0.270	0.101	0.004	0.116	0.047
Cucumber	5	3.67	15.0	8.00	4.56	24.8	10.2	0.075	0.601	0.259	0.031	0.309	0.120
Aubergine	7	0.94	8.73	5.33	0.977	12.9	5.78	0.030	0.268	0.150	0.014	0.085	0.058
Capsicum	3	1.06	2.41	1.52	0.742	1.66	1.13	0.020	0.061	0.041	0.015	0.030	0.022
Beans	11	0.865	409	41.5	0.634	278	29.7	0.028	7.62	0.789	0.011	5.63	0.556
Green pea	1	10.8	10.8	10.8	10.1	10.1	10.1	0.324	0.324	0.324	0.136	0.136	0.136

Table 3.16. Continued

Plant type	n	Se_{sol} – TF			Se_{ads} – TF			Se_{TMAH} - TF			Se_{T} - TF		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Tubers		***0.670***	***17.1***	***7.26***	***1.040***	***16.6***	***7.04***	***0.016***	***0.403***	***0.183***	***0.008***	***0.169***	***0.084***
Radish	1	13.5	13.5	13.5	12.6	12.6	12.6	0.403	0.403	0.403	0.169	0.169	0.169
Carrot	4	1.07	8.59	3.44	1.58	8.96	3.89	0.033	0.275	0.106	0.010	0.132	0.047
Onion	12	0.670	8.94	3.33	1.11	6.17	3.06	0.024	0.258	0.086	0.013	0.106	0.041
Potato	11	0.819	17.1	4.71	1.04	16.6	4.74	0.016	0.334	0.111	0.008	0.121	0.045
Turnip	2	6.57	16.1	11.3	6.88	14.9	10.9	0.205	0.211	0.208	0.101	0.137	0.119
Fruits		***0.30***	***7.75***	***2.53***	***0.22***	***8.23***	***2.74***	***0.01***	***0.260***	***0.08***	***0.00***	***0.130***	***0.04***
Grapes	1	4.06	4.06	4.06	6.72	6.72	6.72	0.163	0.163	0.163	0.084	0.084	0.084
Ber	1	6.63	6.63	6.63	6.22	6.22	6.22	0.258	0.258	0.258	0.128	0.128	0.128
Mulberry	3	1.68	3.48	2.40	1.14	3.22	2.22	0.031	0.070	0.048	0.023	0.030	0.026
Fig	3	1.98	4.16	2.91	1.37	4.35	2.81	0.050	0.083	0.072	0.025	0.040	0.030
Pear	11	0.449	2.63	1.15	0.300	2.74	1.11	0.009	0.084	0.028	0.003	0.040	0.013
Apricot	19	0.497	4.88	1.81	0.424	4.55	1.72	0.011	0.146	0.049	0.007	0.061	0.022
Peach	4	0.378	1.06	0.762	0.355	1.18	0.815	0.012	0.027	0.017	0.006	0.013	0.009
Apple	18	0.301	7.75	2.09	0.221	8.23	2.20	0.009	0.168	0.052	0.004	0.087	0.024
Persimmon	3	0.688	1.14	0.973	0.719	1.16	0.883	0.013	0.021	0.018	0.004	0.016	0.011
Nuts		***-***	***-***	***10.7***	***-***	***-***	***7.34***	***-***	***-***	***0.22***	***-***	***-***	***0.15***
Almond	1	2.43	2.43	2.43	1.78	1.78	1.78	0.078	0.078	0.078	0.033	0.033	0.033
Walnut	1	19.0	19.0	19.0	12.9	12.9	12.9	0.355	0.355	0.355	0.262	0.262	0.262

3.4 Conclusions

Iodine

The median concentration of I_w in all water samples was 0.227 µg L^{-1} which is close to the bottom of the worldwide range for fresh water (0.01 – 212 µg L^{-1}) and is nearly two orders of magnitude below the WHO recommended maximum concentration of 18 µg L^{-1} in drinking water. The speciation analysis revealed the presence of I_w in both inorganic (~70%) and organic (~30%) forms. The inorganic I was largely present as IO_3^- (>80%) reflecting the high pH of water in the area.

The average I_T in GB soil was 0.685 mg kg^{-1}, which is lower than global average of soil I_T (2.6 mg kg^{-1}) and most of the reported values for I_w in other parts of the world (Figure 3.40). The alkaline nature, coarse texture and low organic carbon have probably contributed to low retention of I_T in GB soils. The adsorption of I on metal (Fe and Al) oxyhydroxides is low under alkaline conditions and the input to soil I_T from irrigation water is likely to be minimal because of the low concentration of I in irrigation water. The soluble and adsorbed fractions of soil I, which are considered to be available for plant uptake, accounted for <7% of total soil I content. Furthermore, I in the soluble and adsorbed fractions was predominantly (≥90%) present as organic species which may not be available to plants. The inorganic I (c. 10%) was largely present as I^-.

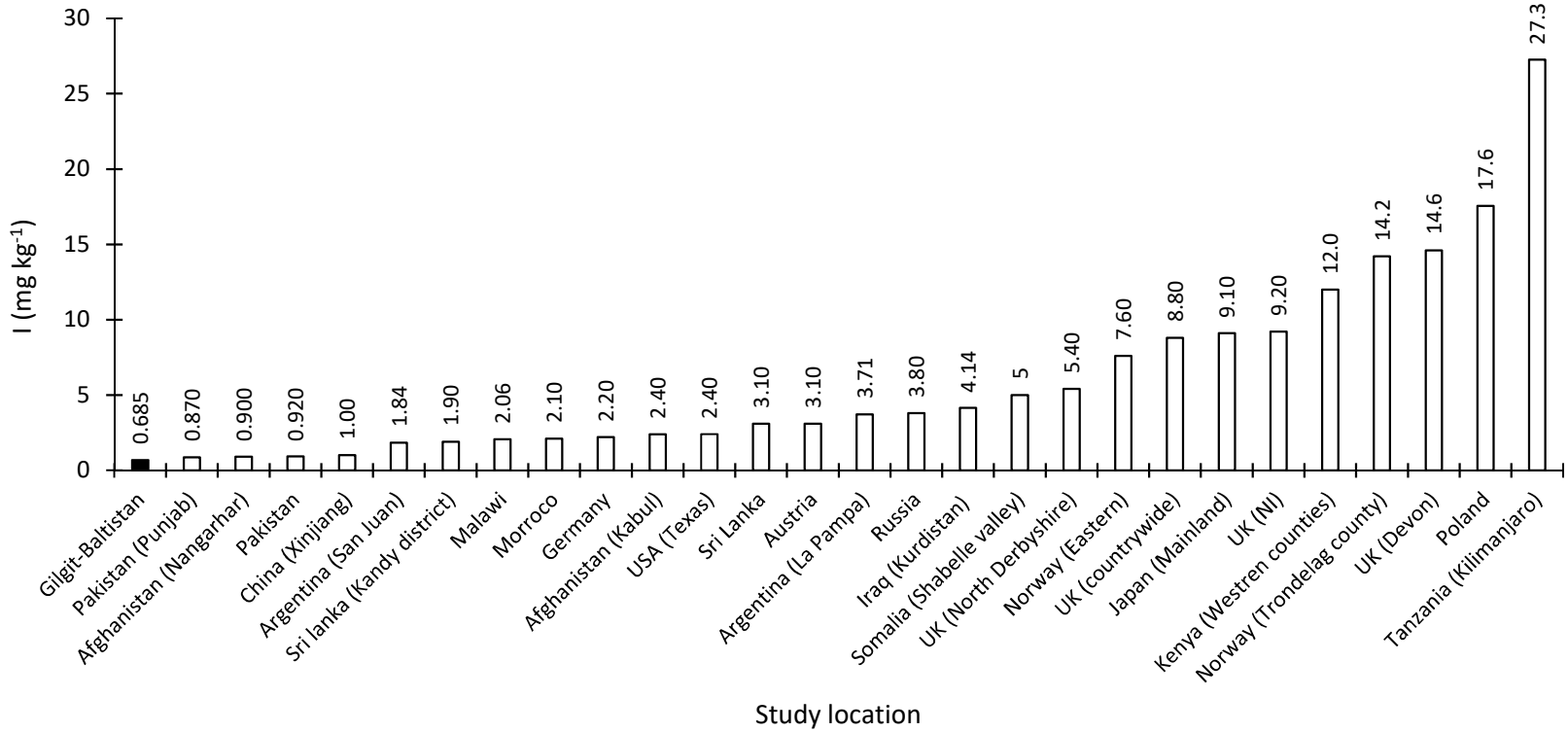


Figure 3.40. Comparison of mean GB soil I_T with average soil I concentration from other studies worldwide as well as in Pakistan. The dark colour bar represents I concentration in GB soil

(Sources: Låg and Steinnes, 1976; Fuge and Long, 1989; Dissanayake and Chandrajith; 1996, Gerzabek et al., 1999; Fordyce et al., 2000; Johnson et al., 2002; Johnson et al., 2003; Ashworth, 2009; Watts and Mitchell, 2009; Watts et al., 2010; Bowley, 2013; Zia et al., 2014; Watts et al., 2015; Yamasaki et al., 2015; Watts et al., 2019a; Unpublished data, 2020)

The distribution coefficient (kd) for I (Eq. 3.2) was very low (1.27 ± 0.214) suggesting very limited buffering of available I against leaching losses and plant uptake.

$$kd = \frac{I_{Ads}}{I_{Sol}} \quad (3.2)$$

where I_{Ads} and I_{Sol} are I concentrations (µg kg^{-1}) in the adsorbed and soluble fractions. The above factors demonstrate the low status of I in the GB environment.

The low concentration of I in GB soil and water is also apparent in locally grown crops. All plants had low I_P compared to similar samples from other parts of the world. Among plant groups, leafy vegetables consistently had higher I_P across all districts but concentrations were still low compared to published studies conducted elsewhere (Figure 3.41). Wheat which contributes to >75% of daily energy intake had low I_P compared to some other parts of the world but wheat grain is not a good accumulator of I (Figure 3.42).

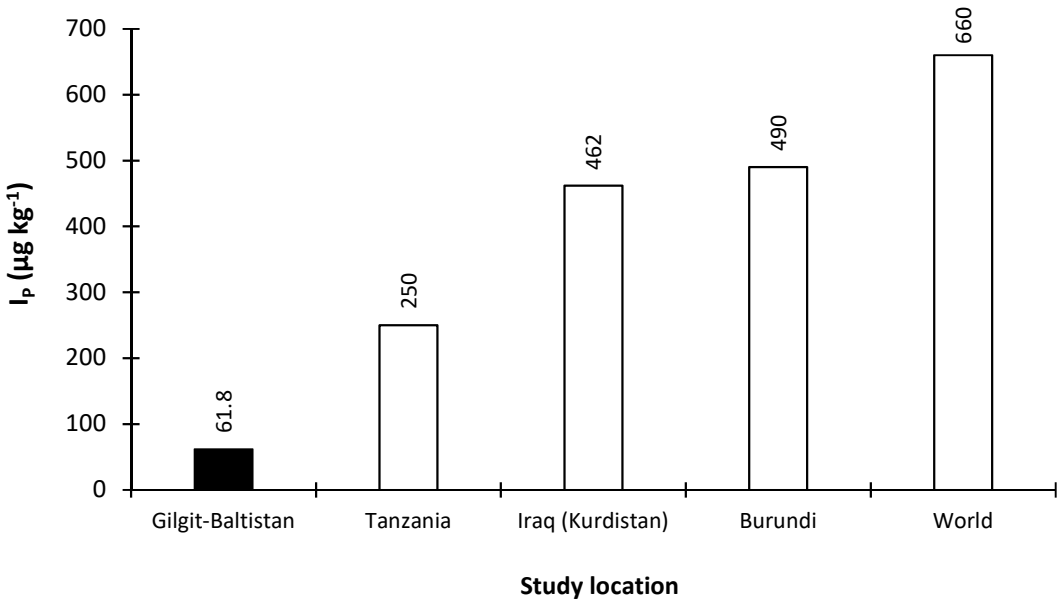


Figure 3.41. Comparison of iodine concentration in spinach samples from GB with published studies

(Sources: Eckhoff and Maage, 1997; Fordyce, 2003; Karim, 2018)

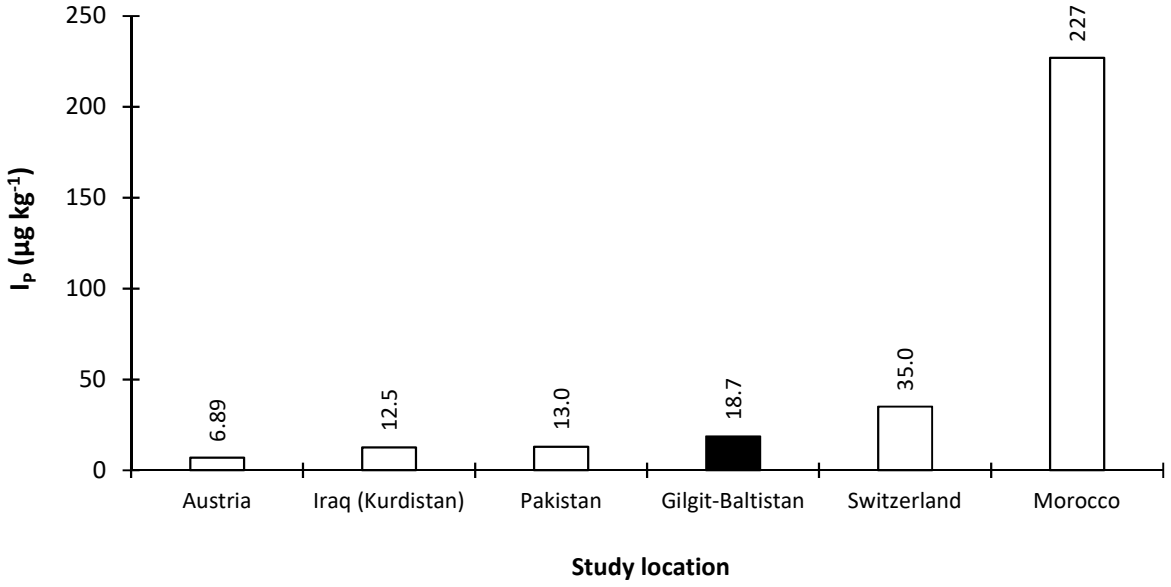


Figure 3.42. Comparison of iodine concentration in wheat samples from GB with published studies

(Sources: Aquaron et al., 1993; Shinonaga et al., 2001; Haldimann et al., 2005; Zia et al., 2014; Karim, 2018)

Selenium

The median concentration of Se_w in all samples was 0.246 μg L^{-1}, which is very low compared with (i) the WHO recommended value of 10 – 40 μg L^{-1} Se in drinking water and (ii) the FAO recommended maximum permissible concentration of 20 μg L^{-1} Se in irrigation water. Speciation analysis indicated that Se_w was present in both organic (~35%) and inorganic (~65%) forms. The only inorganic species detected across all samples was Se^{VI} reflecting its highly soluble nature compared with Se^{IV}.

The average soil Se_T measured in the GB region was 209 μg kg^{-1} which is low compared to a global mean of 400 μg kg^{-1} and the majority of published studies conducted elsewhere (Figure 3.43). A predominantly coarse soil texture and low organic carbon are likely to have contributed to low Se retention by soils in GB. The contribution of Se from seasonal irrigation water to Se_T in soils is likely to be low because of the low Se_w concentrations in irrigation water and the predominance of soluble Se^{VI}. The reactive soil Se (soluble Se_{Sol} and adsorbed Se_{Ads} fractions) which may be available for plant uptake accounted for <3% of Se_T. Soluble and adsorbed fractions had both organic and inorganic species; the majority of the inorganic Se was Se^{IV}. The large presence of Se^{IV} at high pH is unexpected as other studies suggest that Se^{VI} is likely to be the major species at high pH. Selenite is strongly adsorbed on soil surfaces compared to Se^{VI} but the distribution coefficient (kd) for Se^{IV} (Eq. 3.3) was very low (2.04 ± 0.663) due to the coarse texture and low humus content of the soil.

$$kd = \frac{Se^{IV}_{Ads}}{Se^{IV}_{Sol}} \qquad (3.3)$$

where Se^{IV}_{Ads} and Se^{IV}_{Sol} are Se^{IV} concentrations (μg kg^{-1}) in the adsorbed and soluble fractions.

The low kd value reflects the lack of a substantial buffer power for available Se. Not only is the soluble Se_{Sol} very low but the ability of the soil to replenish Se in solution from Se_{Ads} following depletion by leaching or plant uptake is also very poor. Taking all the factors above into account it is clear that the Se status of GB region is exceptionally low.

The low Se status of GB was also reflected in locally grown crops. All plant samples analysed had low Se_P though different plant species varied in their Se concentration. Wheat, as the local staple carbohydrate crop and constituting almost a compulsory portion of every meal in GB, and in Pakistan generally, had extremely low concentrations of Se compared to wheat from other parts of the world (Figure 3.44).

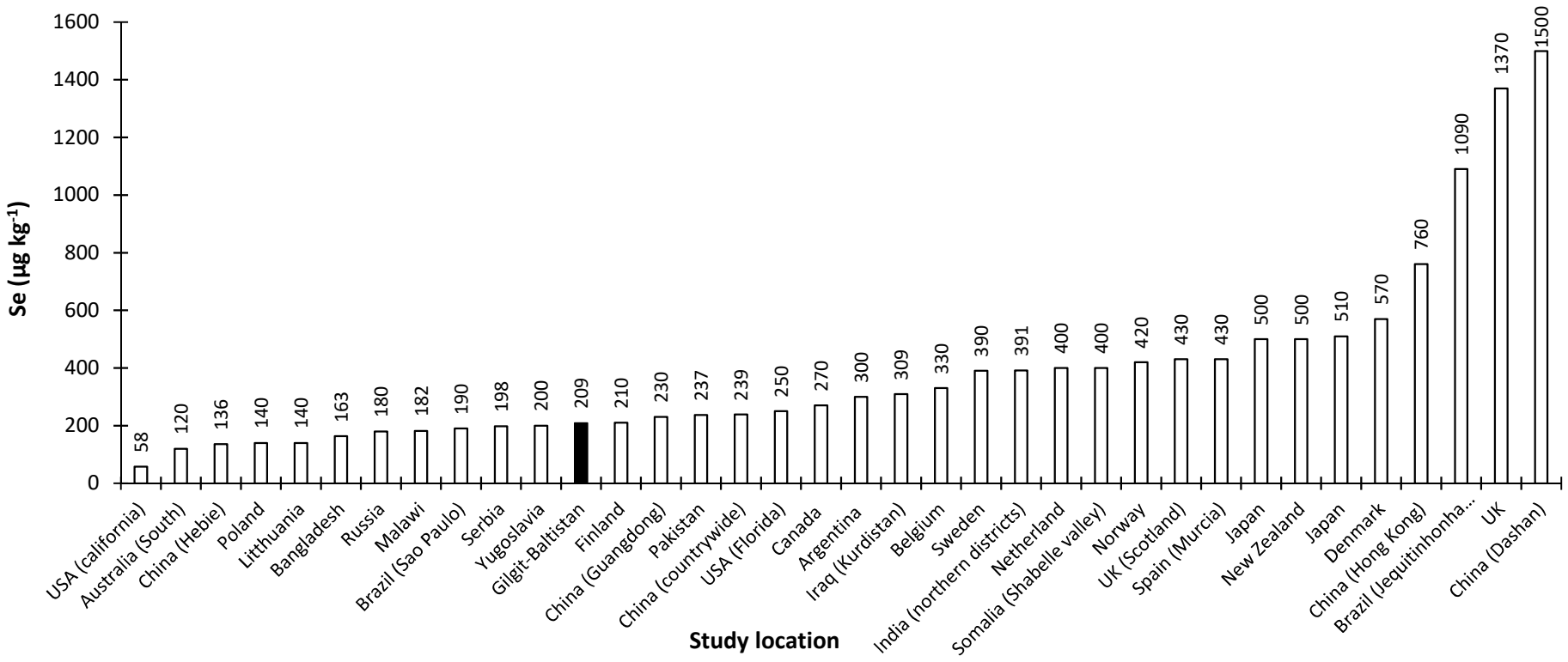


Figure 3.43. Comparison of mean GB soil Se concentration with average soil Se concentration from other studies worldwide as well as in Pakistan. The dark colour bar represents Se concentration in GB soil

(Sources: Girling, 1984; Maksimovic et al., 1992; Johnson et al., 2002; Tan et al., 2002; Fordyce et al., 2003; Yadav et al., 2005; Kabata-Pendias and Mukherjee, 2007; Watts et al., 2010; Rahman et al., 2013; De Temmerman et al., 2014; Mathers, 2015; Xing et al., 2015; Supriatin et al., 2016; Lopes et al., 2017; Matos et al., 2017; Karim, 2018; Unpublished data, 2020)

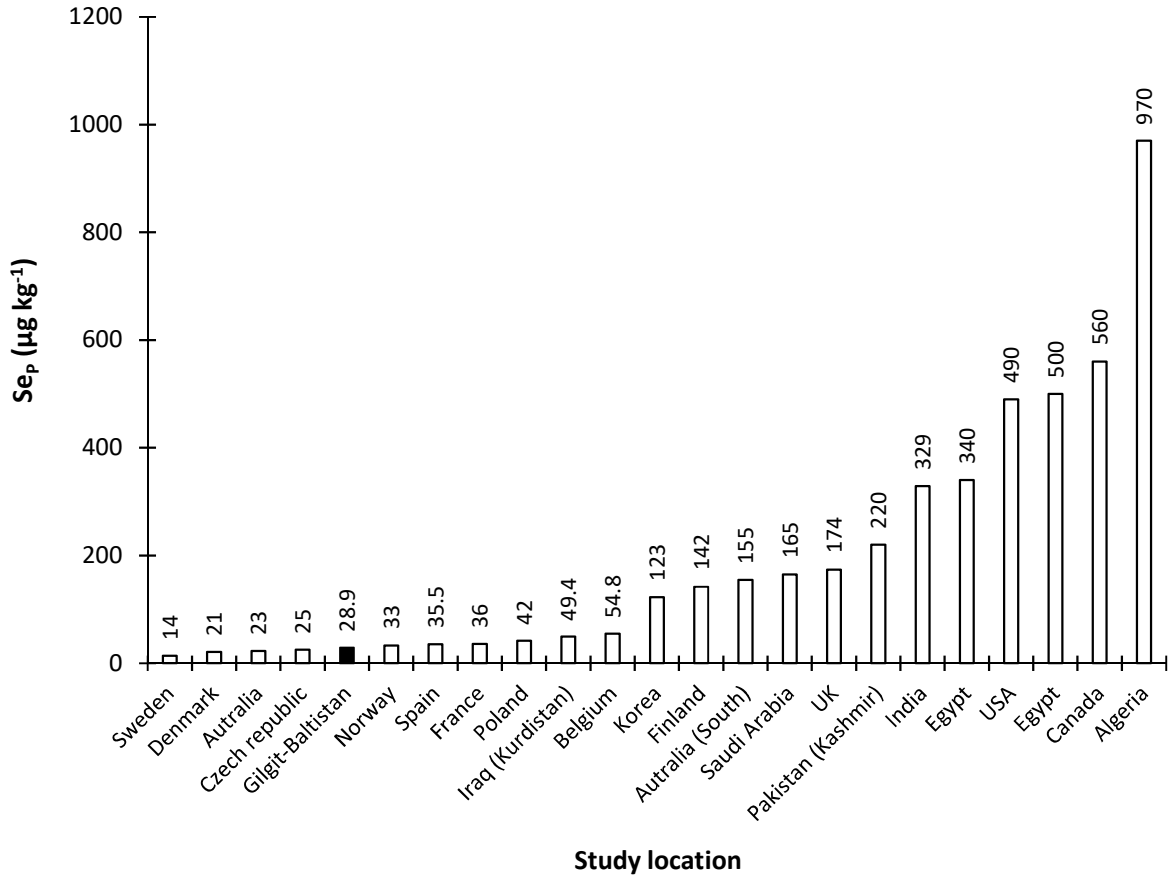


Figure 3.44. Comparison of GB wheat Se concentration with other studies conducted worldwide

(Sources: Arthur, 1972; Askar and Bielig, 1983; Díaz-Alarcón et al., 1996; Waheed et al., 2002; Singh and Garg, 2006; Kabata-Pendias and Mukherjee, 2007; Al-Ahmary, 2009; Choi et al., 2009; De Temmerman et al., 2014; Karim, 2018; Mathers et al., 2017)

From the results of this study it can be estimated that, based on a recommended daily intake of two litres, an individual will obtain 1.09 µg I and 0.74 µg Se per day from drinking water. Furthermore, as GB is a remote mountainous area, the local population largely consume locally grown agricultural produce. Therefore, based on a typical Pakistani food basket, the consumption of locally produced food items will only provide 47.5 µg day^{-1} of I (32% of RDA; Table 3.17) and 33.8 µg day^{-1} of Se (61%

of RDA; Table 3.18). Thus, the combined intakes of I and Se from both drinking water and food sources are substantially below recommended levels. Therefore, this study suggests that it is likely that the population of GB is living with a considerable risk of I and Se deficiency.

Table 3.17. An estimate of individual daily I intake from various dietary sources calculated based on the recommended intake of 150 µg day^{-1} of iodine

Food item	Consumption (g day^{-1})	I concentration (µg kg^{-1})	Dietary I intake (µg day^{-1})	% of daily requirement
Wheat**	350	18.7	6.55	4.37
Rice*	60	14.7	0.881	0.59
Maize**	15	24.3	0.365	0.24
Pulses**	30	5.26	0.158	0.11
Meat products*	40	183	7.32	4.88
Dairy products*	150	195	29.2	19.5
Green vegetables**	100	23.7	2.37	1.58
Fruits**	100	6.51	0.651	0.43
			47.5	**31.7**

** = I concentration values obtained from samples analysed in the current study
* = I concentration values obtained from the literature (Sources: Government of Pakistan, 2001; Fordyce, 2003; Karim, 2018)

Table 3.18. An estimate of individual daily Se intake from various dietary sources based on the recommended intake of 55 µg day^{-1} of Se

Food item	Consumption day^{-1} (g)	Se concentration (µg kg^{-1})	Dietary Se intake day^{-1} (µg)	% of daily requirement
Wheat**	350	28.9	10.1	18.4
Rice*	60	100	6.00	10.9
Maize**	15	19.0	0.29	0.53
Pulses**	30	108	3.24	5.89
Meat products*	40	98.9	3.96	7.20
Dairy products*	150	31.2	4.68	8.51
Green vegetables**	100	50.6	5.06	9.2
Fruits**	100	4.39	0.44	0.80
			33.8	**61.4**

** = Se concentration values obtained from samples analysed in the current study
* = Se concentration values obtained from the literature (Sources: Government of Pakistan, 2001; Iqbal et al., 2008; Karim, 2018; USDA food composition database, 2017)

4 SELENIUM BIOFORTIFICATION FIELD TRIAL

4.1 Introduction

Selenium (Se) is a crucial dietary micronutrient for human and animal health (Lyons et al., 2004, Schrauzer and Surai, 2009; Stroud et al., 2010b; Wang et al., 2017). It is a vital component of more than 30 selenenoproteins (Zarczynska et al., 2012) which play an important role in regulating various body functions, such as metabolism of thyroid hormones and protecting cells from damage by free radicals (Rayman, 2000). Its deficiency is associated with various health disorders such as cardiovascular diseases, cancer and reduced fertility (Broadley et al., 2007). The main sources of Se for humans and animals are foods such as cereals, poultry, meat and fish; contributions from drinking water and other non-food sources are nominal (Rayman, 2008; Broadley et al., 2010; Watts et al., 2019b).

In intermediate and low-income countries, cereals provide a large proportion (50 – 80%) of daily calorific intake (WHO, 2019). In Pakistan cereals, primarily wheat, account for 75% of energy supply in an average daily diet (Zia et al., 2014). A survey of Se concentrations in crops grown in Gilgit-Baltistan (GB), described in chapter 3, suggest that the Se concentration in cereals (wheat) is insufficient to meet the WHO recommended daily allowance (RDA) of Se (50 - 70 µg day^{-1}) for an adult. The average concentration of Se in locally grown wheat was found to be 28.9 µg kg^{-1} which would supply <20% (8.67 µg Se day^{-1}) of the RDA. Deficiency of Se in human populations can be addressed in multiple ways such as taking Se supplements, dietary diversification,

food fortification and crop biofortification through agronomic or genetic interventions (Broadley et al., 2007; White and Broadley, 2009; Broadley et al., 2010; Chilimba et al., 2011). Most of these strategies have various shortcomings associated with them while the efficacy of others, such as crop improvement and genetic modification, is not yet clear. However, in the context of Pakistan, and particularly GB, where the population is largely dependent on indigenous agricultural produce, crop biofortification is the most feasible approach. Furthermore, crop biofortification with fertilizers can easily enhance plant Se content (Broadley et al., 2010) and has been tested successfully in other countries, such as Finland, the UK and Malawi. Finland has adopted crop biofortification with Se-enriched fertilizers and has successfully enhanced Se concentration in the Finnish food supply (Eurola et al., 1991; Broadley et al., 2007; Chilimba et al., 2012a; Alfthan et al., 2015). The efficiency of Se biofortification varies with climatic conditions, agricultural practices and soil type (Broadley et al., 2010). Therefore, experience gained in other countries may not be applicable to the study area of this project. Furthermore, the uptake of Se by plants depends on its concentration, speciation, soil pH and other competing species present in soil such as anions of sulphur and phosphorus (White et al., 2004; Gupta and Gupta, 2017). Another consideration is that the range between Se deficiency and toxicity is rather narrow (Bolt et al., 2011; Gupta and Gupta, 2017). Thus, it is important to carefully assess the amount of Se required in fertilizer to elevate crop Se content to a level beneficial to humans and animals, which has not been done for GB nor for Pakistan. Due to the toxic nature of Se, large or extended applications

might have consequences for groundwater quality. Therefore, it is also vital to understand the fate of residual Se.

This experiment aimed to achieve the following objectives:

i. to study the feasibility of Se biofortification using a single application to wheat of an enriched ^{77}Se stable isotope to discriminate between soil-derived and fertilizer-derived Se;

ii. to determine the efficiency of different inorganic species (selenite and selenate) in wheat biofortification in the alkaline soils of GB;

iii. to evaluate the fate of residual Se in a cereal rotation, i.e. wheat-maize-wheat, practiced in GB.

4.2 Materials and Methods

4.2.1 Overview

A rotational field trial was undertaken over three consecutive cropping seasons (2017/18, 2018/19) in GB. The crops chosen were wheat followed by maize and then wheat again. Selenium inorganic species, selenite (Se^{IV}) and selenate (Se^{VI}), were applied as isotopically enriched ^{77}Se; each species was applied at two different levels (10 and 20 g ha^{-1}) to the first wheat crop in March 2018. After wheat harvest, a maize crop was planted on the site, grown and subsequently sampled; a second wheat crop was then planted and harvested as before. Soil was sampled at each harvest and analysed for 'soluble', 'adsorbed', 'organic' and total ^{77}Se; plant analysis included grain, chaff and straw.

4.2.2 Site selection and preparation

An agricultural field at the Mountain Agriculture Research Centre (MARC) Gilgit station (35.68157 N, 74.62981 E) was selected (Figure 4.1). The total area of the experimental field was 268 m^2 (17.6 x 15.2 m). Twenty plots (2 m x 2 m) were established with 0.4 m between consecutive plots in the central 178 m^2 (14.6 x 12.2 m) of the experimental field. All plots were labelled by erecting a signboard in the corner of each plot. No experimental plotting was done in the outer 3 m on all 4 sides of the selected area but the area was cultivated in the same way as the rest of the field. The purpose of this was to minimise possible interference from passing human and animal traffic because the field was situated at the side of an access road to other fields and a nearby village. Four replicates of each treatment were randomly distributed in a randomised block design. All plots received the same basal fertilizer and irrigation water. Before sowing the first wheat crop, the soil was ploughed and seeds were sown by hand in straight lines in each plot according to the local agriculture practice. However, for sowing the 2nd (maize) and 3rd (wheat) crop the soil was not ploughed and seeds were planted with minimum tillage in all the selected areas.



Figure 4.1. Photographs of field trial site at Mountain Agriculture Research Centre (MARC) Gilgit station

4.2.3 Treatment solution preparation and application

Enriched ^{77}Se treatment solutions of both Se^{IV} and Se^{VI} were prepared from elemental ^{77}Se according to the methods described in (Mathers et al., 2017). The treatment solution for each field was applied at the early stem extension stage (Zadoks stage 31) (Figure 4.2). The experimental field was flood-irrigated and two days later, when the field was still moist, treatment solution was applied to each plot. Before application, each treatment solution was separately mixed with 6 L irrigation water in a watering can (1.5 L m^{-2}), which was then sprayed evenly over the plot. Each treatment application was followed by spraying with 6 L (1.5 L m^{-2}) irrigation water from the same container to wash any treatment solution off the crop leaves.



Figure 4.2. Application of treatment solution to wheat crop

4.2.4 Soil and plant sampling

Soil sampling was undertaken before the experiment started (H0, Nov-2017) and at the end of each growing season i.e. post wheat harvest1 (H1, June-2018), maize harvest (H2, November-2018) and wheat harvest2 (H3, June-2019) (Figure 4.3). A five point composite sample of topsoil (0-20 cm) was collected from each plot using a stainless steel auger. Soil was air-dried and sieved (< 2 mm) before shipping to the University of Nottingham for further processing and analysis.

At the end of respective growing seasons (H1, H2 and H3), plants were harvested from the central 1 m^2 of each plot (2 m x 2m) at 5 cm above the ground with scythe (Figure 4.3). Plants were subsampled (10% of the total) and then sun dried at the MARC laboratory. Wheat ears were hand threshed to separate grain and chaff. Maize plants were divided into stems, leaves, husks and grains. All the crop samples were separately packed and shipped to the University of Nottingham for further processing and analysis.



Figure 4.3. Photographs of soil and plant sampling after wheat harvest 1

4.2.5 Samples preparation and analysis

After arrival at the University of Nottingham's lab, about 10 g of each soil sample was ball- milled and then acid (HNO_3-$HClO_4$-HF) digested in triplicate (section 2.4.5) for Se and multi elemental analysis. The milled soil was also used for measuring metal oxides (section 2.4.4) and carbon contents (section 2.4.2). Fractionation of Se was performed on sieved soil (< 2 mm) to determine soluble, adsorbed and organically bound Se (section 2.4.7). Soil pH was also measured on sieved soil (section 2.4.1). Soluble and adsorbed fractions were analysed for Se speciation (Se^{IV} and Se^{VI}) by LC-ICP-MS (section 2.7).

The fully dried plant samples were separately milled into finely ground powder using an ultra-centrifugal mill fitted with a 0.5 mm stainless steel sieve. Subsamples from every plot were then microwave acid (HNO_3) digested in triplicate for Se and elemental analysis (section 2.5.1).

4.2.6 Selenium-77 data processing

The raw intensity data of Se isotopes (^{77}Se and ^{80}Se) were exported as counts-per-second (CPS) values from the triple quadrupole ICP-MS (iCAP TQ). Both isotopes were measured in O_2 mode as mass shifted to m/z 93 ($^{77}Se.^{16}O$) and m/z 96 ($^{80}Se.^{16}O$) to reduce interferences from Se and Ge hydrides. The ^{77}Se intensity signals were also corrected for interference from ^{76}Ge hydrides at mass 77 ($^{76}Ge.H$) and mass 93 ($^{76}Ge\text{-}H\text{-}^{16}O$) by predicting the intensity of the interfering Ge signals from analysing a known concentration (5 µg L^{-1}) of Ge in terms of the ratio of m/z 72 (^{72}Ge) to m/z 93 and 96. The intensity signals of ^{77}Se and ^{80}Se were also corrected for instrumental drift by reference to the intensity of the internal standard (Rh). The natural isotopic abundance of both isotopes (^{77}Se and ^{80}Se) was used to calculate their concentration in multi isotopes Se calibration standards (1, 2, 5 and 10 µg L^{-1}). Then the calibration slopes for each isotope was calculated from the corrected intensity signals of calibration standards and their known isotopic concentrations predicted from the natural abundance of each isotope. The blank-subtracted, drift-corrected intensities of each sample were converted to concentration (µg L^{-1}) by multiplying by the dilution factor and dividing by the calibration slope (cps/ppb) for each isotope. The solution concentration was then converted to solid phase concentrations (µg kg^{-1}) by

multiplying by the volume of digest or extract and dividing by the sample weight. The concentration of native ^{77}Se in each sample was calculated from the natural isotopic abundance of ^{77}Se and measured concentration of total Se. Then by subtracting the native ^{77}Se from the total measured ^{77}Se in each sample, the ^{77}Se derived from fertilizer was obtained. The measurement of ^{80}Se was used to calculate total Se concentration.

4.3 Results and discussion

4.3.1 Soil characteristics

Basic soil characteristics including soil pH, organic carbon, $CaCO_3$ and metal oxides (Fe_2O_3, MnO_2 and $Al(OH)_3$) were similar across all the field plots and did not vary significantly (ANOVA, $p>0.05$). The mean soil pH of all plots was 7.84. The soil organic carbon and $CaCO_3$ contents were 1.60% and 1.73% respectively; the average concentrations of Fe, Mn and Al oxides were 3.13, 0.151 and 0.738 g kg^{-1} respectively. The mean total soil Se and TMAH-extractable Se contents were 139 µg kg^{-1} and 94 µg kg^{-1} respectively suggesting a low overall Se concentration and the presence of a non-organic recalcitrant Se phase (c. 50 µg kg^{-1}).

4.3.2 Fertilizer selenium (Se_{Fert}) dynamics in soil

The concentration of fertilizer-derived Se (Se_{Fert}) in soil decreased with time following its application in March 2018 (Table 4.1). Compared to its original application of 10 and 20 g ha^{-1}, the concentration of Se_{Fert} had decreased by 29-39% at wheat harvest (H1; June 2018), by 51-62% at maize harvest (H2; November 2018) and by 60-82% at

the second wheat harvest (H3; June 2019) (Figure 4.4). There was no significant difference in the proportion of Se_{Fert} lost between treatments at any of the three harvests (ANOVA, $p > 0.05$).

The decrease in Se_{Fert} concentration at H1 can be attributed to the combined effects of plant uptake and loss of Se from soil through leaching or volatilisation. The percent of the loss due to plant uptake at H1 varied between treatments and application levels. For Se^{IV} values were 1.36% and 1.46% at 10 g ha^{-1} and 20 g ha^{-1} respectively; equivalent figures for Se^{VI} were higher, at 3.34% and 4.32% due to greater bioavailability. Thus only a small proportion of the loss between application and H1 was due to crop uptake and suggesting leaching in irrigation water shortly after application must have been the main source of loss. Surprisingly the difference in total loss between Se^{IV} and Se^{VI}, mainly due to leaching, was quite small. This may be due to the high pH of the soil at which Se^{IV} adsorption is comparatively weak. Further loss of Se_{Fert}, measured at H2 and H3, cannot be attributed to plant uptake because Se_{Fert} in H2 and H3 crops was below the detection limit for ^{77}Se (c. 0.0025 $\mu g\ L^{-1}$). Losses from soil through leaching are particularly likely because of the high pH, sandy soil texture and low organic carbon content (1.6%) of the soil, characteristics which will reduce the ability of the soil to retain added Se (Gissel-Nielsen and Hamdy, 1977). Speciation analysis of post H1 soils demonstrated that the only inorganic form of Se_{Fert} present in the soluble and adsorbed fractions was Se^{IV}. It is reported that approximately 20% of the Se^{VI} applied to Finnish soils is taken up by plants whereas the remaining Se is reduced to Se^{IV} and immobilised in the soil (Keskinen et al., 2009). The findings of this study are also consistent with (Stroud et al., 2010b) who studied

the fate of Se added as Se^{VI} to soils in the UK and reported that Se^{VI} was not detectable in soil samples either before or after fertiliser application. It was initially thought that the calcareous conditions of the GB soil might ensure survival of some of the Se_{Fert} as Se^{VI}, at least at H1, but this was not the case.

The added Se_{Fert} did not significantly increase total soil Se because addition of 10 and 20 g ha^{-1} would only result in an increase in concentration of 4 and 8 μg kg^{-1} respectively, assuming 2500 t ha^{-1} of soil. By contrast, the average concentration of the native soil Se was 139 ± 9.12 μg kg^{-1}.

Table 4.1. Status of Fertilizer Se (Se_{Fert}) in soil after each harvest (wheat harvest = H1, maize harvest = H2, second wheat harvest = H3). Concentrations presented are the average of 4 replicate plots.

* Treatment types and level of application (g ha^{-1})	Plant uptake (g ha^{-1})			Se_{Fert} remaining in soil (g ha^{-1})			Loss (g ha^{-1})		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
10-Se^{IV}	0.136	0.00	0.00	6.37	4.15	1.81	3.49	5.85	8.19
10-Se^{VI}	0.334	0.00	0.00	5.82	4.29	3.14	3.85	5.71	6.86
20-Se^{IV}	0.287	0.00	0.00	13.9	9.75	8.02	5.80	10.2	12.0
20-Se^{VI}	0.864	0.00	0.00	12.4	7.61	5.17	6.74	12.4	14.8

* The numbers (10 and 20 g ha^{-1}) before the treatment type represent the level of Se_{Fert} application

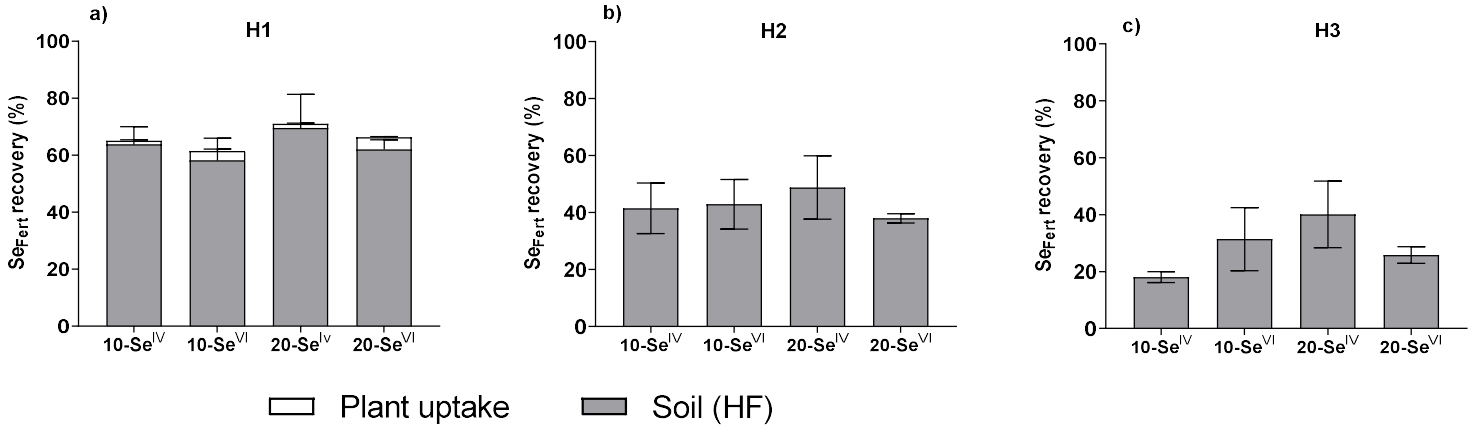


Figure 4.4. Fate of fertilizer added Se in soil after a) first wheat harvest (H1), b) maize harvest (H2) and c) second wheat harvest (H3)

4.3.3 Fractionation of selenium

4.3.3.1 Native soil Se (Se_{Nat})

The three-step extraction procedure demonstrated that the fractionation of Se_{Nat} was consistent across all four sampling events (H0, H1, H2 and H3) (Figure 4.5). The soluble and adsorbed fractions of Se_{Nat} were low and accounted for <2.5% (0.30-3.27 µg kg^{-1}) and 2% (1.12-2.61 µg kg^{-1}) respectively across all samples. The organically bound Se was typically constant at 60% (75-88 µg kg^{-1}) in H0, H1 and H2 samples while it substantially reduced to 43% (60 µg kg^{-1}) in H3 samples. The remaining Se_{Nat} of 40-57% (47.4-78.2 µg kg^{-1}) may be regarded as a 'recalcitrant' fraction of Se. The recalcitrant fraction is present in a non extractable form and may be locked up within mineral structures (Mathers, 2015). The concentration of the recalcitrant pool of Se as a proportion of total Se is broadly consistent with Se fractionation data from other districts in GB as described in section 3.3.11 of chapter 3.

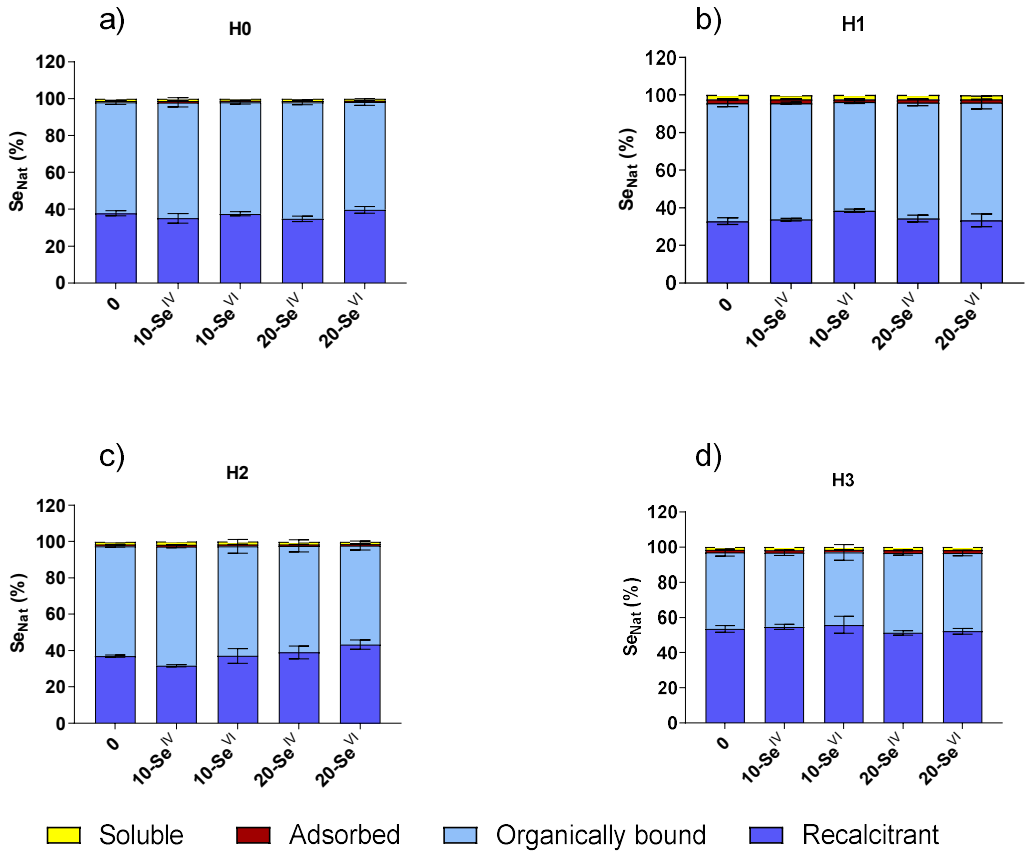


Figure 4.5. Fractionation (%) of Se_{Nat} in soils - a) pre experiment (H0), b) post wheat harvest 1 (H1), c) post maize harvest (H2), d) post wheat harvest2 (H3). Error bars represent standard error of means (n = 4)

The chemical forms of recalcitrant Se are not known. The following two possibilities can be envisaged in explaining the nature of recalcitrant Se: (i) a proportion of Se added to soil from different sources such as rock weathering, atmospheric deposition or irrigation water could be slowly converted to a recalcitrant phase by absorption into $CaCO_3$; (ii) the Se could be originally present in a form in parent material which is not extractable with TMAH but slowly weathers and contributes to soil organic Se with time. Therefore, it's not clear whether the recalcitrant Se acts as a sink or a source of Se.

4.3.3.2 Fertilizer Se (Se_{Fert})

The fractionation results for Se_{Fert} are presented in Table 4.2 and Figure 4.6. The soluble and adsorbed fractions of Se_{Fert} in post-H1 soils accounted for 7.4-10.3% (0.379-0.738 g ha^{-1}) and 4.1-8.0% (0.258-0.482 g ha^{-1}) respectively in 10 g ha^{-1} application treatments whereas for 20 g ha^{-1} applications the soluble and adsorbed fractions accounted for 5.4-10.5% (0.617-1.380 g ha^{-1}) and 2.8-6.9% (0.407-1.25 g ha^{-1}) respectively. The average concentration of plant-available fractions (soluble and adsorbed) combined for both application rates (10 and 20 g ha^{-1}) varied with time and decreased to 4.54% (0.297 $\pm$ 0.15 g ha^{-1}) and 3.03% (0.201 $\pm$ 0.11 g ha^{-1}) but slightly increased to 9.80% (0.369 $\pm$ 0.266 g ha^{-1}) and 6.26% (0.254 $\pm$ 0.173 g ha^{-1}) in H3 soils.

The remaining amount of Se_{Fert} in H1, H2 and H3 samples was all present in an organically bound form; all the remaining Se_{Fert} was extractable with TMAH and so there were insignificant concentrations present in a 'recalcitrant' pool. Within analytical error, the sum of all extractable fractions (soluble, adsorbed and organically bound) in each sample was equal to the total concentration of Se_{Fert} in soil after each harvest but the total concentration decreased with time compared to the original application (Figure 4.7). A decrease of ≥ 30% was observed in Se_{Fert} concentration after 72 days (H1) since the initial application; the remaining Se_{Fert} decreased further to <50% and <40% after 224 days (H2) and 439 days (H3) respectively.

Table 4.2. Fractionation of Se_{Fert} after crop harvest (H1 = wheat harvest, H2 = maize harvest, H3 = second wheat harvest)

Treatments and level of application (g ha^{-1})	Total Se_{Fert} remaining in soil (g ha^{-1})			Soluble (g ha^{-1})			Adsorbed (g ha^{-1})			Organically bound (g ha^{-1})		
	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
10-Se^{IV}	6.37	4.15	1.81	0.545	0.170	0.146	0.34	0.112	0.096	7.07	4.15	2.07
10-Se^{VI}	5.82	4.29	3.14	0.498	0.215	0.251	0.365	0.135	0.176	5.21	4.26	3.04
20-Se^{IV}	13.9	9.75	8.02	1.02	0.410	0.503	0.849	0.292	0.37	13.7	10.1	7.07
20-Se^{VI}	12.4	7.61	5.17	0.978	0.392	0.577	0.501	0.265	0.372	10.3	7.87	6.85

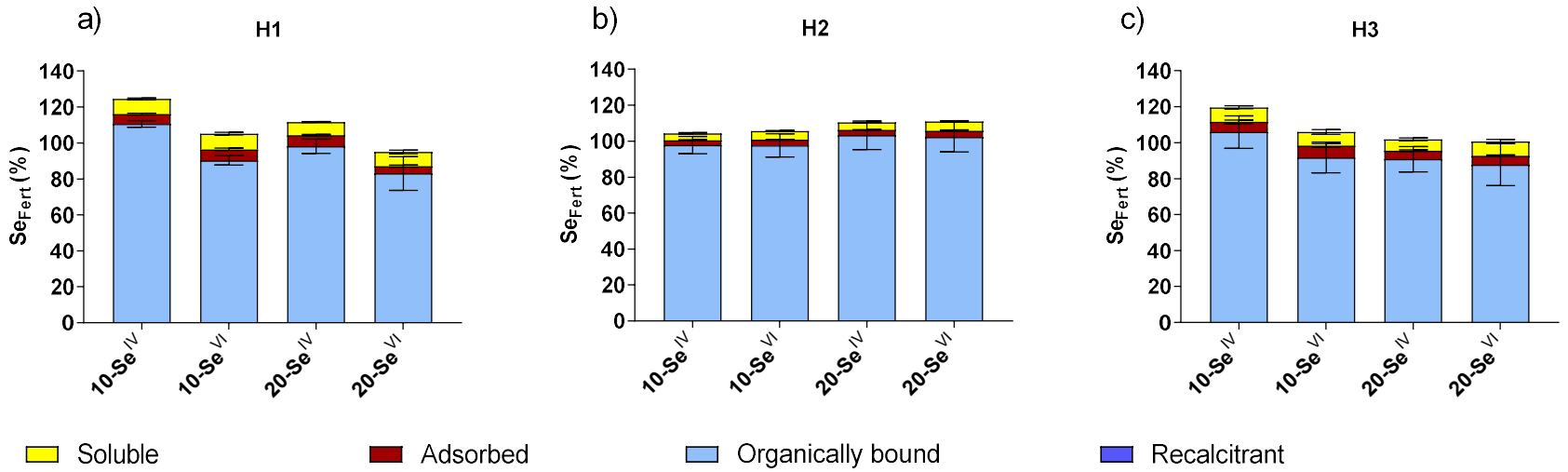


Figure 4.6. Fractionation of Se_{Fert} in soil representing proportional (%) distribution of Se_{Fert} fractions in soil after a) wheat harvest –H1, b) maize harvest – H2, c) repeat wheat harvest –H3. Error bars represent standard error of means (n=4)

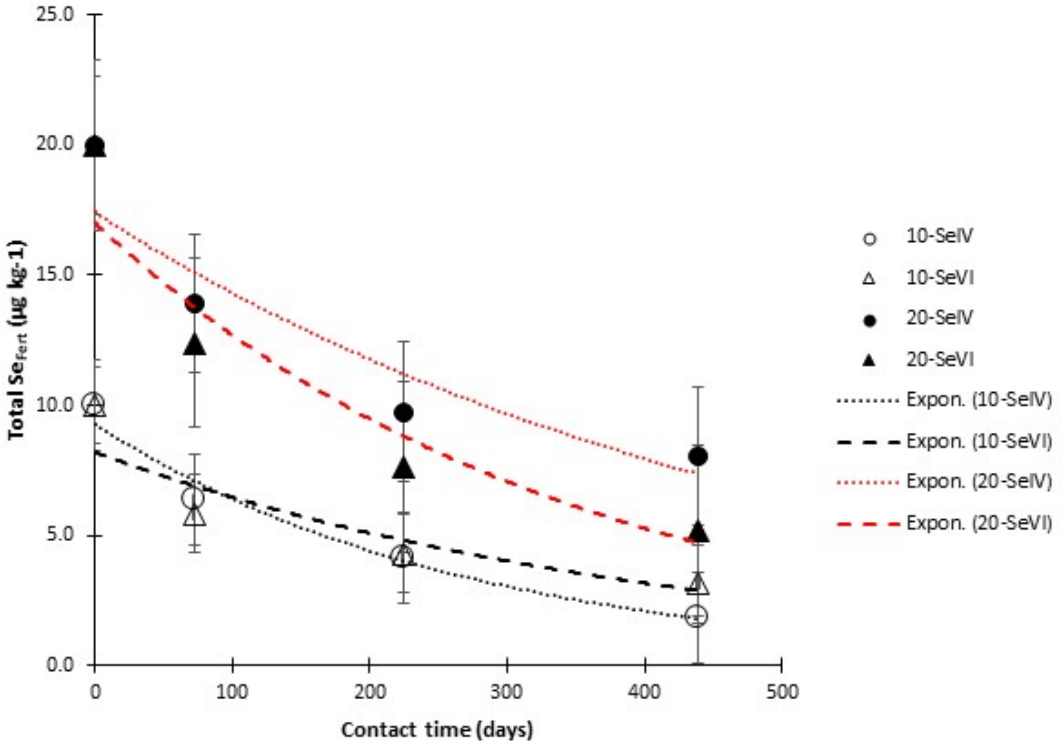


Figure 4.7. Reduction in concentration of Se_{Fert} (10-Se^{IV}, R^2 = 0.9886; 10-Se^{VI}, R^2= 0.878; 20-Se^{IV}, R^2= 0.8907; 20-Se^{VI}, R^2 = 0.9344) with time since application. The numbers (10 and 20) before Se species in the legend represent the application rate in g ha^{-1}

As there was a continuing loss of Se_{Fert} from the soil which followed an approximate exponential decline, it is possible to estimate a residential half-life from Eq. 4.1.

$$Se_t = Se_0\, e^{(-k\ )} \qquad (4.1)$$

where Se_0 is the initial concentration of Se_{Fert} (g ha^{-1}) applied to soil, Se_t is the Se_{Fert} concentration (g ha^{-1}) still remaining in the soil at time (t), k is the rate of loss and t is the time elapsed since the application of Se_{Fert} (days). The slope values for different Se_{Fert} species applied at different levels from Figure 4.8 provide the values of the rate constant (k) in Eq. 4.1.

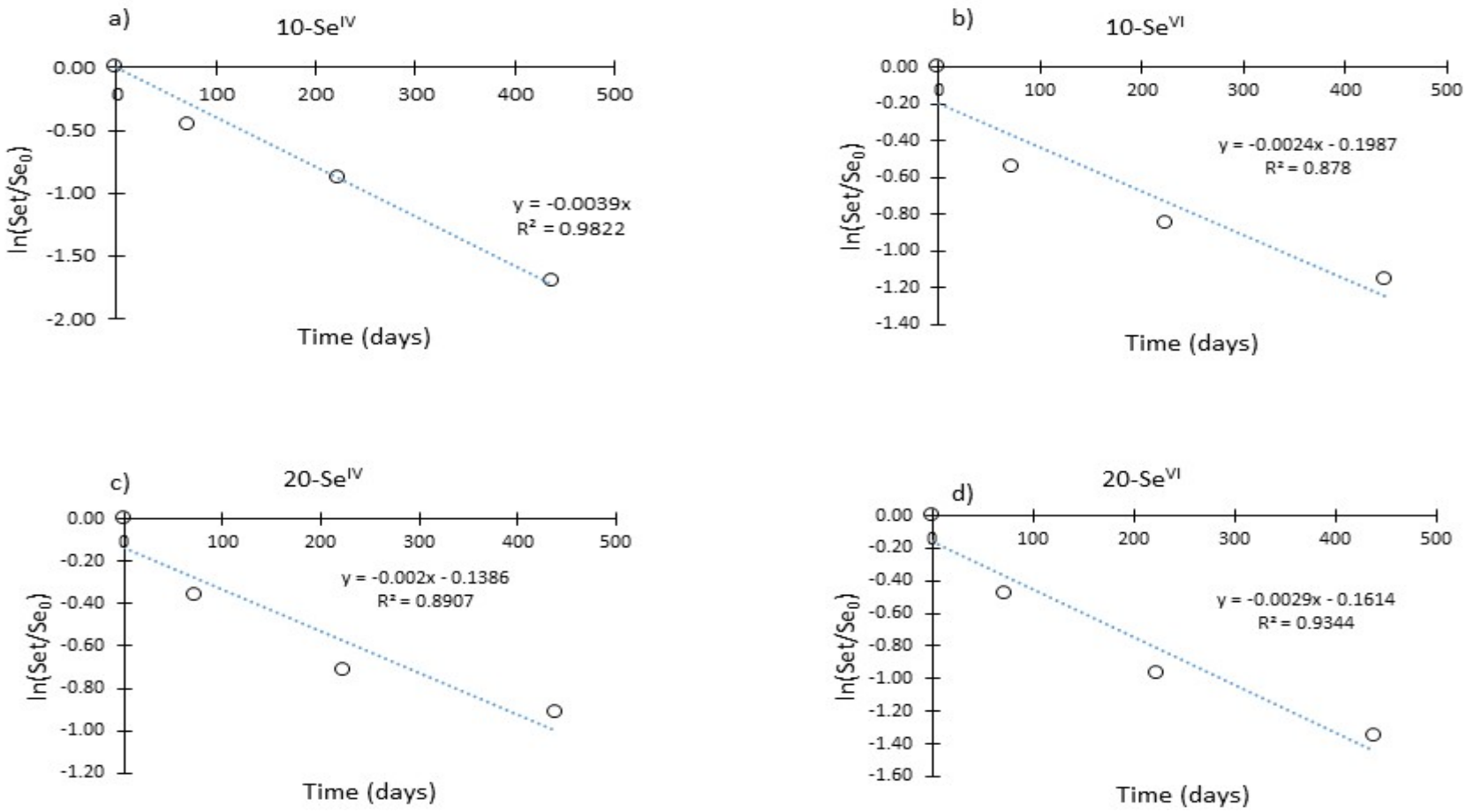


Figure 4.8. The rate of loss in Se_{Fert} with time. The Y-axis represents the natural log of the ratio of Se remaining in soil (Se_t) to the initial application of Se (Se_0)

The half residence times of Se^{IV}_{Fert} applied at 10 and 20 g ha^{-1} were 186 and 353 days respectively while the equivalent values for Se^{VI}_{Fert} were 291 and 236 days.

The continued loss of Se_{Fert} from soil to groundwater potentially poses a risk of contamination to drinking water in the catchment area. Therefore, a simple risk assessment was performed for the loss of Se to groundwater by considering the use of 6 million L ha^{-1} irrigation water, calculated based on a total of 12 irrigation events during the wheat growing period and a water depth of 50 mm for each event, which was practiced during this field trial. The irrigation water calculated for this field trial is consistent with the FAO guideline value of 4.5-6.5 million L ha^{-1} for wheat growth in one season (Brouwer and Heibloem, 1986). An estimated calculation suggests that approximately 30% (~3 and 6 g ha^{-1}) loss of Se_{Fert} measured at H1 for the application rates of 10 g ha^{-1} and 20 g ha^{-1} could result in 0.50 μg L^{-1} and 1.0 μg L^{-1} Se contribution to groundwater respectively after the wheat growing season. Alternatively, in the worst case scenario if it is assumed that all the Se_{Fert} applied (10 and 20 g ha^{-1}) to the wheat crop was lost with the first irrigation, it would result in 20 and 40 μg L^{-1} of Se in drainage water based on the use of 0.5 million L ha^{-1} irrigation water, supplied at a depth of 50 mm. Both of the above values, 20 and 40 μg L^{-1}, are still below the US EPA maximum contamination level of 50 μg L^{-1} for Se in drinking water and neither of these scenarios allows for the dilution of drainage water that would occur following egress of Se-enriched water from the soil into surface water systems.

The results of sequential extraction for Se_{Fert} and Se_{Nat} demonstrated that a comparatively larger proportion of freshly added Se was present in the bioavailable (soluble and adsorbed) fraction at H1 compared to post H2 and H3 soils (Figure 4.6).

However, available Se_{Fert} was too low to contribute to plant Se uptake in H2 and H3 crops which is consistent with the findings of Gissel-Nielsen et al. (1984) who found a minimal residual availability of Se in pasture systems. Similarly Chilimba et al. (2012b) and Mathers et al. (2017) reported minimal recovery of residual Se in maize and wheat crops respectively.

4.3.3.3 Distribution coefficient of Se_{Nat} and Se_{Fert} in soil

The distribution coefficient (kd) is the ratio of Adsorbed Se (Se_{Ads}) to Soluble Se (Se_{Sol}) and was calculated both for Se_{Nat} and Se_{Fert} respectively, from Eq. 4.2.

$$kd = \frac{Se_{Ads}}{Se_{Sol}} \quad (4.2)$$

where Se_{Ads} and Se_{Sol} are the concentrations of soil Se (µg kg^{-1}) in the Adsorbed and Soluble fractions. The kd values for Se_{Nat} and Se_{Fert} were very low with similar mean values of 0.76 ± 0.141 and 0.70 ± 0.199 demonstrating a very low absorption capacity in this soil to retain Se. This is expected because the soil has a coarse texture, high pH and low organic carbon content. The similarity of the kd values for Se_{Nat} and Se_{Fert} (there was no significant difference observed; ANOVA, $p > 0.05$) was expected because the reactive Se fractions (Se_{Sol} and Se_{Ads}) are dictated by soil properties which equally applies to both Se_{Nat} and Se_{Fert}. When Se is added to soil, some proportion of it will gradually transform into organically bound or recalcitrant phases but the reactive pools should reach equilibrium rapidly. The low kd values and low retention ability of this soil also advocate seasonal application of Se to each crop.

4.3.4 Selenium in the wheat crop at Harvest 1 (H1)

The average grain yield was 4.0 t ha^{-1} which is slightly higher than the typical yield of 3.5 t ha^{-1} in irrigated fields in Pakistan (Masood, 2015). The reason for the relatively high yield compared to the national average was probably the intensive management of the field trial in terms of irrigation timing, application of fertilizer and weeding. However, the yield is low compared to the UK where average wheat yield is 8.5 t ha^{-1} (DEFRA, 2017). The low wheat yield in the study area compared to the UK is probably due to climate, limited use of fertilizers and pest control, and cultivation of low yielding wheat varieties.

The application of different levels and species of Se had no effect on crop yield (Figure 4.9) which is consistent with other investigations (Ducsay and Ložek, 2006; Grant et al., 2007; Curtin et al., 2008; Broadley et al., 2010; Mathers et al., 2017). The average yields of straw and chaff together, and grain based on harvest from the central 1 m^2 of each plot were 4.8 and 4.0 t ha^{-1}.

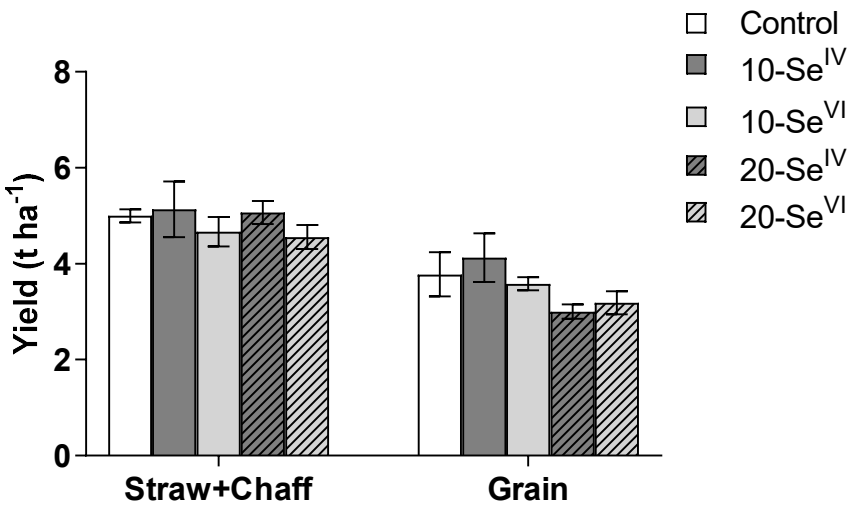


Figure 4.9. Wheat straw+chaff and grain yield after harvest (H1) in June 2018. Error bars represent the standard error of means (n = 4)

Both Se_{Nat} and Se_{Fert} contributed to plant Se (Table 4.3). However, the concentrations of Se_{Nat} (sum of straw, chaff and grain) were small compared to Se_{Fert} and similar across all plots; there was no significant variation in the Se_{Nat} concentrations among different treatments (ANOVA, $p > 0.05$). However, the concentration of Se_{Nat} varied between plant tissues (straw, chaff and grain) in all treatments (Figure 4.10). Chaff had the highest average concentration of Se_{Nat} at 7.15 µg kg^{-1} followed by straw (2.87 µg kg^{-1}) and grain (1.14 µg kg^{-1}) in all cases. The native Se concentration in chaff and grain was constant across all treatments. However, concentration of Se_{Nat} in straw revealed variation between different treatments (ANOVA, $p < 0.05$); the reason for this is not clear.

Table 4.3. Concentration of Se in wheat (sum of chaff, grain and straw) at H1 originating from soil and fertilizer, and plant enrichment factor (the proportional increase of Se in the plant above native levels originating from the application of Se_{Fert}). The number in treatments before Se species (selenite (Se^{IV}) and selenate (Se^{VI})) indicate the Se application rate (10 and 20 g ha^{-1}).

Treatments	Plant total Se	Soil derived Se (Se_{Nat})	Fertilizer derived Se (Se_{Fert})	Plant enrichment factor
	($\mu g\ kg^{-1}$)			
Control	10.9	10.2	0.740	
10-Se^{IV}	53.5	11.4	42.1	4.89
10-Se^{VI}	132	12.4	120	12.1
20-Se^{IV}	117	11.2	105	10.7
20-Se^{VI}	348	10.5	337	31.8

The concentration of Se was significantly greater in all plant tissues due to fertiliser Se application (Figure 4.10). Both species of Se_{Fert} (Se^{IV}_{Fert} and Se^{VI}_{Fert}) enhanced Se concentration in the wheat plant (grain, chaff and straw) at harvest but Se^{VI}_{Fert} was more effective compared to Se^{IV}_{Fert}. A single application of 10 and 20 g ha^{-1} of Se^{IV}_{Fert} resulted in a 14 and 32-fold increase in grain Se compared to an extremely low grain Se concentration of 1.42 $\mu g\ kg^{-1}$ in control plots. The same application rates of Se^{VI}_{Fert} produced a 35 and 95-fold increase in grain Se over control plots.

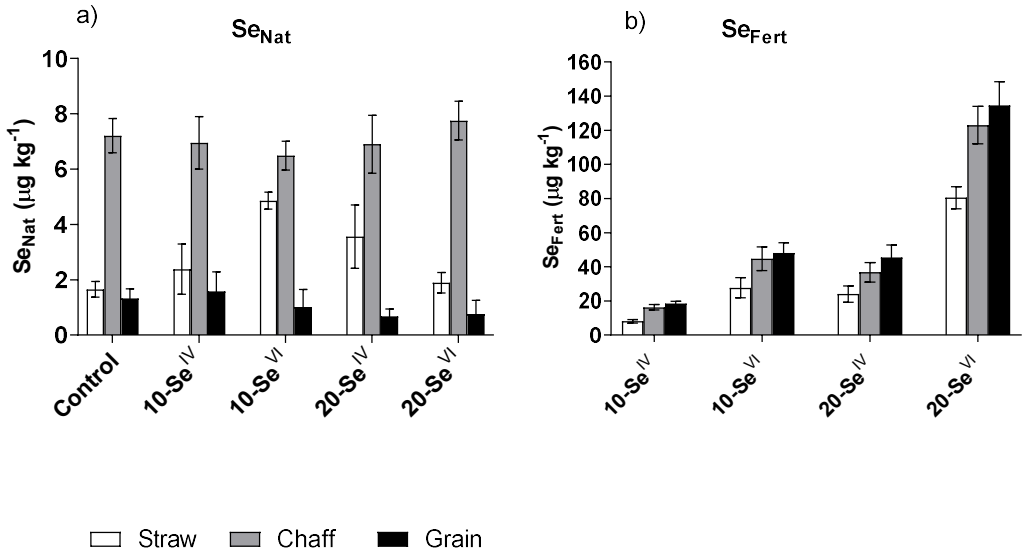


Figure 4.10. Selenium concentration in plant tissues at harvest 1 (H1) originating from a) soil native Se (Se_{Nat}) and b) Fertilizer derived Se (Se_{Fert}). Error bars represent standard error of means (n = 4). Note the different scales in Figures 4.10a and 4.10b.

The greater efficiency of Se^{VI} in enhancing plant Se content observed in this study is consistent with other investigations. Gupta and Winter (1989) reported that Se^{VI} applications resulted in 5-18 times greater Se concentrations in forages and barley grain compared to the same application of Se^{IV}. Chen et al. (2002) reported a 9-fold increase in rice Se in China, compared to a 6.6-fold increase, as a result of 20 g ha^{-1} application of Se^{VI} and Se^{IV} respectively. Boldrin et al. (2013) applied Se^{IV} and Se^{VI} to soil, as a foliar spray, and found that Se^{VI} was more effective in raising rice Se concentrations in Brazil for both methods of application. Ros et al. (2016) compared a large set of data on Se biofortification and found that on average Se^{VI} is 33 times more effective in increasing plant Se contents compared to Se^{IV} at the same rate of application.

In common with other studies, Se^{VI} was more efficient in increasing plant Se content in this experiment. However, for each g ha^{-1} of Se^{VI} applied the grain Se concentration was increased by only 4.76-6.70 µg kg^{-1}, which is less efficient compared to other investigations. Broadley et al. (2010), Chilimba et al. (2012a) and Mathers et al. (2017), respectively, reported increases in grain Se concentration of 16-26 µg kg^{-1}, 15-21 µg kg^{-1} and 12.1-17.3 µg kg^{-1} for each g ha^{-1} of Se^{VI} applied.

4.3.5 Recovery of Se_{Fert} species in plants at H1

The recovery of Se_{Fert} by wheat plants (sum of straw, chaff and grain) from soil was different for the two Se species (Table 4.4). Selenate recovery was 33-40% greater than that of Se^{IV}_{Fert} at application rates of both 10 and 20 g ha^{-1}. The average recoveries of Se^{IV}_{Fert} by wheat plants was only 1.36% and 1.43% for 10 and 20 g ha^{-1} application respectively (Table 4.4); the equivalent recoveries for Se^{VI}_{Fert} were 3.34% and 4.32% respectively. For both species, the recovery was greater at the higher application rate (20 g ha^{-1}). The recovery of Se_{Fert} species also varied between plant tissues (Table 4.4 and Figure 4.11); grain had higher recovery in all cases followed by chaff and straw.

Compared to other investigations on field-grown wheat and other cereal crops, the recovery of both species was low. Mathers et al. (2017) reported 25.9-44.5% recovery in wheat plants grown on three contrasting sites in the UK with an application of 10 g ha^{-1} of $^{77}Se^{VI}$. Broadley et al. (2010) observed recoveries of 19.6-34.7% in a wheat crop (grain+straw) following Se^{VI} (aqueous Na_2SeO_4) application at six different rates (ranging from 5 – 100 g ha^{-1}) in the UK. However, Lyons et al. (2004) reported slightly

smaller recoveries of 1.8-9.3% for foliar application of Se^{VI} at four different rates (10, 30, 100 and 300 g ha^{-1}) to a wheat crop in Australia. Similarly, Ducsay and Ložek (2006) reported plant uptake of 2.4-9.3% for foliar application of Se to a wheat crop in Slovakia.

Table 4.4. Recovery (% of application) of Se_{Fert} in different plant tissues at wheat harvest (H1). Treatments below indicate the Se application rate (10 and 20 g ha^{-1}) and the Se species (selenite (Se^{IV}) and selenate (Se^{VI})).

Plant tissue	Se_{Fert} recovery in wheat crop (% of application)			
	10-Se^{IV}	10-Se^{VI}	20-Se^{IV}	20-Se^{VI}
Straw	0.250	0.797	0.393	1.05
Chaff	0.345	0.821	0.353	1.17
Grain	0.768	1.72	0.686	2.10
*Total Plant	1.36	3.34	1.43	4.32

* 'Total plant' represents the sum of straw, chaff and grain; roots were not sampled.

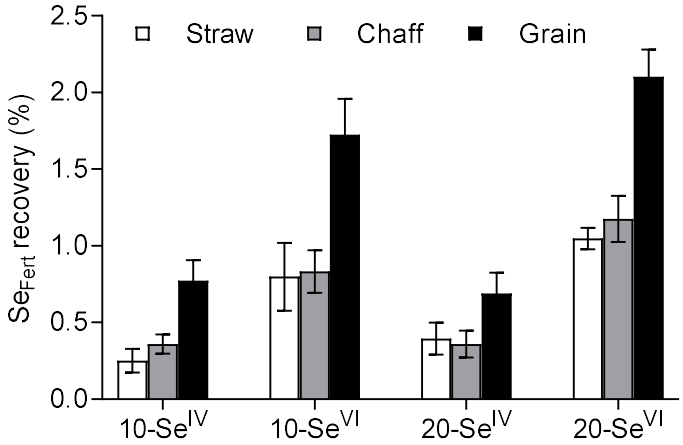


Figure 4.11. Se_{Fert} recovery (% application) in plant tissues for different Se treatments

The recovery of Se_{Fert} in wheat grain observed for different treatments (Table 4.4) was smaller than values reported for wheat in the literature; 12.4-15.2% (Mathers et al., 2017), 10-17.3% (Broadley et al., 2010), 12.7-17% (Curtin et al., 2008), 6.5% (Lyons et al., 2004), 2-6% (Stephen et al., 1989). These values were also less than the 6.5-10.8% recoveries reported by Chilimba et al. (2012a) for a maize crop.

The low Se_{Fert} recoveries in this study compared to other investigations conducted elsewhere could be due to low crop yield, soil characteristics and possibly a limited period of Se_{Fert} availability for plant uptake. Soil texture was sandy with low $CaCO_3$ and organic carbon contents, which may reduce nutrient retention and thereby render the Se_{Fert} more prone to leaching after irrigation.

4.3.6 Soil-to-plant transfer factor

The soil-to-plant transfer factor (TF) is formulated as the ratio of Se_{Nat} or Se_{Fert} in the plant to their respective concentrations in soil and is a convenient index of bioavailability. The TF values for the wheat crop at H1 for both species of Se_{Fert} (Se^{IV} and Se^{VI}) and Se_{Nat} were calculated from Eq. 4.3.

$$Se_{TF} \quad \frac{Se_{plant}}{Se_{soil}} \qquad (4.3)$$

Where Se_{plant} is Se concentration (μg kg^{-1}) in each fraction (straw, chaff and grain) of wheat plant respectively and Se_{soil} is total Se_{Nat} or Se_{Fert} concentration (μg kg^{-1}) in the corresponding soils. The total Se_{Nat} concentration used in Eq. 4.3 was measured in the soil HF digests while the total Se_{Fert} concentrations in soil of 4 and 8 μg kg^{-1} were calculated from the application rates of 10 and 20 g ha^{-1} respectively assuming a topsoil mass of 2500 t ha^{-1}.

Values of TF for Se_{Fert} (Se^{IV} and Se^{VI}) were significantly larger than those of Se_{Nat} (Figure 4.12). This is consistent with findings from Mathers et al. (2017) who observed a higher TF for wheat plants fertilized with Se^{VI} compared to their control plots. Furthermore, as expected the transfer factor of Se^{VI}_{Fert} was higher than that of Se^{IV}_{Fert} (Figure 4.12) due to greater solubility (Peng et al., 2017). The other reason for differences in TF between Se^{IV} and Se^{VI} may lie in the mechanisms governing root uptake from soil and subsequent translocation to aerial parts of the plant. Selenate is freely absorbed by plant roots via sulphate transporters and then transported through xylem vessels into plant stems and leaves whereas a fraction of Se^{IV} is likely to be converted to Se^{VI} or organic Se before being transported to other parts of the plant (White, 2016; Ali et al., 2017). The TF also varied between different parts of the plant in the same treatment as the TF values for Se_{Fert} in grain and chaff were similar but substantially higher than for straw due to different abilities of tissue in various parts of the plant to accumulate or retain Se. The other reason for higher concentration of Se in grain is the transportation of Se in phloem from leaves to grains.

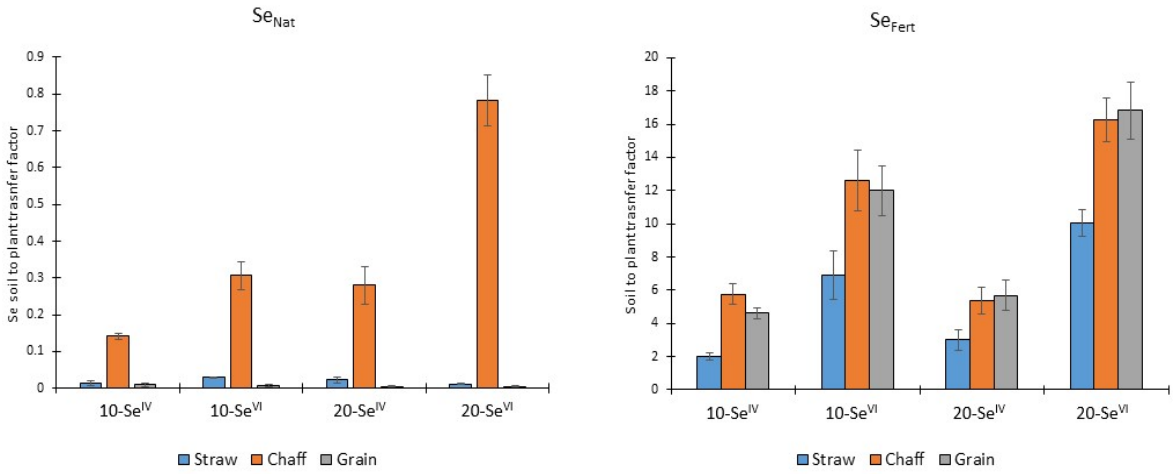


Figure 4.12. Soil-to-plant transfer factor of native Se and fertilizer derived Se in different parts of wheat plants. Error bars represent standard error of means (n = 4). Note the different scales in Figures 4.12a and 4.12b. The numbers (10 and 20) before the Se species symbol represents the amount of Se applied in g ha^{-1}

Differences in TF for Se_{Fert} and Se_{Nat} and for Se_{Fert} species of Se^{IV} and Se^{VI} reflect differences in bioavailability. The *relative* bioavailability of Se_{Fert} (Se^{IV} and Se^{VI}) and Se_{Nat} to the H1 wheat crop was calculated as a 'bioavailability ratio' (B_R) from Eq. 4.4.

$$B_R = \frac{TF_{Fert}}{TF_{Nat}} \quad (4.4)$$

Where, TF_{Fert} represents the transfer factor for fertilizer-derived Se and TF_{Nat} is the transfer factor for soil-derived Se in the plant.

The average B_R of Se_{Fert} : Se_{Nat} to the H1 wheat crop confirmed the large difference in bioavailability of Se_{Fert} compared to Se_{Nat} to all tissues of the wheat (Table 4.5); in particular, the bioavailability of $Se^{VI}{}_{Fert}$ to wheat grain was 764 and 1261 times that of soil-derived Se at applications of 10 and 20 g ha^{-1} respectively. The B_R values of Se_{Fert} : Se_{Nat} were comparable with other Se biofortification investigations (Stroud et al., 2010b, Chilimba et al., 2012a, Galinha et al., 2012) as cited in Mathers et al. (2017)

and confirmed the higher bioavailability of Se_{Fert}^{VI} (Table 4.5). Ali et al. (2017) studied the bioavailability of Se^{IV} and Se^{VI} to wheat plant in a pot experiment and found that mobility and availability of Se^{VI} for plant uptake was 40-90% higher compared to that of Se^{IV}. Similarly, Fan et al. (2015) from his studies on transformation and bioavailability of Se (Se^{IV} and Se^{VI}) applied to tobacco plants reported that Se^{VI} was 4.3 - 7.9 times more bioavailable than Se^{IV}.

Table 4.5. Bioavailability ratio (B_R) of different concentrations of fertilizer-derived selenite (Se^{IV}) and selenate (Se^{VI}) to soil-derived Se (Se_{Nat}) in plant tissues of H1 wheat crop

Plant tissue	Bioavailability ratio of Se_{Fert} : Se_{Nat}			
	10-Se^{IV}	10-Se^{VI}	20-Se^{IV}	20-Se^{VI}
Straw	69.4	91.8	72	376
Chaff	34.8	111	41.4	136
Grain	187	764	490	1261

The number (10 and 20) before the Se species symbol represents the amount of Se applied in g ha^{-1}

It was observed that BR of Se_{Fert} : Se_{Nat} varied between different parts of the plant (Table 4.5). The BR of Se_{Fert} ; Se_{Nat} in grain was significantly greater than those of chaff and straw (ANOVA, $p < 0.001$). However, there was no significant difference between the BR values of chaff and straw (ANOVA, $p > 0.05$). The possible reasons for higher BR in grain are the timing, mode of Se_{Fert} application and species of Se_{Fert}. The Se_{Fert} was applied at early stem extension stage and as described earlier that Se_{Fert} was more bioavailable compared to Se_{Nat} so comparatively higher concentration of Se_{Fert} is taken up by plant and reserved in leaves which is then supplied to grain when its formation begins. The concentration of Se is generally higher in leaves before

anthesis and then starts to decrease when its translocation from leaves to reproductive organs begin (White, 2016). Furthermore, because Se_{Fert} was sprayed on the plant canopy it is likely that some of it may have been absorbed into leaves and translocated via phloem from leaves to grain. The BR values for Se^{VI} in plant parts including grain is higher compared to Se^{IV} because Se^{VI} is readily translocated from roots to other parts of the plant while Se^{IV} tends to accumulate in roots (Li et al., 2008).

4.3.7 Consequences of biofortification for dietary Se intake

Wheat is the largest staple crop in Pakistan (Raza, 2018) and forms an essential component of the daily diet for the majority (~80%) of the local population (Zia et al., 2014). The average daily consumption of wheat-based food in Pakistan is 350 g $person^{-1}$ and accounts for 75% of the daily calorific intake for an individual (Zia et al., 2014). Considering the average daily wheat consumption, the Se content of wheat grown on the control plots of this experiment would provide only 0.5 µg $person^{-1}$ day^{-1} which accounts for < 1% of the WHO-recommended (55-70 µg day^{-1}) daily Se intake (Table 4.6). However, the biofortified wheat grain produced in this study with the application of 10 and 20 g ha^{-1} Se^{VI} would provide 17.2 and 47.4 µg day^{-1} accounting for 31.2% and 86.2% of the daily requirement. The application of Se^{IV} at the same level as that of Se^{VI} would provide only 7 and 16.1 µg $person^{-1}$ day^{-1} contributing 12.7% and 29.3% towards the recommended daily Se intake. Based on Se concentrations in a typical Pakistani food basket from previous research, and values reported in the literature, Se contribution from other sources is around 25 µg day^{-1}

(Hussain, 2001, Iqbal et al., 2008, USDA, 2019). Therefore, the consumption of wheat flour obtained from 10 g and 20 ha^{-1} Se^{VI} application would increase Se intake to 42.2 and 72.4 µg day^{-1} $person^{-1}$ for individuals with diverse food sources, assuming 25 µg day^{-1} has come from other sources.

The results of this study suggest that wheat biofortification with just 20 g ha^{-1} of Se^{VI} would be a successful strategy for boosting daily per-capita Se intake into the correct range to avoid Se deficiency. Chilimba et al. (2012a) presented a brief economic analysis, based on the addition of 5 g ha^{-1} of Se to Malawi soils, in which the per capita cost was just 1.6 -3.5 US cents per annum. A full economic analysis for introducing Se into compound fertilizers in GB is beyond the scope of this thesis but the Chilimba analysis suggests that the cost is likely to be equally trivial.

Table 4.6. Comparison of the increase in grain Se concentration resulting from different treatments compared to reliance on native soil-derived Se. Treatments below indicate the Se application rate (10 and 20 g ha^{-1}) and the Se species (selenite (Se^{IV}) and selenate (Se^{VI})).

Treatments	Se concentration in grain (µg kg^{-1})		Adult daily Se intake from grain (µg $person^{-1}$)		Proportion (%) of recommended adult daily Se intake
	Mean	Standard Error	Mean	Standard Error	
Controls	1.42	0.26	0.50	0.09	0.904
10-Se^{IV}	19.99	1.86	7.00	0.65	12.7
10-Se^{VI}	49.05	6.58	17.2	2.30	31.2
20-Se^{IV}	46.02	7.54	16.1	2.64	29.3
20-Se^{VI}	135.40	14.20	47.4	4.97	86.2

4.3.8 Residual Se in crop at H2 and H3

Se_{Fert} could not be detected in the first rotation crop (maize) at H2 or the 2nd wheat crop at H3. Thus, within analytical error, all the Se measured in all parts of the maize

(grain, husk, leaf and stem) and wheat (straw, chaff and grain) was soil-derived. This is consistent with the observation that the major proportion of Se_{Fert} remaining in the soil had become organically bound and unavailable for plant uptake. Whatever Se_{Fert} was still available in the soluble and adsorbed fractions was at too low concentration to measurably contribute to plant uptake.

The negligible recovery of residual Se in following crops is consistent with other investigations. Mathers et al. (2017) observed a negligible amount of residual Se in follow up crops in his experiment on ^{77}Se application to winter wheat at sites in the UK with 10 g ha^{-1} Se^{VI} applications. Stroud et al. (2010b) reported that no significant difference was found in Se concentration of wheat grain from control plots compared to those that were previously treated with 10 or 20 g ha^{-1} of Se as Na_2SeO_4. Similarly, Gupta et al. (1993) also reported no effect of residual Se on barley grain Se content in Canada with applications at three different rates (10, 20 and 40 g ha^{-1}) of Se^{IV} and Se^{VI} respectively. Chilimba et al. (2012b) reported a very small recovery (0.78% and 2%) of isotopically labelled residual ^{74}Se in maize grain at two different field sites in Malawi, treated with 10 g ha^{-1} $^{74}Se^{VI}$ the previous year. In addition, Wang et al. (2017) also reported a 77.9% and 91.2% reduction in Se concentration in wheat and maize grain from plots, which were treated with 30 and 60 g ha^{-1} of Se in previous cropping season in China.

4.4 Conclusions

Both species of Se_{Fert} (Se^{IV} and Se^{VI}) at application rates of 10 and 20 g ha^{-1} increased Se concentration in a wheat crop. However, Se^{VI} was more efficient in increasing

wheat grain Se at H1 and produced 33-40% greater recovery compared to Se^{IV}. A one-time application of 20 g ha^{-1} Se^{VI} increased Se concentration in the wheat grain to 135 µg kg^{-1} compared to an extremely low Se concentration of 1.42 µg kg^{-1} from control plots. Considering the average per capita wheat consumption of 350 g day^{-1} in Pakistan, this will provide c. 47 µg $person^{-1}$ day^{-1} Se, which should be enough to avoid Se deficiency in GB population. There was no Se_{Fert} detected in H2 (maize) and H3 (wheat) crops.

On average 71.1 ± 16.9% of Se_{Fert} was lost from the soil at the time of final soil sampling (H3-July 2019). The Se_{Fert} which remained in soil after every harvest was largely (≥90%) present in organically bound form which rendered it completely unavailable for plant uptake so that Se_{Fert} was not detected in H2 and H3 crops. Therefore, it is recommended that Se application to crops in every growing season would be required to obtain an increased concentration of Se in crops. However further research is required to assess (i) the effect of Se lost from soil on water quality immediately following application and (first) irrigation and (ii) any longer term residual effects from repeated applications as the capacity of these soils to continue fixing Se into organic forms is unknown.

5 IODINE AND SELENIUM STATUS OF A SAMPLE POPULATION IN GILGIT-BALTISTAN

5.1 Introduction

Iodine (I) and selenium (Se) are important micronutrients for human health and play a vital role in thyroid gland functioning and thyroid hormone synthesis and metabolism (Schomburg and Köhrle, 2008; Wu et al., 2015; Jin et al., 2019). Low dietary I causes health complications, collectively known as iodine deficiency disorders (IDD) (Fuge and Johnson, 2015), which are some of the most prevalent public health problems worldwide (Kapil, 2007; Gizak et al., 2017). Iodine deficiency can affect any stage of human development including foetus, neonate, child, adolescent and adult (FAO and WHO, 2002; Hetzel, 2000; Zimmermann et al., 2008), however the consequences are more serious during foetal development and childhood (Obican et al., 2012; Bath et al., 2013; Watts et al., 2019a; Velasco et al., 2018). Globally, approximately 2 billion people are living at risk of IDD, which also include 240 million school age children (Watts et al., 2015; Watts et al., 2019a). Iodine deficiency was widespread in Pakistan prior to the start of the 'Universal Salt Iodisation' (USI) programme in 1994 with goitre prevalence >70% in some parts of the country (Khattak et al., 2017). It is reported that the USI programme has resulted in up to a 50% decrease in IDD but it is suspected that this decline may be non-uniform and possibly over-estimated (Khattak et al., 2017). The study area of this project, Gilgit-Baltistan (GB), has long been known as an endemic region for IDDs (Stewart, 1990), which are still considered a major public health problem in this

mountainous region (Shah et al., 2014). The fourth national nutritional survey of Pakistan has reported a urinary I concentration (UIC) of 62 and 64 μg L^{-1} in mothers and children from GB (Government of Pakistan, 2011), which is well below the adequate range of 100 – 199 μg L^{-1}.

Selenium deficiency is known to be associated with a range of health problems including cardiovascular and myodegenerative diseases, infertility and cognitive decline (Shreenath and Dooley, 2019). There are approximately 0.5 to 1.0 billion people affected from Se deficiency globally (Haug et al., 2007; Jones et al., 2017). It is reported that a coexisting deficiency of both I and Se aggravates hypothyroidism and may result in myxoedematous cretinism (Arthur et al., 1999; Rayman, 2000). Currently, there appears to be no literature available on the Se status of the GB population.

Both I and Se deficiencies occur when there is inadequate dietary intake of these essential elements (Kapil, 2007; Shreenath and Dooley, 2019). The I nutritional status of a population can be estimated from UIC, which is a well-established, cost effective and easily obtainable biomarker (WHO, 2013). Selenium concentration in blood plasma is the trusted biomarker for assessing Se level at both individual and population level (Fairweather-Tait et al., 2011) but blood plasma samples are more difficult to obtain, for obvious reasons. However, Phiri et al. (2020) have reported urinary Se concentration (USeC) as a plausible alternative to blood plasma to assess population level Se status.

All the locally grown agricultural produce collected from GB revealed very small concentrations of I and Se (reported in Chapter 3). It is understood that both I and Se

are largely supplied from dietary sources and that the population of GB consumes mainly locally grown agricultural produce. Therefore, there are grounds to suspect that the GB population is living at risk of I and Se deficiency.

This study aimed to (i) evaluate the I and Se status of people residing in the Gilgit district of GB using urinary concentrations (UIC and USeC) as biomarkers and (ii) study the role of iodised salt in improving the I status of the local population.

5.2 Materials and Methods

5.2.1 Survey overview

The University of Nottingham Research Ethics Committee granted ethical approval for (i) urine sampling of individuals representing different age groups in GB and (ii) undertaking a household questionnaire survey to estimate salt consumption in the local population. Individuals (n = 450) of different age groups and gender were drawn from the Gilgit district of GB. Following analysis of urine samples, 86 individuals were excluded based on the WHO criteria of urine hydration status. According to WHO, a urine sample taken for I analysis should be excluded from consideration if the creatinine concentration is too dilute (<30 mg dL^{-1}) or too concentrated (>300 mg dL^{-1}) (Barr et al., 2005). Creatinine is a chemical waste product produced from the breakdown of creatinine phosphate in muscles and exclusively excreted in urine at a relatively constant rate (Hessels et al., 2018).

Therefore, with the exclusion of 86 samples, the number of samples that are discussed in this study was 364; details are provided in Table 5.1.

Table 5.1. Number (n) and proportion (%) of each age group in the GB surveyed population

Age group (y)	Total participants	Gender	
	n (%)	Male (n)	Female (n)
< 5	4 (1.10)	2	4
5-16	130 (35.7)	65	91
17 - 24	69 (19.0)	30	39
25 - 44	102 (28.0)	44	70
45 - 64	48 (13.2)	29	25
≥65	11 (3.02)	10	7

Of the 364 viable samples, male and female participants were 46 and 54% respectively and 35% of the surveyed population were women of reproductive age (WRA) (15-49 y).

5.2.2 Salt intake survey

A nominated member in each household completed a household salt intake questionnaire to ascertain information relating to the purchase and storage of salt (iodised/non-iodised) and its daily consumption rate.

5.2.3 Salt sampling

A domestic salt sample was collected from each participating household in a plastic bag which was kept sealed and stored at < 5^{o}C in the dark at the Mountain Agriculture Research Centre (MARC) and then shipped to the University of Nottingham for chemical analysis.

5.2.4 Urine sampling

A first morning void urine sample was obtained from each participant in an 8 mL capacity polypropylene tube. Upon collection, samples were kept in a cool box and transported to the MARC lab where they were frozen and stored at -20 ^{o}C. Frozen urine samples were then shipped to the University of Nottingham for elemental and hydration adjustment analyses.

5.2.5 Chemical analysis

Salt samples

Duplicate samples of each salt (1.25 g) were dissolved in 25mL of Milli-Q water and then diluted 1-in-10 with 1% TMAH prior to analysis by ICP-MS.

Urine samples

Elemental analyses were performed on all urine samples with ICP-MS. Iodine analysis was conducted in an alkaline matrix following a 1-in-20 dilution (0.5 ml urine + 9.5 ml 1% TMAH) whereas multi elemental analyses, including Se and sodium Na, were performed in an acidic matrix (0.5 ml + 9.5 ml 2% HNO_3). Hydration correction of urinary analyte concentrations was undertaken with urinary creatinine and specific gravity to minimise the effect of hydration status in samples. Urinary creatinine was measured spectrophotometrically as described in Karim (2018) and Watts et al. (2019a). Specific gravity was measured with a handheld pocket refractometer (PAL-10S, Atago, Japan) on 300 µL of urine as described in (Phiri et al., 2020).

Equation 5.1 was used to adjust urinary analyte concentration for variation in creatinine concentration.

$$C_{cre-adj} = \frac{C_m}{C_{cre}} \quad (5.1)$$

where $C_{cre-adj}$ is the adjusted concentration (μg g^{-1}), C_m is the uncorrected concentration (μg L^{-1}) of analyte in urine and C_{cre} represents creatinine concentration (g L^{-1}).

For each sample, correction with specific gravity was also undertaken, using Eq. 5.2.

$$C_{sg-ad} = C_m \times \frac{(sg_{mean}-1)}{sg_{meas}-1)} \quad (5.2)$$

Where C_{sg-adj} is the adjusted concentration (μg L^{-1}), sg_{mean} represents the average specific gravity of all the samples analysed in the study and sg_{meas} is the measured specific gravity.

5.2.6 Quality control

Two certified reference materials (CRM) (Seronorm™ Trace Elements Urine, L-1 and L-2) for urine trace elements were analysed simultaneously with urine samples to assess the accuracy of analysis. The recoveries (%) of I in Seronorm L-1 (certified value: 105 μg L^{-1}, measured value: 98.7 ± 2.93 μg L^{-1}, n = 4) and Seronorm L-2 (certified value: 297 μg L^{-1}, measured value: 297 ± 9.68 μg L^{-1}, n = 4) were 94% and 100% respectively.

5.3 Results and discussion

5.3.1 Questionnaire survey

The results of the household (n =76) questionnaire survey revealed that 85% (n = 65) of households were using iodised salt which demonstrates high availability and awareness of iodised salt in the region. The current use of iodised salt at household level was slightly lower than previously reported (90.6%) for GB but higher than the nationwide average (79.6%) and the average from rural areas of Pakistan reported in the 5th national nutritional survey of Pakistan (Government of Pakistan, 2018).

Based on the amount of salt purchased by each household, per capita salt consumption was estimated as 52.8 g day^{-1}, which is unrealistically higher than normal salt consumption of 5 to 15 g day^{-1} across different cultures (Medeiros-Neto and Rubio, 2016). This is also ten times higher than the WHO recommended salt intake of 5 g day^{-1} (WHO, 2012). The higher salt consumption estimated from salt purchase can be erroneous as the salt purchase does not necessarily reflect its consumption by human. The majority of households (95%) reported storing salt in lidded containers at home, which helps to reduce salt moisture content and consequent loss of its I content.

5.3.2 Salt Iodine concentration

Iodine concentration in salt samples, whether numerical or log transformed, was not normally distributed ($p < 0.005$) (Figure 5.1). It ranged from 0.03 – 45.8 mg kg^{-1} with arithmetic mean and median values of 8.85 ± 10.4 and 4.16 mg kg^{-1} respectively. The I concentration in the majority of GB salt samples (82.9%) was well below the

minimum level of the WHO recommended range of 20-40 mg I per kg salt (WHO, 2001; WHO, 2007). The salt I concentration observed in this study differed from the concentration reported in the fourth national nutrition survey of Pakistan which found that 29.9% of iodised salt collected from GB had 'inadequate' I by using 15 mg I kg^{-1} of salt as the minimum threshold for adequately iodised salt (Government of Pakistan, 2011).

The I content of salt is reported to decrease over time due to various factors including humidity and temperature. Jayashree and Naik (2000) reported a loss of 8.4 to 23.3% I in different types of iodised salts stored in different lidded containers over a period of 15 days. Biber et al. (2002) reported a loss of 58.5% I content in ~3.5 years from salt stored at room temperature with a relative humidity of 30-45% in their study on the stability of I content in iodised salt. This demonstrates that even if the salt is stored in a lidded container its I content can be reduced, although the rate of loss appears to be slow compared to the likely rate of consumption.

The reasons for such low concentrations of I in the iodised salt used in GB are not known. However, possible reasons may include initial addition of inadequate I, poor mixing which results in uneven I in different bags and batches, storage conditions (light, humidity, temperature). Further investigations are required to establish the reasons for low I content of iodised salt in the region.

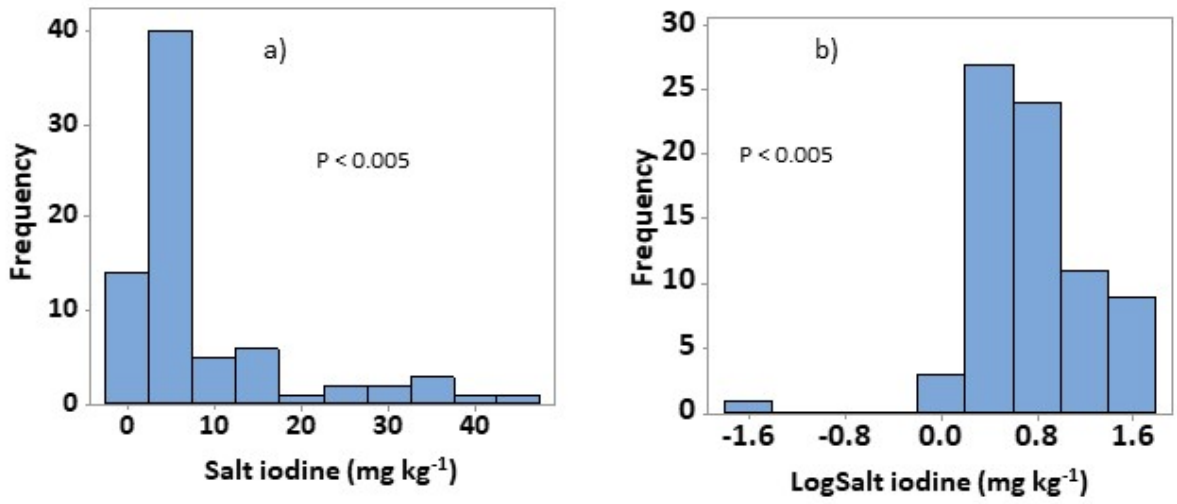


Figure 5.1. Histograms of salt iodine concentration: a) numerical data; b) log transformed data. The P value ≤ 0.05 represent that data is not normally distributed.

5.3.3 Urinary hydration corrections

It is important to apply urinary hydration correction of analytes measured in order to compare data with other investigations which may have dealt with populations that differed in hydration status (Watts et al., 2019a). Remer et al. (2006) reported that using uncorrected UIC to asses population I status can distort comparisons between populations due to changes in hydration status. In this study, urinary hydration correction for the measured analytes was undertaken using measurements of creatinine concentration and specific gravity. Hydration correction with both creatinine and specific gravity are prone to interferences. Creatinine excretion varies among demographic groups depending on protein intake, incidence of malnutrition, muscle mass and exercise (Middleton et al., 2016b; Watts et al., 2019a). The specific gravity measurements made with a refractometer could be affected by the presence of urinary solutes such as protein, glucose and ketones (Middleton et al., 2016b).

However, in the context of this study, corrections with creatinine appear to be more questionable due to the prevalence of malnutrition and low protein intake within the Pakistani population (Aziz and Hosain, 2014, FAO and Government of Pakistan, 2018; Government of Pakistan, 2018). By contrast, correction with specific gravity may be a more reliable approach as it is also considered a proxy for osmolality – the most reliable approach for urinary dilution correction (Watts et al., 2019a).

5.3.4 Urinary sodium concentration (UNaC)

The details of UNaC, uncorrected ($UNaC_{uncor}$) or corrected for creatinine ($UNaC_{cre}$) or specific gravity ($UNaC_{sg}$), are provided in Table 5.2. The normal range of UNaC excretion is reported as 40-220 mmol day^{-1} and >20 mmol in spot urine samples (Karim, 2018; Mir et al., 2019). The median $UNaC_{sg}$ concentration of all participants together was 140 mmol L^{-1}, which is equivalent to 196 mmol day^{-1} considering a urinary volume of 1.4 L in 24 h. Urinary Na excretion of 196 mmol day^{-1} just falls within the normal UNa excretion range of 40 -220 mmol day^{-1}. The urinary volume (1.4 L day^{-1}) was calculated based on reports from the literature which suggest that normal range of 24 h urine volume is 0.8 – 2 L provided there is a 2 L fluid intake by individuals (Karim, 2018; MedlinePlus, 2019).

The daily salt intake estimated from UNaC seems to be more reliable compared to salt intake calculated from monthly or weekly salt purchase. The household salt intake estimation from monthly purchases have some shortcomings, such as loss of salt through spillage or losses in cooking water, or the use of salt for purposes than cooking; for example some people in GB use salt for preserving meat particularly

during Eid ul Adha (a religious festival when people sacrifice animal). Therefore, salt use may be higher in months when salt has been used for preserving meat.

It is reported that ~90% of consumed Na is excreted in urine (McLean, 2014) and based on this approach participants salt intake was calculated from Eq. 5.3 as reported in Chen et al. (2018), who used 24 h urine to estimate Na intake. However, this study has used spot urine samples for estimating Na intake from Eq. 5.3 as it has been reported that spot urine samples can be used as a plausible alternative in determining population salt intake (Huang et al., 2016).

$$Salt - NaCl\ (g\ day^{-}) = C_s \times MW \qquad (5.3)$$

where C_s is the amount of Na (mmol) in 24 h urine and MW is the molar mass of NaCl (g mol^{-1}).

From $UNaC_{sg}$ it was estimated that the average salt intake in the study area was 13.0 $\pm$ 5.64 g day^{-1} (median 12.7 g day^{-1}, range 0.51-31.0 g day^{-1}) which largely falls within the usual range for daily salt consumption (5-15 g) but is much lower than the consumption rate estimated from the salt intake questionnaire survey. However, it still indicates a higher salt consumption than the WHO-recommended amount of 5 g day (WHO, 2012). It is also higher than average global salt intake of 10.1 g day^{-1} (Powles et al., 2013). High salt (Na) intake is associated with adverse health effects including high blood pressure and cardiovascular diseases (Partearroyo et al., 2019).

Table 5.2. Summary statistics of urinary Na concentrations (UNaC): uncorrected urinary Na ($UNaC_{uncor}$); creatinine-corrected urinary Na ($UNaC_{cre}$); specific gravity corrected urinary Na ($UNaC_{sg}$) for different age groups of the surveyed population

Age group (Years)	Sample population (%)	$UNaC_{uncor}$ (mmol L^{-1})				$UNaC_{cre}$ (mmol g^{-1})				$UNaC_{sg}$ (mmol L^{-1})			
		Median	Mean	Std. dev.	Range	Median	Mean	Std. dev.	Range	Median	Mean	Std. dev.	Range
< 5	1.10	138	174	130	68.7-351	262	295	222	67.5-589	159	153	92.0	44.1-250
5 -16	35.7	136	143	72.0	8.1-395	233	236	138	6.5-646	142	142	59.7	19.9-296
17 - 24	19.0	145	156	70.9	53.2-376	171	186	112	31.7-498	141	146	58.0	39.9-284
25 - 44	28.0	146	152	70.5	8.3-367	157	184	117	4.38-762	142	149	65.0	5.63-341
45 - 64	13.2	130	130	56.7	6.2-246	140	198	144	11.5-602	117	133	66.0	12.7-315
≥ 65	3.02	129	112	60.4	10.6-173	115	119	78.6	13.2-261	98.6	101	51.9	12.6-180
All age groups	100	139	146	70.3	6.19-395	178	204	130	4.38-762	140	143	62.1	5.63-341
WRA (15-49)*	35	134	146	75.0	8.3-376	165	193	127	4.38-762	138	141	64.1	5.63-341

* This number represents participants from different age groups and mean 35% of the total surveyed population

The salt intake estimated from UNaC is comparable with salt consumption reported in other countries in the region such as Bangladesh and India, who also have a similar cuisine. A daily salt consumption of 13.4 and 11 g have been reported for Bangladesh and India respectively (Zaman et al., 2017; Johnson et al., 2019). However, the daily salt consumption found for GB in this study is higher compared to other countries; for example salt consumption of 6.5, 8.4 10.0, 8.3 and 8 g day^{-1} have been reported for Malaysia, Thailand, Vietnam, Singapore and the UK respectively (Amarra and Khor, 2015; Bates et al., 2016).

5.3.5 Urinary iodine concentration (UIC)

Figure 5.2 demonstrates the distribution of numerical and log-transformed data for uncorrected UIC (UIC_{uncor}), creatinine-corrected UIC (UIC_{cre}) and specific gravity corrected UIC (UIC_{sg}). The mean and median UIC_{uncor} values for all participants (n = 364) were 120 $\pm$ 111 and 85.0 $\mu g\ L^{-1}$, respectively (range 7.53 – 773 $\mu g\ L^{-1}$), which were similar to UIC_{sg} values (mean 114 ± 99 $\mu g\ L^{-1}$; median 82 $\mu g\ L^{-1}$; range 12 – 631 $\mu g\ L^{-1}$) but smaller than UIC_{cre} (mean 155 ± 153 $\mu g\ L^{-1}$; median 109 $\mu g\ L^{-1}$; range 6.05 – 973 $\mu g\ L^{-1}$) (Table 5.3). Participants aged <5 years had the highest median UIC (corrected and uncorrected) while participants ≥ 65 years had the lowest median values in both uncorrected and corrected UIC indicating a decrease in UIC with age (Table 5.3). However, there was no correlation ($p > 0.05$) observed between UIC (UIC_{uncor}, UIC_{cre}, UIC_{sg}) and age for all participants together as one dataset. There was no significant difference observed between the UIC (UIC_{uncor}, UIC_{cre}, UIC_{sg}) of male and female participants at household level or different age groups (ANOVA, $p > 0.05$).

According to WHO, a UIC value of <100 $\mu g\ L^{-1}$ represents I deficiency and ≥300 $\mu g\ L^{-1}$ shows an excessive I intake which can also result in health complications, including hyperthyroidism

and autoimmune thyroid diseases (Zimmermann and Andersson, 2012; WHO, 2013; Sun et al., 2014). Considering the WHO limits for UIC, 59% of the surveyed GB population were I deficient (UIC < 100 μg L^{-1}) and 7.14% had excessive I intake (UIC > 300 μg L^{-1}) based on UIC_{uncor}. While creatinine-corrected UIC, revealed that only 44.8% of the population had I deficiency and 11.0% had excess I; correction with specific gravity did not show a significant change in population I status compared to what was described based on UIC_{uncor} data (62.9% < 100 μg L^{-1}; 7.14% >300 μg L^{-1}).

According to WHO epidemiological criteria for evaluating I nutrition in different age groups, the severity of IDD varies with UIC (Zimmermann and Andersson, 2012; WHO, 2013; Hussain et al., 2019). Table 5.4 describes I nutrition status in all age groups based on WHO criteria of population I nutrition status and prevalence of IDD using UIC_{uncor} and corrected (UIC_{cre} and UIC_{sg}) data.

Assessing the I status of school age children (SAC), based on UIC_{sg} with WHO criteria, it was observed that only 25% children had adequate I intake and a small proportion (8%) had excessive I intake. A large proportion (60%) of participants had insufficient I intake with varying levels of I deficiencies consisting of severe (1%), moderate (17%) and mild (42%) (Table 5.4). This deficiency in 60% participants from the SAC group is slightly smaller than what was found by the 4th national nutritional survey which reported I deficiency in 69.9% children (age 6-12 years) from GB (Government of Pakistan, 2011).

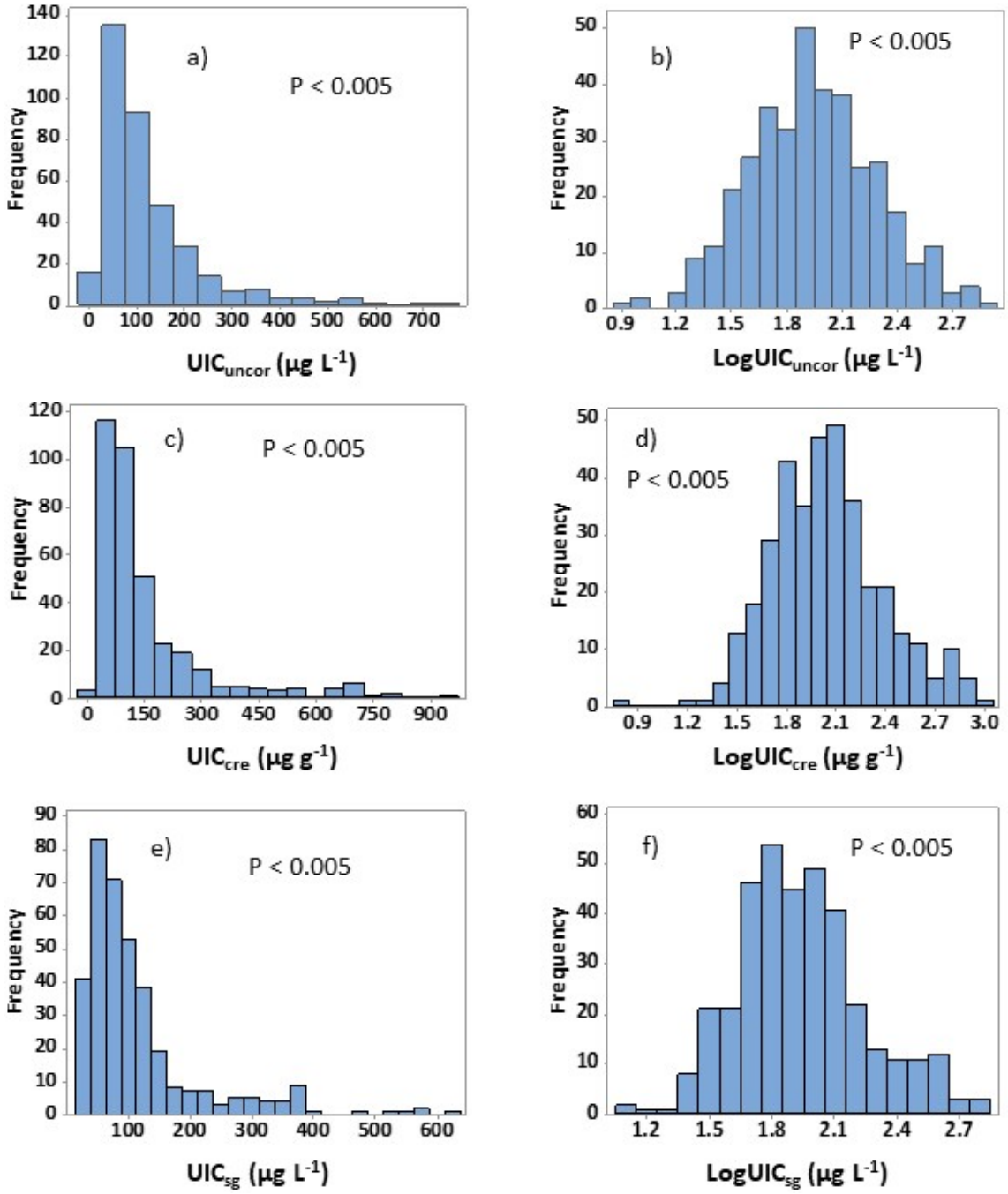


Figure 5.2. Histograms of UIC: UIC_{uncor}; UIC_{cre}; UIC_{sg} numerical and log transformed data. a) UIC_{uncor} numerical data; b) UIC_{uncor} log transformed data; c) UIC_{cre} numerical data; d) UIC_{cre} log transformed data; e) UIC_{sg} numerical data; f) UIC_{sg} log transformed data. The P value ≤ 0.05 represent that data is not normally distributed.

Table 5.3. Summary statistics of urinary iodine concentration (UIC) (uncorrected urinary I; UIC_{uncor}, creatinine corrected urinary I; UIC_{cre}, specific gravity corrected urinary I; UIC_{sg}) in surveyed population

Age group (Years)	Sample population (%)	UIC_{uncor} (μg L^{-1})				UIC_{cre} adjusted (μg g^{-1})				UIC_{sg} adjusted (μg L^{-1})			
		Median	Mean	Std. dev.	Range	Median	Mean	Std. dev.	Range	Median	Mean	Std. dev.	Range
< 5	1.1	174	185	44.1	145 - 246	344	316	132	148 - 428	178	179	84.7	96.6 - 262
5 -16	35.7	84.1	122	116	7.5 - 696	121	183	172	6.1 - 973	84.8	119	108	13.4 - 631
17 - 24	19.0	81.5	111	93.8	14.5 - 566	88.4	123	124	22.1 - 767	79.6	101	80.0	17.0 - 386
25 – 44	28.0	89.7	132	130	8.9 - 773	106	142	141	28.0 - 811	84.3	118	100	27.4 - 567
45 - 64	13.2	81.1	108	87.4	10.1 - 442	97.5	157	159	27.8 - 710	78.6	114	108	12.8 - 538
≥ 65	3.02	52.8	70.3	51.6	16.1 - 192	60.0	72.1	47.7	32.5 - 183	58.9	64.6	38.5	22.9 - 155
All age groups	100	85.0	120	111	7.53 - 773	109	155	153	6.05 - 973	82.0	114	99.0	12.8-631
WRA (15-49)	35	77.2	116	119	8.56 - 773	95.6	138	143	22.1 - 811	70.4	106	100	11.3 - 567

Table 5.4. Urinary iodine concentration (UIC) level in different age groups of the surveyed population and their I intake status based on WHO guidelines

Median UIC	Iodine intake	Number (%) of participants in each age group							
		All age groups	< 5 y	5 -16 y (SAC)	17 - 24 y	25 - 44 y	45 - 64 y	≥ 65 y	Women of reproductive age WRA (15-49 y)
Uncorrected (μg L^{-1})									
< 20	Severe deficient	9 (2)	0 (0)	4 (3)	1 (1)	1 (1)	2 (4)	1 (9)	4 (3)
20 – 49	Moderate deficient	81 (22)	0 (0)	27 (21)	14 (20)	27 (26)	9 (19)	4 (36)	32 (25)
50 – 99	Mild deficient	123 (34)	0 (0)	48 (37)	26 (38)	28 (27)	18 (38)	3 (27)	49 (38)
100 – 199	Adequate	95 (26)	3 (75)	30 (23)	18 (26)	28 (27)	13 (27)	3 (27)	23 (18)
200 – 299	Above requirements	30 (8)	1 (25)	11 (8)	6 (9)	8 (8)	4 (8)	0 (0)	11 (9)
≥ 300	Excessive	26 (7)	0 (0)	10 (8)	4 (6)	10 (10)	2 (4)	0 (0)	10 (8)
Creatinine corrected (μg g^{-1})									
< 20	Severe deficient	2 (1)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
20 – 49	Moderate deficient	52 (14)	0 (0)	10 (8)	14 (20)	15 (15)	8 (17)	5 (45)	24 (19)
50 – 99	Mild deficient	109 (30)	0 (0)	33 (25)	22 (32)	34 (33)	17 (35)	3 (27)	44 (34)
100 – 199	Adequate	126 (35)	1 (25)	49 (38)	25 (36)	36 (35)	12 (25)	3 (27)	41 (32)
200 – 299	Above requirements	35 (10)	1 (25)	16 (12)	5 (7)	8 (8)	5 (10)	0 (0)	10 (8)
≥ 300	Excessive	40 (11)	2 (50)	20 (15)	3 (4)	9 (9)	6 (13)	0 (0)	10 (8)
Specific gravity corrected (μg L^{-1})									
< 20	Severe deficient	3 (1)	0 (0)	1 (1)	1 (1)	0 (0)	1 (2)	0 (0)	2 (2)
20 – 49	Moderate deficient	67 (18)	0 (0)	22 (17)	14 (20)	15 (15)	12 (25)	4 (36)	28 (22)
50 – 99	Mild deficient	159 (44)	1 (25)	55 (42)	31 (45)	46 (45)	20 (42)	6 (55)	64 (50)
100 – 199	Adequate	88 (24)	1 (25)	32 (25)	17 (25)	28 (27)	9 (19)	1 (9)	20 (16)
200 – 299	Above requirements	21 (6)	2 (50)	9 (7)	2 (3)	6 (6)	2 (4)	0 (0)	6 (5)
≥ 300	Excessive	26 (7)	0 (0)	11 (8)	4 (6)	7 (7)	4 (8)	0 (0)	9 (7)

A large proportion (74%) of the WRA group appeared to demonstrate some degree of I deficiency: severe (2%), moderate (22%) and mild (50%) based on UIC_{sg} (Table 5.4). A small proportion of WRA group participants (7%) were living at risk of potentially adverse health consequences due to excessive intake while 16% had optimal intake (Table 5.4). Out of all the WRA group participants, 11% were pregnant or lactating and their median UIC_{sg} was <100 µg L^{-1} indicating I deficiency. The optimal I nutrition status in pregnancy and lactation is indicated by a median UIC of 150-249 µg L^{-1} and ≥100 µg L^{-1} respectively (Zimmermann and Andersson, 2012). The rate of I deficiency observed in women participants is lower than reported in the 4th national nutritional survey of Pakistan which found a deficiency rate of 75.6% in women from GB and 47.4% in women nationwide (Government of Pakistan, 2011). However, a recently conducted 5th national nutrition survey reported that 17.5% of women of WRA have I deficiency across Pakistan (Government of Pakistan, 2018). Based on reported figures of the 5th national nutrition survey conducted in 2018 it appears that I deficiency has been significantly reduced nationwide. However, according to the findings of the current project the I status of the WRA group from GB is almost the same as that reported in the 4th national nutrition survey in 2011 (Government of Pakistan, 2011).

There was no correlation observed between salt intake and I status at a household or individual level ($p > 0.05$). The reason for this is not known and it implies that iodised salt may not be the main source of I in the population of the study area. It might be expected that when iodised salt is the primary source of I in individuals a relationship between UNaC and UIC is apparent. The literature reports that up to 90% of Na and I are excreted in urine and Karim (2018) reported a good relationship between UIC and UNaC in a Kurdistan population where iodised salt is the main source of I in the population.

5.3.6 Urinary selenium concentration (USeC)

The summary statistics of uncorrected urinary Se concentration ($USeC_{uncor}$), creatinine corrected urinary Se concentration ($USeC_{cre}$) and specific gravity corrected urinary Se concentration ($USeC_{sg}$) of all surveyed population are shown in Table 5.5. The distribution of USeC ($USeC_{uncor}$, $USeC_{cre}$, $USeC_{sg}$) of all participants as one data set is provided in Figure 5.3. There was no significant difference observed between $USeC_{sg}$ for different age groups as well as between male and female participants in each household (ANOVA, $p > 0.05$). Specific gravity corrected USeC for SAC and WRA groups were comparable to $USeC_{sg}$ values reported by Phiri et al. (2020) for a Malawian population who were reported to be Se deficient based on their plasma Se status.

There was no correlation ($p > 0.05$) found between UIC and USeC which contradicts the findings of Karim (2018) who found a correlation between $USeC_{uncor}$ and UIC_{uncor} for spot urine sampling ($n = 410$) in the Kurdistan region of Iraq. This also contradicts the findings of Thomson et al. (1996) who also found a correlation between $USeC_{uncor}$ and UIC_{uncor} in both spot and 24 h urine ($n = 62$) samples in a New Zealand population. The lack of correlation between UIC and USeC probably demonstrate the supply of these essential nutrients from different food sources.

Urinary Se excretion is one of the most effective indicators of Se intake (Rodríguez Rodríguez Elena et al., 1995; Hawkes et al., 2003; Yoneyama et al., 2008) which suggest that USeC can be used to predict the Se intake of populations (Longnecker et al., 1996; Nakamura et al., 2019). Alaejos and Romero (1993) found a strong correlation ($r = 0.962$, $p < 0.001$) between USeC and dietary Se intake dietary over a wide range of diets from across the world and also reported that approximately 50 – 70% of Se ingested is excreted in urine. Based on this

approach the daily Se intake in the surveyed population was found to vary between 37.1 ± 13.2 to 52.0 ± 13.7 μg day^{-1} and 64-90% of the participants had daily Se intakes that were less than the recommended daily allowance (RDA) of 55 μg day^{-1}. The European Food Safety Authority (2014) reported that USeC reflects Se intake and approximately 40 – 60% of Se intake is excreted in urine which, in the context of current study, means that 32-82% of the surveyed population is living at risk of Se deficiency. Yoneyama et al. (2008) and Nakamura et al. (2019) estimated daily Se intake in a Japanese population from 24 h urinary excreted Se and found that 73 and 77% of ingested Se is excreted via urine in men and women respectively. If urine samples collected in this study are assumed equivalent to 24 h urine sampling then Se intake based on $USeC_{sg}$ in males and females of the surveyed population will be 34.4 and 32.4 μg day^{-1} respectively, which is less than the recommended daily Se intake. Calculating population Se intake based on the above approaches, it is concluded that Se intake in the Gilgit population appears to be quite low. However, further research is required to quantify Se status and daily Se intake from the consumption of different food items and its estimation from blood plasma and urinary Se in Gilgit. Currently there are no cut-off points available to assess the severity of Se deficiency based on USeC, however once these are established, USeC can be used as an effective, non-invasive and cost effective alternative to blood plasma.

Table 5.5. Summary statistics of urinary Se concentrations (USeC) (uncorrected urinary Se; $USeC_{uncor}$, creatinine corrected urinary Se; $USeC_{cre}$, specific gravity corrected urinary Se; $USeC_{sg}$) in different age group of the surveyed population

Age group (Years)	Sample population (%)	$USeC_{uncor}$ (µg L^{-1})				$USeC_{cre}$ (µg g^{-1})				$USeC_{sg}$ (µg L^{-1})			
		Median	**Mean**	**Std. dev.**	**Range**	**Median**	**Mean**	**Std. dev.**	**Range**	**Median**	**Mean**	**Std. dev.**	**Range**
< 5	1.1	35.7	33.9	17.3	14.1-50.4	41.7	50.4	18.3	40.4-77.9	26.5	27.4	4.06	23.4-33.0
5 -16	35.7	17.6	20.3	11.4	1.33-61.3	27.3	28.9	11.7	1.07-85.0	18.0	19.0	6.33	7.65-41.4
17 - 24	19.0	18.8	20.9	11.3	5.32-62.7	19.7	20.7	6.23	6.09-40.1	17.8	17.9	5.21	9.11-30.0
25 - 44	28.0	18.5	20.5	11.7	0.95-73.1	19.6	21.8	11.3	2.97-80.6	16.7	18.4	7.65	6.59-49.4
45 - 64	13.2	16.6	18.5	11.0	5.49-56.1	20.8	22.9	8.81	10.3-47.8	17.0	17.8	8.14	7.42-52.7
≥ 65	3.02	23.8	21.1	10.1	5.47-36.3	18.0	20.9	7.06	14.4-37.8	17.4	19.2	7.07	10.3-36.2
All age groups	100	18.1	20	12	0.95-73.1	22.4	24.6	11.2	1.07-85.0	17.8	18.6	6.84	6.59 - 52.7
WRA (15 -49)	35*	17.9	20.0	11.0	0.95-55.1	21.0	23.1	9.20	3.0-61.6	17.1	18.0	6.30	6.6-41.4

* This number represents participants from different age groups and mean 35% of the total surveyed population

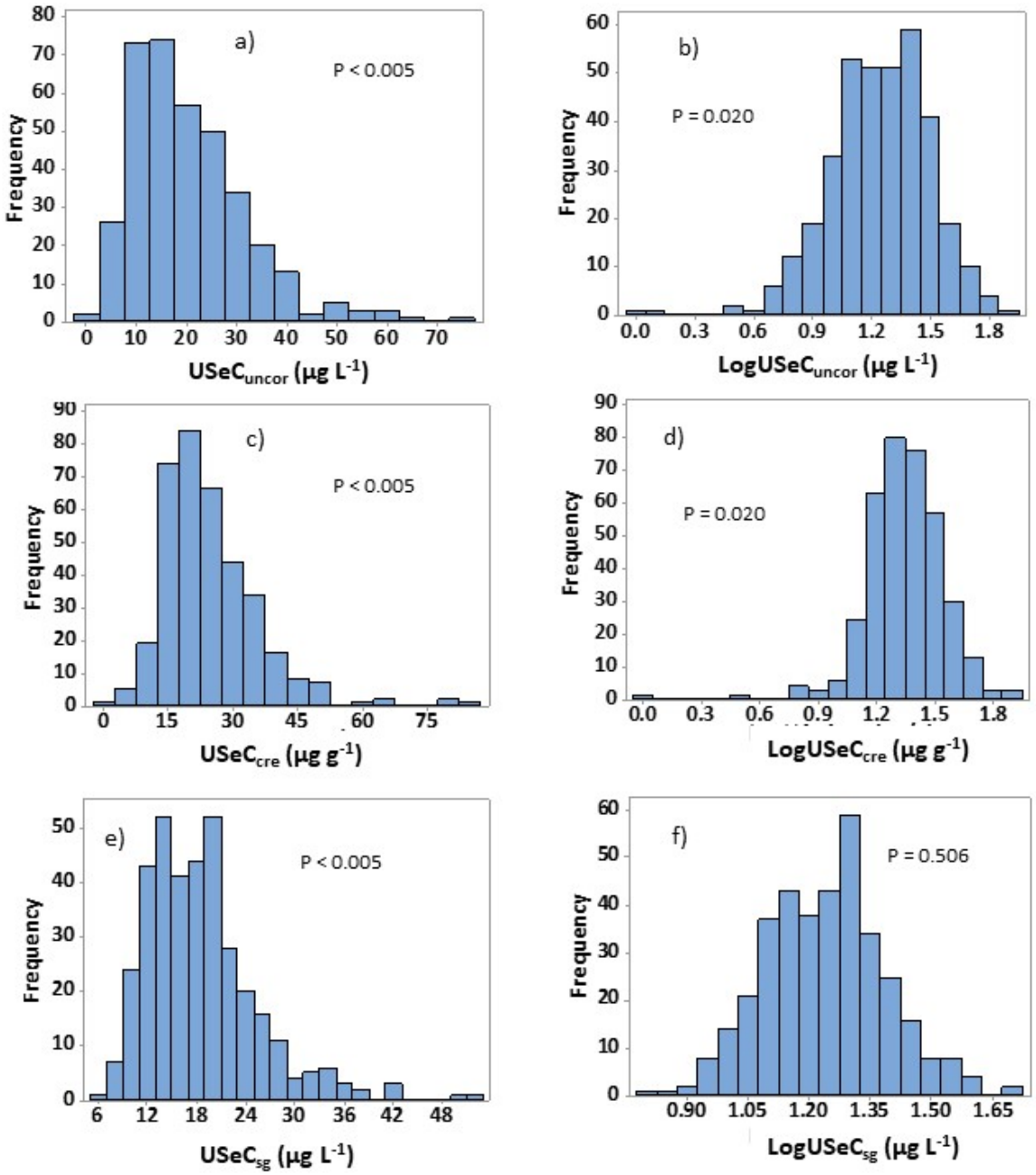


Figure 5.3. Histograms of USeC: USeC$_{uncor}$; USeC$_{cre}$; USeC$_{sg}$ numerical and log transformed data. a) USeC$_{uncor}$ numerical data; b) USeC$_{uncor}$ log transformed data; c) USeC$_{cre}$ numerical data; d) USeC$_{cre}$ log transformed data; e) USeC$_{sg}$ numerical data; f) USeC$_{sg}$ log transformed data. The P value ≤ 0.05 represent that data is not normally distributed.

5.4 Conclusions

This study suggests that individual daily salt consumption in the GB population is almost 3 times higher than the limit of WHO recommended daily salt intake. The majority of the GB population is using iodised salt but a higher proportion of salt samples (85.5%) were inadequately iodised as they had less I than the WHO-recommended minimum concentration of 20 mg kg^{-1} of I in iodised salt.

Potentially the GB population is living at risk of I deficiency as their median UIC_{sg} was <100 μg L^{-1}. Of the different age groups, participants aged ≥ 65 and WRA groups had the highest percentage of I deficiency: 94% and 74% respectively. The problem of I deficiency could be partially resolved if the salt was adequately iodised - although this study did not find any correlation between the salt intake of individuals and their UIC. Almost all the population was using iodised salt but it was inadequately iodised. However, as reported earlier, individual salt intake was quite high (13 g day^{-1}). Therefore, any change in salt iodisation would need to be carefully monitored because if an individual was consuming 13 g salt day^{-1}, then their daily I intake would be 260 μg day^{-1} from salt alone, if the salt was (correctly) iodised with 20 mg kg^{-1}. The WHO recommended adequate requirement for normal functioning of an adult individual is 150 μg day^{-1}. Iodine is also available in different food items and, as reported in Chapter 3 (Section 3.4), an average individual in GB receives 47.5 μg day^{-1} from food alone. Therefore, keeping in mind the high salt intake of the GB population, increased salt iodisation would have to be supported with an educational extension programme aimed at moderating salt intake. It is known that high salt (Na) intake can result in hypertension and cardiovascular diseases, therefore in the long

run it might be wise to introduce a salt reduction awareness campaign in the area. It would be important to implement both measures simultaneously to avoid aggravating population I deficiency resulting from a reduction in salt intake.

Average daily Se intake estimated from USeC, suggests that Se intake in the GB population is below the recommended daily intake of 55 μg day^{-1}. However, further research is recommended to assess the population Se status based on blood plasma Se concentration. As discussed previously, wheat is the primary staple carbohydrate crop in GB and constitutes an essential and consistent item in all daily meals. Therefore, as shown in Chapter 4, wheat biofortification with selenate treated fertilizers would be the best solution to rectify Se deficiency in the GB population.

6 CONCLUSIONS AND FUTURE WORK

6.1 Overview

Historically iodine (I) deficiency has been reported in the population of Gilgit-Baltistan (GB) while currently there is no data available on the Se status of the environment and population of the area. It is known from the literature that health complications arising from I deficiency are greater with simultaneous deficiency of selenium (Se). The deficiency of these essential micronutrients is dietary in origin and their intake by local populations are largely affected by their concentrations in the environment. This is particularly true in regions such as GB where individuals typically consume locally grown agricultural produce. Therefore, this study was undertaken to explore the current status of I and Se in soils and the availability of I and Se to the local population of GB. The scope of the investigation included a survey of soil, plant and water, used for irrigation and drinking, in various districts of GB to understand the factors responsible for controlling I and Se bioavailability in the soils of this area. The plant uptake of Se and feasibility of biofortification was investigated with a field trial using different Se species (Se^{IV} and Se^{VI}) of isotopically enriched ^{77}Se as a tracer. A salt iodisation programme has been running in the area to counter I deficiency, therefore a survey was undertaken to evaluate the efficiency of this salt iodisation programme. Finally, a survey of I and Se in human urine was undertaken to ascertain the current I and Se status of the local population.

6.2 Iodine

The median concentration of I in water (I_w) from surface and groundwater was 0.227 µg L^{-1} which is typically smaller than I_w of similar mountainous regions. It is also less than the median I_w of 7.3 µg L^{-1} reported from other parts of Pakistan. Groundwater had relatively higher I_w than surface waters. The majority of I_w (~70%) was present in inorganic forms (iodide and iodate) with iodate being the major species reflecting the high pH of water in the area and possibly differences in solubility between I species within the catchment soils.

The average total I in soil (I_T) was 685 µg kg^{-1} which is just 26% of the global average I_T (2,600 µg kg^{-1}). Soil samples had a coarse texture, low organic carbon concentration and high pH, which have contributed to low soil I_T. However, I_T is not a good indicator of I availability for plants, which is probably better represented by the reactive fractions, soluble I (I_{sol}) and adsorbed I (I_{ads}). Reactive soil I was only a small proportion (<7%) of I_T, consisting of <3% as I_{sol} and <4% as I_{ads}. Furthermore, speciation analysis indicated that >90% of reactive I was present in organic forms, which are unlikely to be available for plant uptake. Furthermore, the solid-solution distribution coefficient ($Kd = I_{ads}/I_{sol}$) was very low which indicates a low buffering capability of I_{sol} against depletion by leaching and uptake. The above factors all suggest a low availability of I in GB soils.

The low concentration of I in GB water and soil was also reflected in all plant samples as typically all crops surveyed showed smaller I concentrations (I_P) compared to similar species from other parts of the world. However, among plant groups, leafy vegetables consistently had greater I_P across the whole area, likely from rainwater or

dry deposition from the atmosphere. The average concentrations (µg kg^{-1}) of I_P in descending order, over all districts, were 36.3 in leafy vegetables, 21.8 in grain, 16.9 in crop vegetables, 10.0 in tubers and 6.6 in nuts. Iodine concentrations in plants were not correlated with any simple measure of I availability, including I_w, I_{sol}, I_{ads} and I_T. Furthermore, soil characteristics, including pH, % soil organic carbon (%SOC) and % soil inorganic carbon (%SIC) were not correlated with I_P. This lack of correlation with I_P could be due to bioavailable control by transient environmental factors such as irregular rainfall and atmospheric deposition or occasional but infrequent irrigation of crops. Furthermore, the narrow range of soil characteristics (soil pH and carbon) could be another reason for the lack of correlation between soil characteristics and I_P.

Based on a daily consumption of 2 L of drinking water, the average direct intake of I from water would be 1.09 µg day^{-1}. By contrast, based on a typical Pakistani food basket, it is estimated that the consumption of locally produced food items would supply approximately 47.5 µg day^{-1}. The combined intake of I in GB would therefore provide only 32% of the recommended daily allowance (RDA – 150 µg day^{-1}). An iodised salt programme is operational in the area to resolve the problem of I deficiency. However, analysis of iodised salt revealed a very low concentration of I (median 4.2 mg kg^{-1}) compared to the WHO recommended range (20 – 40 mg kg^{-1}) of I in iodised salt. The average salt consumption estimated from urinary sodium concentration (UNaC) suggested a mean individual salt intake of 13.0 ± 5.6 µg day^{-1}. Considering the average daily intake of salt and its I content, it was found that iodised salt currently as used in GB would contribute only 52.8 µg day of I (35% of the RDA).

Combining contributions from water, food and salt, it is apparent that the population of GB are probably not receiving adequate I nutrition from their diet. Furthermore, it was also noted that salt intake was higher than the WHO recommended daily intake of 5 g day^{-1}. Iodine nutrition status was assessed with urinary I concentration (UIC) and it was found that 44.0- 62.9% of all surveyed population had UIC <100 μg L^{-1} classifying them as having insufficient daily I intake based on WHO criteria of I deficiency.

This study recommended enhancing I concentration in iodised salt accompanied with a salt reduction awareness programme in the area. If I concentration was increased in salt to the WHO recommended range, and the salt intake remained at the current level, then the population I intake would rise to a level regarded as 'above requirement' (200-299 μg day^{-1}) or even to an 'excessive level' (>300 μg day^{-1}), which could lead to potential health consequences. Therefore, any approach to eliminating I deficiency with iodised salt in GB needs to be carefully assessed in terms of likely response by the local population before implementation.

6.3 Selenium

The concentration of Se in water (Se_w) (mean 0.371 μg L^{-1}, median 0.246 μg L^{-1}) measured in the GB region lies at the bottom of the range for global fresh water (0.1-100 μg L^{-}). As found for I_w, groundwater had greater Se_w than surface waters which could be due to the larger contact time between rocks and water in the aquifer or simply differences in the Se concentration in source rocks. The results of speciation analysis demonstrated that Se_w was largely present in inorganic forms (~65%); in fact

Se^{VI} was the only detectable species demonstrating its high solubility in water compared with Se^{IV}.

The mean total concentration of soil Se (Se_T = 209 µg kg^{-1}) was low compared to a global mean of 400 µg kg^{-1}. The coarse texture, low organic carbon and alkaline nature of soil have probably contributed to the limited accumulation of Se in soils of GB. The reactive Se, soluble Se (Se_{sol}) and adsorbed Se (Se_{ads}) fractions, accounted for <5% of Se_T suggesting very limited availability of Se for plant uptake. Furthermore, speciation analysis indicated that ~67% and ~40% of Se in soluble and adsorbed fractions were present as organic species. Selenite accounted for >80% of inorganic Se in both soluble and adsorbed fractions respectively. The above observations indicate that the availability of Se for plant uptake is very low in the GB region. On average ~47% of Se_T was extractable with TMAH (Se_{TMAH}) which means that a large proportion (>50%) was present in non-extractable form. The origin and form of Se_T which was not extractable with TMAH is not known. It may be present as elemental Se or physically occluded in a mineral structure, which stops it from dissolving in a strong alkaline media. Soil organic carbon exhibited a positive correlation with Se_T and Se_{TMAH} for data combined from all districts. However, this was not observed when data from individual districts was considered, probably due to the smaller individual sample sizes and a narrow range of %SOC within each district. Other soil characteristics, including soil pH and texture, also showed a positive correlation with Se_T and fractions of Se_T.

The concentration of Se in crop plants (Se_P) from GB was low possibly reflecting smaller concentrations of available Se in the environment of study area. Leafy

vegetables had the highest concentration of Se_P and fruits had the smallest concentration. The average concentrations of Se_P in leafy vegetables, crop vegetables, nuts, grains, tubers and fruits across all districts were 105, 34.2, 28.5, 23.8, 12.9 and 4.4 µg kg^{-1} respectively. Values of Se_P for selected species of plant groups showed a positive correlation with several soil characteristics including soil pH, %clay and metal oxides and while %sand provided a negative correlation with grain Se. Both Se_{sol} and Se_{ads} were positively correlated with Se_P in species of all plant groups however Se_w have shown correlation only with Se_P in vegetables and grains.

From the results of this study, it was estimated that, based on the recommended 2 litre daily intake of drinking water, an individual would consume 0.742 µg Se per day from drinking water, which is equal to 1.35% of the RDA (55 µg). Based on a typical Pakistani food basket, an individual would only obtain 61% (33.6 µg day^{-1}) of the RDA for Se. Overall, these findings suggest that the GB population is living at risk of Se deficiency. This was supported by data (USeC) from the urinary Se survey which suggested that 64-90% of the sampled population appeared to have a daily Se intake which was less than the recommended daily allowance of 55 µg day^{-1}.

To explore a possible solution to the risk of Se deficiency in the GB population, a field trial was undertaken to assess the feasibility of Se biofortification in a wheat crop. Wheat is the staple carbohydrate crop in GB and accounts for 75% of the daily energy intake. The application of 20 g ha^{-1} of Se^{VI} increased the Se concentration in the wheat grain to 135 µg kg^{-1}. Considering the average per capita consumption of wheat (350 g day^{-1}) in the GB population, this should be enough to avoid Se deficiency (c. 47 µg day^{-1}). However, the field trial also demonstrated that, notwithstanding the coarse

texture of the soils and their high pH, Se application to crops would be required in every growing season as the residual Se had no effect on Se concentration on crops in the following two growing seasons. The majority (60-80%) of Se was lost from soil by the end of the third growing season.

6.4 Future work

From the results of the current work, some areas of research that would benefit from further investigations are listed below.

i. A wider survey of soil and water I and Se concentration in all districts of GB would provide a more comprehensive understanding of the I and Se status in the whole region and a fuller picture of the geochemical associations of soil characteristics, geology, elevation and topography.

ii. A detailed survey of all the food items including dairy products and meat and fish products for I and Se concentrations is required as the current survey is limited to locally grown agriculture produce only. In particular, it would be useful to investigate the potential role of fish farms in GB as a useful dietary source of Se and I.

iii. As a large proportion of native soil Se was not extractable with TMAH (in contrast to fertilizer-derived Se), further research is needed to understand the nature and origin of non-extractable soil Se and to establish whether this apparently unavailable pool of Se can act as a source or sink of bioavailable Se. It is possible, for example that application of acidifying fertilizers (e.g.

ammonium sulphate) may liberate Se through dissolution of Se-containing calcite matrices.

iv. A re-assessment of the GB population Se status with a more established biomarker (blood plasma) is required to improve the assessment of possible Se deficiency and to improve understanding of the relationship between USeC and blood plasma Se.

v. An investigation of the loss of fertilizer-added Se from soil and its long term impact on groundwater quality would be advisable if Se biofortification of wheat is to be adopted.

vi. It would be worth determining the reasons for such low concentrations of I in iodised salt at the point of consumption in GB.

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APPENDICES

Appendix 1: Characteristics of water samples (surface and ground water) and concentration of major and minor elements

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)	($mg\ L^{-1}$)			($\mu g\ L^{-1}$)												
G01	Stream	8.86	0.096	0.487	7.41	61.7	411	197	3251	2.64	1215	1028	3.54	3374	1401	10349	0.419	19.97	0.814
G02	Stream	7.52	0.086	0.890	6.70	55.8	391	294	2728	3.66	1119	660	4.33	2604	1368	11622	0.252	11.04	0.979
G04	River	8.25	0.187	0.000	17.30	144.1	555	173	4464	10.70	1102	4005	31.50	4833	2593	28558	5.342	432.81	1.463
G05	River	8.22	0.157	1.888	15.29	127.4	296	100	6288	6.10	1113	3787	5.68	6149	2801	25925	4.741	76.45	0.270
G06	Stream	8.12	0.202	0.025	13.77	114.8	320	170	13729	1.99	901	1523	4.80	12916	2763	34435	0.589	66.02	0.290
G07	Stream	7.63	0.118	1.271	4.22	35.2	328	90	5976	3.02	987	414	3.86	5516	2188	11768	0.338	7.05	0.519
G08	Stream	7.81	0.094	1.484	4.00	33.3	256	105	5690	3.11	1049	546	1.08	5638	2847	10800	0.426	7.29	0.526
G09	Stream	7.56	0.094	1.084	5.24	43.6	346	308	3370	2.55	1132	912	0.66	2980	1508	10337	0.401	7.34	0.714
G11	Stream	7.87	0.095	1.375	5.49	45.8	357	229	3740	2.70	1265	1044	0.25	3800	1596	11575	0.422	12.32	0.768
G14	Stream	8.08	0.128	1.191	8.01	66.7	533	244	5504	3.61	1232	1059	1.95	5099	2317	17521	0.551	15.68	0.755
G15	Stream	7.68	0.093	1.612	5.95	49.6	838	421	2904	2.50	1230	632	11.69	2619	2172	11956	0.369	17.13	0.594
G17	Stream	7.89	0.110	1.450	6.69	55.8	303	234	3589	2.73	1082	803	0.57	3220	2217	13414	0.465	16.56	0.641
G18	Stream	7.84	0.106	1.067	5.14	42.9	161	11	1156	0.98	290	201	2.37	729	621	9650	0.156	14.00	0.373
G19	Stream	8.53	0.088	1.655	6.46	53.8	257	151	2480	2.92	883	544	1.33	2120	1704	11166	0.655	15.10	1.068
G20	Stream	8.19	0.353	1.669	13.41	111.8	324	0.00	48462	4.36	4613	6820	0.00	39138	6115	56027	1.217	4.30	2.922
G21	Stream	8.11	0.184	0.614	13.04	108.7	0.00	0.00	12549	2.15	940	3617	2.17	11533	2175	29665	0.586	28.52	0.124
G22	Stream	7.76	0.240	0.957	5.14	42.9	0.00	0.00	34916	2.78	1376	1523	1.94	29515	3600	40025	0.278	17.85	0.259
G23	Stream	7.89	0.377	0.921	17.01	141.7	1752	119	51710	2.95	2874	8247	3.16	41961	5997	63012	2.448	5.73	0.940
G24	Stream	7.94	0.370	2.516	15.32	127.7	410	318	49807	3.09	2789	8241	3.79	42123	5977	62993	2.387	4.25	0.953
G03	Spring	7.68	0.086	0.590	5.90	49.2	311	286	2442	3.47	881	534	1.09	2392	1153	11519	0.213	11.52	0.836

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
		(µg L^{-1})																
G01	Stream	0.100	0.470	11.136	0.015	0.124	0.542	4.703	0.255	1.754	27.626	0.735	0.007	0.017	7.851	0.004	0.164	0.096
G02	Stream	0.094	0.101	3.700	0.012	0.222	0.708	2.858	1.391	1.622	24.809	1.704	0.001	0.011	5.108	0.003	0.086	0.086
G04	River	0.823	20.234	788.844	0.383	0.663	1.013	1.920	2.654	10.416	94.917	2.234	0.008	0.233	12.064	0.027	1.409	0.051
G05	River	0.091	1.174	54.723	0.027	0.077	0.388	0.233	1.223	7.619	90.621	2.529	0.002	0.081	9.089	0.020	1.050	0.054
G06	Stream	0.071	0.671	12.615	0.011	0.010	0.724	0.062	0.126	2.534	126.022	2.966	0.004	0.004	5.318	0.005	0.108	0.041
G07	Stream	0.064	0.040	1.778	0.015	0.150	1.254	5.452	0.099	2.884	24.800	2.349	0.004	0.016	9.723	0.004	0.019	0.077
G08	Stream	0.056	0.271	2.891	0.022	0.159	1.622	66.805	0.094	3.829	22.978	3.514	0.008	0.026	8.931	0.005	0.041	0.084
G09	Stream	0.055	0.094	2.276	0.010	0.144	0.401	0.719	0.217	1.893	26.892	0.754	0.000	0.017	10.072	0.004	0.098	0.091
G11	Stream	0.073	0.124	3.941	0.008	0.110	0.364	0.687	0.244	1.835	30.068	0.840	0.001	0.020	10.238	0.005	0.329	0.096
G14	Stream	0.045	0.181	5.362	0.014	0.195	0.656	0.483	0.208	2.089	41.323	1.284	0.003	0.013	22.396	0.005	0.353	0.077
G15	Stream	0.067	0.448	14.914	0.037	0.665	1.199	2.884	0.167	2.483	30.240	0.945	0.002	0.025	17.786	0.007	0.066	0.094
G17	Stream	0.056	0.138	7.645	0.013	0.145	0.573	0.261	0.202	2.958	33.261	0.949	0.001	0.033	21.306	0.009	0.272	0.078
G18	Stream	0.017	0.094	2.745	0.004	0.102	0.331	0.294	0.057	0.963	21.226	0.317	0.002	0.009	16.717	0.004	0.216	0.025
G19	Stream	0.067	0.048	1.558	0.038	0.154	0.724	1.538	0.373	1.976	38.145	0.589	0.000	0.009	8.076	0.003	0.083	0.070
G20	Stream	0.252	0.020	0.150	0.010	0.122	0.221	0.477	0.302	1.646	183.144	7.857	0.005	0.006	14.806	0.002	0.660	0.155
G21	Stream	0.065	4.409	4.467	0.017	0.064	0.490	0.495	0.552	1.650	104.846	2.396	0.005	0.012	5.425	0.003	0.095	0.043
G22	Stream	0.018	0.115	0.521	0.006	0.117	0.356	1.022	0.060	5.110	61.504	5.547	0.006	0.013	10.346	0.009	0.079	0.058
G23	Stream	0.555	0.093	1.486	0.006	0.076	0.231	0.925	0.297	0.555	120.192	4.661	0.002	0.003	15.330	0.000	0.222	0.090
G24	Stream	0.571	0.048	1.588	0.004	0.076	0.243	1.417	0.450	0.521	118.349	4.773	0.003	0.003	15.489	0.000	0.166	0.088
G03	Spring	0.066	0.035	0.663	0.006	0.183	0.287	38.770	1.487	1.489	22.855	1.646	0.003	0.015	4.323	0.003	0.141	0.069

Appendix 1: Continued

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)	($mg\ L^{-1}$)			($\mu g\ L^{-1}$)												
G10	Spring	7.58	0.320	3.958	22.78	189.8	2163	774	19129	10.85	7020	5284	4.59	18818	5678	52518	1.929	3.45	2.382
G12	Spring	7.88	0.270	1.389	17.66	147.2	925	282	16105	8.05	3208	3265	2.28	15063	4280	42399	1.100	6.98	1.399
G13	Spring	8.04	0.285	1.895	17.49	145.8	1168	402	20804	8.49	3609	3736	3.51	18329	4341	43558	1.316	4.81	1.577
G16	Spring	7.64	0.243	0.852	20.84	173.7	1230	614	10389	5.79	2729	2017	1.82	9434	4127	40879	0.814	2.87	1.493
D30	Stream	8.22	0.245	1.931	15.91	132.6	489	233	19523	8.09	2061	1447	0.41	16598	6884	42879	3.304	22.25	0.539
D31	stream	8.23	0.297	3.656	12.53	104.4	460	121	31662	7.30	2272	3235	1.99	25673	6079	45429	0.957	46.49	0.732
D33	Stream	8.23	0.242	0.634	10.34	86.2	362	0.00	26427	78.74	5072	3752	0.00	21822	2545	33849	23.832	3.84	0.405
D35	Stream	8.43	0.220	3.506	17.03	142.0	2112	141	9230	22.75	5251	3840	0.00	8092	4210	29217	0.548	18.65	3.123
D36	Stream	8.07	0.094	0.982	4.65	38.7	74	164	5303	17.29	1433	1158	0.00	4383	1494	10175	7.217	14.28	0.210
D37	Stream	8.32	0.248	0.902	9.72	81.0	383	207	13799	13.62	5154	2024	0.00	11397	2665	23163	7.594	54.60	0.443
D39	Stream	7.93	0.156	1.166	9.28	77.3	224	166	11155	19.09	1563	1815	0.73	10462	4049	24508	5.422	72.78	0.764
D40	Stream	7.86	0.063	1.777	4.02	33.5	571	265	1449	8.82	1582	792	0.75	1065	1449	5721	0.112	4.51	1.002
D41	Stream	7.84	0.077	1.547	3.02	25.2	222	111	357	5.54	696	412	4.21	0	638	3724	0.081	6.34	0.494
D42	Stream	8.17	0.192	0.554	14.28	119.0	1133	441	8598	11.57	2659	3337	0.73	8286	2430	26612	2.136	6.31	0.703
D45	Stream	8.43	0.087	1.370	5.60	46.6	740	725	1846	8.46	1953	1230	2.00	1404	1801	8577	0.184	7.86	1.060
D46	Stream	8.17	0.129	1.483	10.04	83.7	1354	717	2151	9.14	2946	2877	0.00	1857	886	15159	0.168	5.51	1.254
D48	Stream	8.26	0.140	1.082	10.70	89.2	4928	747	1767	9.12	5554	2199	3.10	1481	1344	14543	0.262	10.64	1.450
D51	Stream	8.14	0.105	0.960	9.14	76.2	742	349	1962	7.47	2222	1822	4.19	1593	713	12360	0.141	3.20	1.330
D32	Spring	8.88	0.947	6.320	48.24	402.0	53225	0.00	67653	4670.34	104380	42	0.00	22837	1916	1403	366.095	26.09	0.022
D34	spring	7.86	0.368	1.209	25.18	209.8	1030	361	32612	40.63	6279	7004	0.00	25650	5272	55450	42.399	4.63	1.268

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
										(µg L^{-1})								
G10	Spring	0.399	0.032	1.034	0.010	0.244	0.437	2.048	0.298	0.602	137.745	2.954	0.005	0.001	22.528	0.001	9.715	0.247
G12	Spring	0.154	0.046	0.922	0.011	0.123	0.292	0.781	0.315	0.904	87.239	2.743	0.004	0.009	36.330	0.002	1.457	0.128
G13	Spring	0.156	0.154	5.458	0.017	0.128	0.260	1.136	0.318	0.285	93.843	2.755	0.002	0.001	26.736	0.000	1.925	0.141
G16	Spring	0.172	0.017	1.068	0.010	0.140	0.278	1.582	0.228	0.228	73.634	1.818	0.003	0.001	26.992	0.000	1.067	0.113
D30	Stream	0.049	0.558	0.635	0.033	0.572	0.590	2.686	4.803	15.323	92.704	21.371	0.015	0.136	28.843	0.014	9.235	0.084
D31	stream	0.031	0.624	1.886	0.030	0.527	1.464	2.099	0.345	6.478	147.307	6.599	0.003	0.034	21.310	0.008	0.500	0.088
D33	Stream	1.046	0.083	3.146	0.018	0.384	0.464	1.101	84.631	4.955	58.040	13.567	0.012	0.191	3.852	0.008	26.011	0.221
D35	Stream	0.055	0.200	14.578	0.044	0.252	2.634	2.315	0.671	1.651	164.080	2.224	0.001	0.008	45.638	0.003	1.351	0.243
D36	Stream	0.063	0.556	4.454	0.011	0.270	0.253	0.987	62.586	2.513	19.148	3.033	0.003	0.222	1.382	0.004	5.249	0.113
D37	Stream	0.130	0.655	41.794	0.028	0.173	0.409	0.480	4.030	4.156	55.341	5.103	0.003	0.026	3.646	0.007	22.004	0.276
D39	Stream	0.177	1.635	110.097	0.130	0.953	1.691	0.615	17.518	4.768	137.568	2.539	0.006	0.043	14.784	0.005	2.715	0.082
D40	Stream	0.048	0.079	3.417	0.010	0.076	1.074	1.431	0.231	1.115	36.334	0.448	0.001	0.002	13.373	0.001	0.061	0.164
D41	Stream	0.019	0.114	6.932	0.012	0.031	0.529	0.840	0.153	0.624	20.058	0.171	0.000	0.004	7.140	0.001	0.035	0.091
D42	Stream	0.133	0.068	2.353	0.010	0.288	0.280	1.168	0.312	3.567	214.894	3.023	0.003	0.035	44.523	0.011	1.122	0.129
D45	Stream	0.065	0.145	3.094	0.009	0.068	0.923	0.294	0.252	1.582	47.707	0.631	0.000	0.003	14.051	0.002	0.045	0.165
D46	Stream	0.167	0.034	0.487	0.012	0.126	0.648	1.059	0.418	0.624	50.927	0.597	0.001	0.002	4.202	0.001	0.154	0.182
D48	Stream	0.434	0.206	5.643	0.024	0.177	0.794	3.200	0.414	1.346	49.944	0.670	0.002	0.004	5.965	0.001	0.049	0.359
D51	Stream	0.184	0.110	1.016	0.011	0.168	0.698	1.936	0.475	0.444	40.252	0.555	0.000	0.001	3.855	0.001	0.070	0.156
D32	Spring	0.000	0.128	0.000	0.001	0.007	0.277	0.000	0.316	40.140	41.294	7.802	0.007	23.959	0.334	0.000	0.125	23.683
D34	spring	0.159	0.025	1.181	0.014	0.146	0.185	2.976	36.696	1.536	131.825	14.303	0.007	0.043	9.831	0.003	127.328	0.211

Appendix 1: Continued

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)		($mg\ L^{-1}$)								($\mu g\ L^{-1}$)						
D38	Spring	7.81	0.727	4.234	29.74	247.8	5937	4634	115711	61.76	58260	18125	0.00	86991	10372	87709	31.853	5.11	2.463
D43	Spring	8.25	0.173	0.000	15.67	130.6	639	728	5204	7.35	5541	3991	1.37	4803	1717	19673	0.096	6.68	11.529
D44	Spring	9.19	0.252	0.975	16.22	135.2	3464	3117	13156	22.08	8635	6783	0.00	12426	2711	28842	0.063	8.01	3.704
D47	Spring	7.91	0.325	4.330	31.78	264.8	4399	1966	5117	34.85	13672	8929	1.12	5245	2672	41543	0.286	4.20	8.868
D49	Spring	7.97	0.150	3.358	12.11	100.9	1559	754	2648	9.80	3530	4526	0.00	2502	911	16795	0.078	3.67	1.053
D50	Spring	7.65	0.121	1.479	9.09	75.8	2463	704	2522	8.82	3827	2243	0.00	2189	747	14068	0.013	5.17	1.579
N52	Stream	8.34	0.211	0.955	17.32	144.4	703	221	12495	9.21	1721	8160	0.00	11221	2009	26717	2.430	39.90	0.229
N53	Stream	8.20	0.205	0.000	16.37	136.4	126	117	11484	3.07	963	973	0.00	10417	1870	36979	0.583	16.25	0.130
H54	Stream	8.04	0.239	0.430	11.85	98.7	0.00	0.00	23512	3.38	1041	4144	0.89	20918	4282	36541	13.408	21.37	0.221
H55	Stream	8.15	0.246	0.485	3.92	32.7	501	0.00	849	2.76	394	257	0.88	416	812	6031	0.744	12.61	0.071
N56	Stream	6.99	0.063	0.720	5.36	44.7	104	159	443	1.73	149	382	0.83	48	712	8989	0.228	9.81	0.067
N57	Stream	8.28	0.245	2.936	26.38	219.8	361	419	5915	6.11	3004	19150	0.45	5872	4739	17850	2.236	15.01	0.187
H59	Stream	8.22	0.383	1.368	17.29	144.1	0.00	0.00	52030	4.97	2686	8256	2.84	43836	5374	67314	53.066	17.19	0.132
H61	Stream	7.89	0.112	1.091	3.06	25.5	105	93	10793	6.37	721	1070	0.00	9761	3975	13161	6.440	11.53	0.217
N62	Stream	7.91	0.143	0.000	10.99	91.6	255	91	7885	2.81	877	3694	1.12	7582	1318	19994	4.695	18.53	0.066
N64	Stream	7.92	0.164	1.431	11.40	95.0	327	0.00	11482	13.25	1221	2805	1.76	10634	3389	25138	6.321	167.05	0.619
N65	Stream	7.90	0.138	1.862	9.21	76.8	144	142	7609	2.53	605	3640	1.34	7167	1257	19399	4.271	29.22	0.077
H69	Stream	8.03	0.117	1.509	9.26	77.2	581	123	5630	13.10	726	1435	5.73	4842	3258	17192	10.822	163.10	0.831
N70	Stream	7.91	0.118	0.803	12.16	101.3	277	149	2455	2.09	464	1715	2.36	2000	1603	18150	0.945	25.59	0.079
N71	Stream	8.10	0.180	0.009	18.49	154.1	293	167	6433	3.89	693	2231	1.91	6034	2014	31835	1.263	17.20	0.074

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
		(µg L^{-1})																
D38	Spring	2.030	0.055	0.361	0.012	0.075	0.502	0.466	4.046	3.427	536.988	21.640	0.012	0.032	16.331	0.005	255.741	1.479
D43	Spring	1.124	0.076	2.160	0.005	0.107	0.161	1.404	1.495	0.243	21.689	0.905	0.004	0.002	0.229	0.000	0.281	0.298
D44	Spring	0.274	0.087	1.537	0.079	0.314	3.356	2.731	0.703	0.970	109.321	1.344	0.002	0.003	4.143	0.001	0.940	0.376
D47	Spring	0.118	0.042	0.469	0.024	0.141	1.736	1.016	2.881	0.610	159.877	1.138	0.001	0.001	8.008	0.001	1.181	0.502
D49	Spring	0.180	0.044	0.615	0.012	0.141	0.306	82.687	0.304	0.446	57.905	0.650	0.003	0.001	2.178	0.001	0.237	0.197
D50	Spring	0.148	0.027	0.975	0.011	0.040	0.441	0.750	0.428	0.398	51.588	0.700	0.001	0.001	0.393	0.000	0.098	0.250
N52	Stream	0.256	0.610	17.849	0.022	0.299	0.516	0.628	1.929	2.129	110.044	1.308	0.001	0.092	8.408	0.004	0.242	0.075
N53	Stream	0.048	0.700	4.644	0.009	0.097	0.712	1.522	1.629	1.486	92.668	2.978	0.003	0.003	20.364	0.002	0.074	0.043
H54	Stream	0.101	0.445	10.058	0.018	1.228	0.699	2.455	25.039	4.887	79.627	5.059	0.009	0.046	13.500	0.007	4.843	0.044
H55	Stream	0.057	2.278	14.013	0.026	0.461	1.303	1.750	1.770	1.313	9.408	0.259	0.014	0.055	4.257	0.004	0.179	0.043
N56	Stream	0.013	0.047	1.265	0.005	0.152	0.985	7.690	0.434	1.440	17.312	0.395	0.002	0.017	4.457	0.004	0.015	0.013
N57	Stream	0.760	0.281	9.415	0.012	0.120	0.922	0.401	0.830	1.380	90.181	4.312	0.004	0.009	26.135	0.001	0.174	0.118
H59	Stream	0.044	0.488	2.893	0.014	0.959	0.199	0.096	4.231	9.384	211.291	9.122	0.007	0.301	11.483	0.009	23.880	0.082
H61	Stream	0.015	0.401	14.885	0.014	0.512	0.267	0.409	0.133	4.993	32.090	6.816	0.007	0.039	20.153	0.009	0.588	0.051
N62	Stream	0.037	0.109	2.858	0.005	0.305	0.785	0.742	2.914	2.496	39.890	1.005	0.001	0.084	3.910	0.006	7.318	0.047
N64	Stream	0.237	36.268	202.134	0.125	0.349	0.461	0.663	2.639	6.333	78.994	2.571	0.008	0.132	9.883	0.024	1.877	0.062
N65	Stream	0.069	24.894	11.389	0.061	0.510	0.212	0.622	3.998	2.298	38.935	0.944	0.005	0.080	3.119	0.005	5.940	0.033
H69	Stream	0.314	14.164	243.014	0.144	0.505	0.425	2.803	4.204	7.013	24.016	5.702	0.008	0.181	7.895	0.019	2.516	0.045
N70	Stream	0.033	16.570	9.001	0.034	0.163	0.466	0.646	0.289	2.108	66.485	0.864	0.003	0.011	10.189	0.004	0.071	0.028
N71	Stream	0.100	1.539	6.342	0.011	0.124	0.453	1.047	0.091	1.701	85.064	1.599	0.002	0.004	6.985	0.003	0.041	0.032

Appendix 1: Continued

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)	($mg\ L^{-1}$)			($\mu g\ L^{-1}$)												
N66	Lake	8.01	0.183	2.504	13.69	114.1	302	90	10414	8.21	1815	6988	0.21	9645	1717	23464	4.974	46.59	0.197
N58	Spring	8.12	0.262	3.114	28.34	236.2	386	307	6806	6.31	3377	21662	0.70	6549	4853	17991	2.535	17.21	0.170
H60	Spring	7.82	0.428	1.458	7.78	64.8	0.00	0.00	78262	13.09	2733	7457	1.79	61576	6471	74417	10.286	1.49	0.661
N63	Spring	7.92	0.243	0.000	15.84	132.0	255	315	21178	7.79	2139	7465	1.29	19362	2383	34271	28.606	3.63	0.072
H67	Spring	8.06	0.290	1.320	16.04	133.7	0.00	0.00	30076	7.54	1914	6696	0.00	26568	5087	44411	21.998	7.56	0.406
H68	Spring	8.15	0.384	5.686	26.12	217.7	458	0.00	40524	57.62	7496	9202	1.95	35734	13223	55148	46.055	3.47	0.527
A73	Stream	8.24	0.348	2.652	13.89	115.8	2492	0.00	41792	8.99	10639	6701	0.00	35027	8508	47031	37.301	11.55	0.591
A77	Stream	7.81	0.290	1.406	7.55	62.9	0.00	0.00	42585	2.42	1513	1812	0.00	36997	2690	53976	6.680	60.33	0.237
A78	Stream	7.99	0.143	0.733	6.96	58.0	1166	173	11210	2.26	1885	1272	0.00	10033	2351	19603	3.799	11.76	0.322
A79	Stream	7.93	0.147	0.665	8.28	69.0	300	211	10969	1.86	1317	1628	0.00	9559	2362	20291	3.292	27.04	0.297
A81	Stream	7.81	0.095	0.806	5.83	48.6	112	60	5489	1.52	786	1180	0.00	4593	1405	12301	0.923	7.18	0.231
A83	Stream	7.05	0.104	1.592	7.69	64.1	213	90	4536	1.85	927	1584	9.88	3856	2074	13934	1.070	6.20	0.202
A84	River	7.14	0.073	0.506	5.51	45.9	337	136	2884	24.67	879	804	0.09	2501	1367	9262	5.209	109.10	0.255
A85	River	7.41	0.067	1.010	5.76	48.0	351	206	2239	3.18	786	885	0.00	1802	704	9207	0.797	9.85	0.260
A86	Stream	7.35	0.089	0.615	6.47	53.9	332	103	4068	19.62	838	1029	5.04	3653	1797	12222	2.907	14.73	0.164
A88	Stream	8.00	0.032	1.034	2.53	21.1	286	43	650	1.60	497	199	1.97	113	905	3444	0.101	65.41	1.055
A90	River	7.61	0.055	0.646	4.43	36.9	229	171	1490	3.88	1013	488	0.00	929	686	7113	0.193	4.96	1.238
A91	Stream	7.11	0.435	1.309	4.32	36.0	0.00	0.00	94688	3.93	5664	5922	0.00	69546	3668	78563	10.695	21.40	0.075
A72	Spring	8.07	0.164	1.431	7.87	65.6	360	81	10196	25.15	2265	1487	3.96	9493	2180	19545	5.190	19.79	0.891
A74	Spring	8.10	0.213	1.668	10.01	83.4	0.00	0.00	18550	6.16	5334	3849	0.00	17300	2881	29064	7.723	10.25	0.568

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
										(µg L^{-1})								
N66	Lake	0.072	4.071	6.203	0.041	0.251	0.152	4.236	3.061	2.928	154.133	4.149	0.004	0.024	6.228	0.006	2.836	0.085
N58	Spring	0.838	0.563	14.527	0.016	0.061	0.324	2.488	0.685	1.227	96.415	4.817	0.003	0.009	29.797	0.001	0.168	0.127
H60	Spring	0.048	0.078	0.742	0.183	3.898	0.440	140.170	0.927	2.644	73.863	19.395	0.030	0.016	11.418	0.003	12.938	0.081
N63	Spring	0.105	1.138	2.114	0.019	0.276	0.883	249.724	11.037	2.367	72.726	2.566	0.015	0.662	2.315	0.002	53.416	0.086
H67	Spring	0.129	0.121	0.974	0.027	1.302	0.656	24.224	14.974	6.966	107.584	12.198	0.014	0.164	12.434	0.008	34.133	0.071
H68	Spring	0.065	0.087	1.735	0.010	0.200	0.195	13.851	14.827	5.887	154.889	29.463	0.021	0.051	18.557	0.007	32.337	0.246
A73	Stream	0.105	0.611	10.619	0.024	0.418	0.620	7.426	0.835	5.164	49.790	19.465	0.016	0.003	6.642	0.006	10.197	0.384
A77	Stream	0.046	7.272	0.660	0.136	1.105	0.378	1.667	4.160	4.639	164.641	2.793	0.020	0.113	6.517	0.005	12.729	0.055
A78	Stream	0.113	0.568	1.585	0.014	0.298	0.926	2.588	4.999	2.632	47.914	2.278	0.002	0.015	4.797	0.005	3.903	0.111
A79	Stream	0.154	1.305	22.867	0.027	0.157	0.333	1.901	1.792	2.371	56.672	1.836	0.004	0.027	3.896	0.006	3.276	0.076
A81	Stream	0.065	0.868	1.063	0.042	0.833	0.293	1.200	1.041	1.437	41.737	1.713	0.003	0.003	8.240	0.003	0.629	0.057
A83	Stream	0.073	0.658	2.497	0.023	0.205	0.502	1.719	0.359	1.641	55.202	1.414	0.003	0.002	11.439	0.003	0.500	0.063
A84	River	0.160	9.095	112.421	0.111	0.544	0.242	1.516	7.534	2.694	18.094	1.210	0.006	0.075	3.085	0.008	2.258	0.074
A85	River	0.130	2.050	3.233	0.040	0.556	0.367	3.412	1.101	1.383	44.350	0.664	0.003	0.012	3.305	0.004	0.565	0.066
A86	Stream	0.088	0.278	8.155	0.010	0.251	0.308	1.946	10.768	2.484	24.817	1.250	0.004	0.013	8.679	0.006	1.322	0.062
A88	Stream	0.133	1.007	21.147	0.042	0.141	1.405	6.273	0.481	1.034	5.610	0.528	0.002	0.005	1.682	0.002	0.034	0.070
A90	River	0.107	0.280	1.086	0.011	0.031	0.267	5.605	0.871	0.640	16.257	0.934	0.000	0.005	3.096	0.001	0.142	0.099
A91	Stream	0.022	53.603	1.515	3.349	28.517	1.121	44.819	0.173	12.116	181.384	2.731	0.106	0.048	3.423	0.012	1.110	0.166
A72	Spring	0.109	0.194	1.065	0.010	0.097	0.499	2.267	3.326	4.481	48.680	2.027	0.002	0.054	4.959	0.003	1.611	0.133
A74	Spring	0.065	0.288	13.169	0.011	0.095	0.120	1.189	0.564	0.676	38.678	19.032	0.013	0.008	5.373	0.001	7.428	0.247

Appendix 1: Continued

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)		($mg\ L^{-1}$)								($\mu g\ L^{-1}$)						
A75	Spring	8.16	0.194	2.666	13.44	112.0	1655	304	9152	7.52	4686	2595	0.00	8547	6284	26169	25.474	5.72	0.367
A76	Spring	7.66	0.434	0.521	35.26	293.8	4358	873	32868	14.49	8526	10755	0.00	29812	14559	67359	41.002	3.28	0.513
A80	Spring	7.61	0.213	1.470	18.21	151.8	645	800	7852	3.85	3391	2969	0.00	7237	4775	32855	9.620	2.21	0.490
A82	Spring	7.84	0.143	0.776	10.74	89.5	336	185	6869	2.36	1716	1899	0.97	5868	1809	21173	1.709	1.37	0.265
A87	Spring	7.70	0.153	1.679	8.00	66.7	141	0.00	12354	2.67	2956	2873	2.32	10856	2238	20469	4.848	1.07	0.114
A89	Spring	7.81	0.035	0.840	2.66	22.2	128	202	578	1.51	378	214	0.00	0.00	660	3614	0.138	19.32	0.546
S92	Stream	7.58	0.106	1.695	6.40	53.4	420	172	4863	61.27	6981	673	0.00	4259	1413	11175	19.203	17.41	0.276
S93	Stream	7.39	0.058	0.376	2.72	22.7	55	176	2960	2.06	865	490	0.00	2319	1256	5812	1.984	100.53	0.307
S95	Stream	7.31	0.585	3.552	39.32	327.7	3037	0.00	74924	208.45	28607	22014	0.00	56570	4968	76869	36.630	1.06	0.609
S96	Stream	8.20	0.147	2.222	14.27	118.9	618	0.00	4030	4.91	2252	2774	0.00	3016	4809	19109	0.343	7.37	2.671
S99	Stream	8.11	0.193	1.080	13.62	113.5	756	173	13167	7.23	1258	4296	0.00	11316	5646	27726	1.151	25.18	0.353
S100	Stream	7.54	0.314	1.933	10.00	83.3	341	231	10207	7.43	1135	2212	0.47	8364	4326	22420	1.649	15.57	1.621
S102	Stream	7.62	0.073	0.516	5.20	43.3	256	189	3436	3.10	601	521	0.00	2460	1076	10343	0.368	12.31	0.556
S104	Stream	8.34	0.238	2.928	21.58	179.8	1143	567	10129	9.79	3226	4996	4.32	8643	3445	38412	0.745	6.87	1.395
S107	Stream	7.91	0.237	3.518	21.88	182.3	1229	232	11361	8.84	3710	4636	3.67	9603	5699	37941	1.037	18.52	0.918
S109	Stream	7.95	0.128	2.190	10.49	87.4	1616	0.00	4336	14.66	2310	2018	3.94	3453	1211	17919	4.017	13.32	0.830
S110	Lake	8.08	0.120	1.796	9.72	81.0	1113	117	4300	14.64	1651	2078	0.00	3712	1103	17988	3.953	11.06	0.838
S111	Lake	8.60	0.113	0.922	9.88	82.3	953	82	4284	13.44	1470	1958	0.00	3502	659	16994	3.601	6.10	0.353
S94	Spring	7.89	0.913	3.964	27.24	227.0	2209	0.00	178099	19.03	32079	26074	0.00	133080	19103	140058	47.286	1.23	1.967

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
										(µg L^{-1})								
A75	Spring	0.051	0.260	2.119	0.021	0.119	1.267	0.781	1.033	1.890	31.593	15.514	0.013	0.005	5.321	0.003	107.359	0.234
A76	Spring	0.244	0.070	0.544	0.020	0.131	0.282	0.768	10.643	2.622	213.624	13.489	0.012	0.011	18.964	0.003	67.336	0.255
A80	Spring	0.261	0.469	0.395	0.017	0.100	0.561	254.268	7.045	0.713	70.082	4.135	0.011	0.002	11.052	0.001	17.567	0.152
A82	Spring	0.063	0.373	0.190	0.011	0.072	0.117	57.021	0.403	0.387	108.197	1.735	0.004	0.001	9.237	0.000	0.919	0.096
A87	Spring	0.013	11.798	28.574	0.054	2.428	0.301	351.221	0.392	2.798	65.445	0.695	0.016	0.010	6.209	0.002	0.813	0.162
A89	Spring	0.033	0.935	11.057	0.025	0.281	1.052	293.926	0.325	0.920	5.420	0.462	0.007	0.006	2.054	0.002	0.030	0.052
S92	Stream	0.024	1.113	3.250	0.017	0.109	0.563	0.855	1.848	8.444	23.773	8.604	0.006	1.610	1.431	0.010	22.698	0.549
S93	Stream	0.232	1.737	113.301	0.077	0.788	0.499	1.451	0.535	2.469	12.568	2.928	0.004	0.026	1.739	0.010	2.303	0.093
S95	Stream	0.342	0.515	0.575	0.009	0.461	0.073	5.825	0.602	0.762	292.633	7.207	0.012	0.006	12.079	0.001	5.711	0.741
S96	Stream	0.166	0.231	1.651	0.016	0.063	0.698	0.928	0.415	1.508	90.269	3.820	0.002	0.002	29.555	0.001	0.848	0.127
S99	Stream	0.054	0.470	1.792	0.010	0.101	0.888	2.542	0.294	3.600	107.066	9.099	0.005	0.007	16.828	0.006	0.436	0.059
S100	Stream	0.097	0.248	1.158	0.014	0.171	0.611	4.900	1.285	5.057	67.733	6.196	0.004	0.042	11.411	0.009	3.301	0.061
S102	Stream	0.072	0.458	5.065	0.011	0.113	0.213	1.772	1.548	1.392	20.949	1.480	0.002	0.014	3.107	0.003	0.192	0.049
S104	Stream	1.138	0.610	0.633	0.012	0.257	0.237	1.510	1.000	1.265	149.597	0.896	0.002	0.012	3.688	0.002	1.172	0.130
S107	Stream	0.422	91.600	33.423	0.667	1.670	0.733	2.521	3.032	2.959	145.521	0.981	0.009	0.008	8.415	0.001	1.039	0.151
S109	Stream	0.176	1.524	2.122	0.019	0.177	0.352	3.300	11.884	1.583	56.924	2.083	0.003	0.015	3.824	0.002	0.869	0.138
S110	Lake	0.159	0.168	1.129	0.004	0.065	0.252	4.014	12.031	1.399	57.119	2.098	0.002	0.021	3.774	0.002	0.904	0.098
S111	Lake	0.146	0.257	0.028	0.004	0.067	0.186	1.220	7.964	1.189	51.213	2.047	0.001	0.026	1.932	0.001	0.802	0.090
S94	Spring	0.108	0.032	0.381	0.012	0.231	0.149	0.063	0.646	3.899	688.898	14.395	0.009	0.011	26.612	0.002	28.973	0.653

Appendix 1: Continued

Sample code	Source	pH	EC	DOC	DIC	$CaCO_3$	Chloride	Nitrate	Sulphate	B	Na	Mg	P	S	K	Ca	Li	Al	V
			($dS\ m^{-1}$)	($mg\ L^{-1}$)			($\mu g\ L^{-1}$)												
S97	Spring	7.61	0.229	2.574	16.96	141.4	1708	266	13761	9.88	5285	4751	0.00	11463	5509	31595	0.581	2.29	4.299
S98	Spring	8.05	0.240	3.024	15.61	130.1	128	0.00	20325	6.59	2375	5616	0.00	16984	4989	37007	2.099	6.95	1.081
S101	Spring	7.90	0.127	1.697	9.74	81.2	555	203	5033	4.68	1265	1031	0.00	3865	1929	19774	0.551	8.11	1.839
S103	Spring	7.83	0.070	0.751	4.81	40.1	165	213	3233	3.23	526	523	0.00	2505	1049	11032	0.371	9.97	0.530
S105	Spring	7.98	0.309	4.816	28.90	240.8	941	510	14348	13.85	4204	9180	0.00	12572	3363	51482	0.583	1.56	1.019
S106	Spring	7.96	0.281	4.006	25.38	211.5	1061	588	12226	11.31	3874	6885	0.00	10094	3112	44975	0.420	1.26	0.973
S112	Spring	8.40	0.116	1.587	9.56	79.7	368	312	4990	5.05	1257	2236	0.54	4205	1563	15917	1.801	10.76	2.127
S108	Well	7.61	0.404	4.238	39.66	330.5	1886	0.00	13018	10.92	6056	15894	1.07	11616	5087	55649	6.434	1.59	0.119

Appendix 1: Continued

Sample code	Source	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs	Ba	Tl	U	SAR
		($\mu g\ L^{-1}$)																
S97	Spring	0.273	0.084	0.101	0.024	0.034	0.348	1.491	0.520	0.652	230.757	5.212	0.001	0.000	14.201	0.000	2.091	0.232
S98	Spring	0.106	0.607	0.862	0.019	0.069	1.059	38.967	0.623	2.592	104.003	10.070	0.012	0.009	27.117	0.003	0.927	0.096
S101	Spring	0.124	0.301	1.309	0.005	0.029	0.252	85.225	0.874	0.071	33.471	2.611	0.007	0.001	1.217	0.000	0.613	0.075
S103	Spring	0.076	0.374	2.054	0.009	0.119	0.237	3.056	1.501	1.303	21.022	1.467	0.002	0.015	3.044	0.002	0.200	0.042
S105	Spring	1.475	0.196	1.204	0.009	0.108	0.118	22.117	0.981	0.626	222.931	0.774	0.002	0.001	5.236	0.000	0.754	0.142
S106	Spring	1.240	0.651	0.259	0.013	0.114	0.660	371.849	0.686	0.497	186.467	0.803	0.006	0.001	4.268	0.000	0.598	0.142
S112	Spring	0.122	0.074	0.225	0.008	0.014	0.091	2.124	21.963	0.100	75.826	2.422	0.002	0.001	3.538	0.000	3.598	0.078
S108	Well	0.000	6.093	79.842	0.054	0.248	0.080	8.068	0.934	6.291	281.431	1.904	0.001	0.028	60.724	0.010	4.902	0.184

Appendix 2: Iodine and selenium concentration in ground and surface water used for drinking or irrigation

Sample code	Village	Source	Use	Total I	Org-I	Inorg-I	I^-	IO_3^-	Total Se	Org-Se	Inorg-Se	Se^{IV}	Se^{VI}
									(µg/L)				
		Surface											
G01	Juglote	Stream	Irrig	0.463	0.250	0.213	0.000	0.213	0.088	0.036	0.052	0.000	0.052
G02	Basin	Stream	Irrig	0.236	0.017	0.219	0.000	0.219	0.067	0.031	0.035	0.000	0.035
G04	Gilgit city	River	Irrig/drin	0.029	0.017	0.012	0.000	0.012	0.143	0.049	0.094	0.000	0.094
G05	Danyor	River	Irrig/drin	0.230	0.016	0.214	0.000	0.214	0.209	0.064	0.145	0.000	0.145
G06	Danyor	Stream	Irrig/drin	0.040	0.012	0.028	0.000	0.028	0.339	0.089	0.250	0.000	0.250
G07	Minawar	Straem	Irrig	0.242	0.037	0.204	0.000	0.204	0.073	0.033	0.040	0.000	0.040
G08	Minawar	Stream	Drin	0.425	0.217	0.208	0.000	0.208	0.127	0.044	0.083	0.000	0.083
G09	Alifabad	Stream	Irrig/drin	0.286	0.075	0.211	0.000	0.211	0.073	0.037	0.036	0.000	0.036
G11	Darot	Stream	Irrig/drin	0.391	0.178	0.213	0.000	0.213	0.104	0.040	0.064	0.000	0.064
G14	Bargin	Stream	Irrig/drin	0.424	0.223	0.200	0.000	0.200	0.161	0.044	0.117	0.000	0.117
G15	Bargin	Stream	Irrig/drin	0.220	0.024	0.197	0.000	0.197	0.109	0.044	0.064	0.000	0.064
G17	Damot	Stream	Irrig/drin	0.225	0.032	0.193	0.000	0.193	0.123	0.054	0.070	0.000	0.070
G18	Chakarkot	Stream	Irrig/drin	0.200	0.006	0.194	0.000	0.194	0.041	0.016	0.025	0.000	0.025
G19	Pari	Stream	Drin	0.219	0.025	0.194	0.015	0.179	0.054	0.035	0.019	0.000	0.019
G20	Shamughar	Stream	Irrig/drin	0.360	0.122	0.238	0.050	0.188	1.147	0.299	0.849	0.000	0.849
G21	Jalal Abad	Stream	Irrig/drin	0.132	0.106	0.026	0.000	0.026	0.461	0.120	0.341	0.000	0.341
G22	Rahim Abad	Stream	Irrig/drin	0.119	0.088	0.031	0.000	0.031	0.204	0.061	0.143	0.000	0.143
G23	Juglote Guru	Stream	Irrig	0.029	0.000	0.029	0.000	0.029	2.089	0.581	1.508	0.000	1.508
G24	Juglote Guru	Stream	Drin	0.337	0.141	0.196	0.000	0.196	2.170	0.606	1.564	0.000	1.564
D30	Singgasal	Stream	Drin	0.392	0.116	0.276	0.108	0.168	0.892	0.283	0.609	0.000	0.609
D31	Talichi	stream	Irrig/drin	0.280	0.093	0.186	0.021	0.165	0.437	0.156	0.281	0.000	0.281
D33	Batsloi Nala	Stream	Irrig/drin	0.012	0.006	0.007	0.005	0.001	0.320	0.123	0.197	0.000	0.197
D35	Darang	Stream	Irrig/drin	1.789	1.602	0.187	0.017	0.171	0.109	0.079	0.030	0.000	0.030
D36	Lacer Nala	Stream	Irrig/drin	0.124	0.023	0.101	0.081	0.020	0.097	0.027	0.070	0.000	0.070

Irrig = irrigation, Drin = drinking

Appendix 2: Continued

Sample code	Village	Source	Use	Total I	Org-I	Inorg-I	I^-	IO_3^-	Total Se	Org-Se	Inorg-Se	Se^{IV}	Se^{VI}
									(µg/L)				
D37	Gonar Farm	Stream	Irrig/drin	0.743	0.334	0.409	0.012	0.397	0.160	0.058	0.103	0.000	0.103
D39	Bonar Das	Stream	Irrig/drin	0.048	0.014	0.034	0.000	0.034	0.149	0.047	0.101	0.000	0.101
D40	Gas Payen	Stream	Irrig/drin	0.572	0.417	0.156	0.000	0.156	0.062	0.041	0.021	0.000	0.021
D41	Gas Bala	Stream	Irrig/drin	0.227	0.067	0.160	0.000	0.160	0.016	0.016	0.000	0.000	0.000
D42	Babsuar Nala	Stream	Irrig/drin	0.241	0.059	0.181	0.022	0.159	0.381	0.145	0.236	0.000	0.236
D45	Kinar nala	Stream	Irrig/drin	0.830	0.653	0.176	0.020	0.157	0.068	0.054	0.014	0.000	0.014
D46	Batuga Nala	Stream	Irrig/drin	0.252	0.075	0.178	0.022	0.155	0.097	0.051	0.046	0.000	0.046
D48	Chillas MARC	Stream	Irrig	0.320	0.163	0.157	0.000	0.157	0.057	0.048	0.009	0.000	0.009
D51	Khai Batuga	Stream	Irrig/drin	0.505	0.331	0.173	0.015	0.158	0.096	0.062	0.034	0.000	0.034
N52	Chalt	Stream	Irrig	0.224	0.048	0.176	0.020	0.156	0.590	0.174	0.416	0.000	0.416
N53	Chalt	Stream	Drin	0.060	0.006	0.053	0.006	0.047	0.473	0.126	0.347	0.000	0.347
H54	Hussain Abad	Stream	Irrig/drin	0.067	0.000	0.067	0.050	0.018	0.444	0.126	0.318	0.000	0.318
H55	Hussain Abad	Stream	Irrig	0.019	0.000	0.019	0.000	0.019	0.026	0.012	0.014	0.000	0.014
N56	Rakaposhi	Stream	Irrig/drin	0.110	0.090	0.021	0.000	0.021	0.039	0.014	0.025	0.000	0.025
N57	Pissan	Stream	Irrig	0.151	0.108	0.043	0.000	0.043	0.584	0.152	0.432	0.000	0.432
H59	Nasir Abad	Stream	Irrig	0.116	0.046	0.070	0.031	0.038	0.455	0.165	0.290	0.000	0.290
H61	Karim Abad	Stream	Irrig	0.071	0.018	0.053	0.026	0.027	0.104	0.030	0.074	0.000	0.074
N62	Smr Khay	Stream	Irrig	0.010	0.001	0.009	0.001	0.009	0.248	0.059	0.189	0.000	0.189
N64	Bara Nagar	Stream	Irrig	0.214	0.042	0.172	0.142	0.029	0.187	0.065	0.122	0.000	0.122
N65	Mamubar-N	Stream	Irrig/drin	0.145	0.020	0.125	0.097	0.028	0.261	0.059	0.202	0.000	0.202
H69	Hassan Abad	Stream	Irrigation	0.400	0.003	0.397	0.255	0.142	0.129	0.048	0.081	0.000	0.081
N70	Qasim Abad	Stream	Irrig/drin	0.345	0.189	0.156	0.016	0.140	0.126	0.042	0.084	0.000	0.084
N71	Nilt	Stream	Irrig/drin	0.062	0.043	0.019	0.000	0.019	0.472	0.119	0.353	0.000	0.353
A73	Donia	Stream	Irrig	0.081	0.003	0.078	0.070	0.008	0.321	0.128	0.194	0.000	0.194
A77	Harchu	Stream	Irrig/drin	0.040	0.000	0.040	0.021	0.019	0.206	0.063	0.143	0.000	0.143

Irrig = irrigation, Drin = drinking

Appendix 2: Continued

Sample code	Village	Source	Use	Total I	Org-I	Inorg-I	I^-	IO_3^-	Total Se	Org-Se	Inorg-Se	Se^{IV}	Se^{VI}
									(µg/L)				
A78	Harchu	Stream	Irrig	0.167	0.051	0.115	0.084	0.031	0.190	0.054	0.136	0.000	0.136
A79	Chungra	Stream	Irrig	0.146	0.065	0.080	0.045	0.036	0.212	0.060	0.152	0.000	0.152
A81	Buland	Stream	Irrig/drin	0.196	0.111	0.085	0.046	0.039	0.246	0.055	0.192	0.000	0.192
A83	Gorikot	Stream	Irrig	0.034	0.001	0.033	0.019	0.015	0.133	0.051	0.082	0.000	0.082
A84	Chori Nala	River	Irrig/drin	0.144	0.000	0.144	0.125	0.019	0.093	0.018	0.075	0.000	0.075
A85	Rato Nala	River	Irrig/drin	0.148	0.098	0.050	0.036	0.014	0.091	0.030	0.060	0.000	0.060
A86	Rahmanpur-B	Stream	Irrig	0.046	0.001	0.045	0.034	0.012	0.119	0.029	0.090	0.000	0.090
A88	Mankyal	Stream	Irrig	0.262	0.186	0.075	0.058	0.018	0.047	0.028	0.019	0.000	0.019
A90	Chahlum Nala	River	Irrig/drin	0.080	0.000	0.079	0.061	0.018	0.068	0.029	0.039	0.000	0.039
A91	Bunji	Stream	Irrig/drin	0.103	0.000	0.103	0.077	0.026	0.398	0.104	0.295	0.000	0.295
S92	Haramosh	Stream	Irrig/drin	0.523	0.172	0.351	0.220	0.131	0.152	0.052	0.099	0.000	0.099
S93	Shatoot	Stream	Irrig/drin	0.161	0.004	0.157	0.137	0.019	0.103	0.029	0.074	0.000	0.074
S95	Astak	Stream	Irrig/drin	0.978	0.000	0.978	0.045	0.933	0.908	0.239	0.669	0.000	0.669
S96	Torki	Stream	Irrig	0.362	0.158	0.203	0.000	0.203	0.236	0.085	0.151	0.000	0.151
S99	Baghicha	Stream	Irrig/drin	0.059	0.000	0.059	0.027	0.032	0.567	0.145	0.421	0.000	0.421
S100	Sari/Chari	Stream	Irrig/drin	0.231	0.154	0.077	0.043	0.035	0.384	0.106	0.278	0.000	0.278
S102	Kachura	Stream	Irrig	0.108	0.000	0.108	0.050	0.058	0.107	0.029	0.078	0.000	0.078
S104	Gamba	Stream	Irrig	1.193	0.066	1.127	0.051	1.076	0.316	0.070	0.246	0.000	0.246
S107	Ranga	Stream	Irrig	0.741	0.108	0.633	0.018	0.615	0.283	0.083	0.200	0.000	0.200
S109	Skardu city	Stream	Irrig	0.074	0.045	0.029	0.006	0.022	0.137	0.055	0.082	0.000	0.082
N66	Atabad Nala	Lake	Irrig	0.218	0.039	0.179	0.140	0.040	0.349	0.082	0.267	0.000	0.267
S110	Skardu city	Lake	Drin	0.152	0.049	0.103	0.063	0.040	0.161	0.041	0.120	0.000	0.120
S111	Sapara	Lake	Irrig/drin	0.134	0.000	0.134	0.097	0.037	0.137	0.042	0.095	0.000	0.095

Irrig = irrigation, Drin = drinking

Appendix 2: Continued

Sample code	Village	Source	Use	Total I	Org-I	Inorg-I	I^-	IO_3^-	Total Se	Org-Se	Inorg-Se	Se^{IV}	Se^{VI}
									(µg/L)				
		Ground											
G03	Basin	Spring	Drin	0.192	0.167	0.025	0.000	0.025	0.081	0.033	0.048	0.000	0.048
G10	Paidan Das	Spring	Drin	2.182	0.524	1.658	0.000	1.658	0.748	0.197	0.551	0.000	0.551
G12	Bargin	Spring	Irrig/drin	1.144	0.390	0.754	0.000	0.754	0.418	0.123	0.294	0.000	0.294
G13	Bargin	Spring	Irrig/drin	1.360	0.353	1.006	0.000	1.006	0.524	0.128	0.395	0.000	0.395
G16	Bargin	Spring	Drin	0.983	0.208	0.775	0.138	0.637	0.335	0.076	0.258	0.000	0.258
D32	Thathapani	Spring	Drin	4.741	3.333	1.408	1.226	0.182	0.000	0.000	0.000	0.000	0.000
D34	Batsloi	spring	Drin	0.430	0.219	0.211	0.043	0.167	0.390	0.094	0.296	0.000	0.296
D38	Gonar Farm	Spring	Drin	10.177	1.541	8.637	0.638	7.999	3.041	0.880	2.161	0.000	2.161
D43	Gene Nala	Spring	Drin	0.983	0.239	0.744	0.000	0.744	0.761	0.159	0.602	0.000	0.602
D44	Dong Nala	Spring	Irrig/drin	1.454	1.187	0.267	0.000	0.267	1.074	0.383	0.691	0.000	0.691
D47	Geach Nala	Spring	Irrig/drin	1.200	1.045	0.155	0.000	0.155	0.398	0.135	0.264	0.000	0.264
D49	Chillas	Spring	Drin	1.149	0.405	0.744	0.022	0.722	0.171	0.050	0.122	0.000	0.122
D50	Khai Batuga	Spring	Drin	1.013	0.260	0.753	0.134	0.619	0.207	0.054	0.154	0.000	0.154
N58	Pissan	Spring	Drin	0.167	0.120	0.048	0.000	0.048	0.668	0.143	0.525	0.000	0.525
H60	Karim Abad	Spring	Drin	0.027	0.002	0.025	0.013	0.012	0.528	0.155	0.373	0.000	0.373
N63	Smr Khay	Spring	Drin	0.390	0.007	0.382	0.037	0.345	0.670	0.171	0.499	0.000	0.499
H67	Aliabad	Spring	Drin	0.102	0.056	0.047	0.008	0.039	1.192	0.344	0.848	0.000	0.848
H68	Hassan Abad	Spring	Drin	1.717	0.308	1.409	0.232	1.177	0.842	0.240	0.603	0.000	0.603
A72	Ramgat	Spring	Drin	0.058	0.000	0.058	0.050	0.008	0.247	0.059	0.187	0.000	0.187
A74	Donia	Spring	Drin	0.116	0.023	0.093	0.060	0.032	0.277	0.075	0.202	0.000	0.202
A75	Mushkin	Spring	Drin	0.126	0.072	0.054	0.028	0.026	0.454	0.130	0.324	0.000	0.324
A76	Dashkin	Spring	Drin	1.270	0.393	0.878	0.230	0.648	1.073	0.283	0.790	0.000	0.790

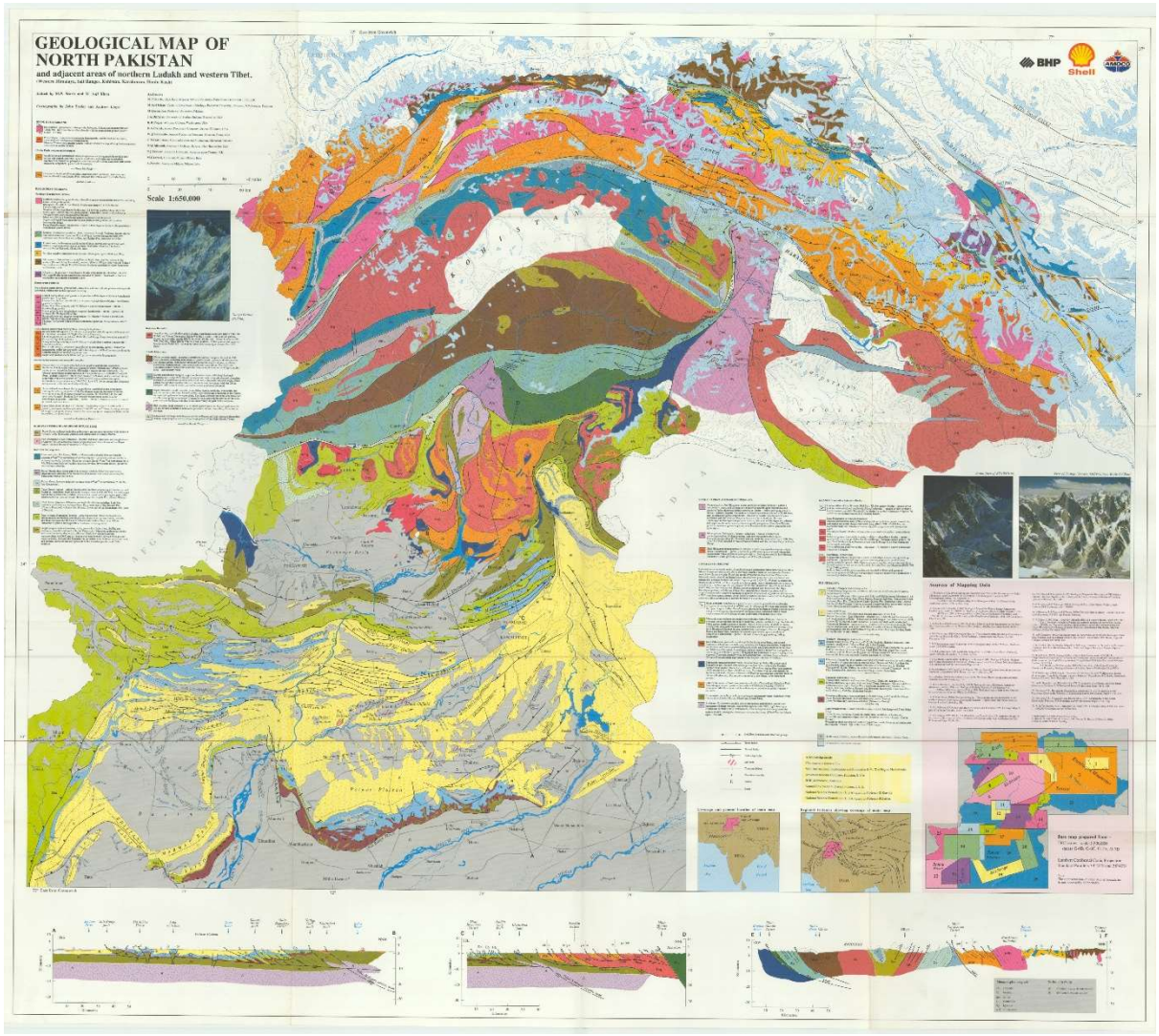
Irrig = irrigation, Drin = drinking

Appendix 2: Continued

Sample code	Village	Source	Use	Total I	Org-I	Inorg-I	I^-	IO_3^-	Total Se	Org-Se	Inorg-Se	Se^{IV}	Se^{VI}
				(µg/L)									
A80	Chungra	Spring	Drin	0.475	0.033	0.442	0.090	0.352	0.307	0.064	0.243	0.000	0.243
A82	Gorikot	Spring	Drin	0.131	0.022	0.109	0.069	0.040	0.319	0.074	0.246	0.000	0.246
A87	Rahmanpur-B	Spring	Drin	0.017	0.000	0.017	0.000	0.017	0.439	0.091	0.349	0.000	0.349
A89	Mankyal	Spring	Drin	0.057	0.000	0.057	0.045	0.012	0.047	0.021	0.027	0.000	0.027
S94	Shungus	Spring	Drin	0.244	0.014	0.229	0.083	0.146	0.296	0.099	0.198	0.000	0.198
S97	Torki	Spring	Drin	2.219	0.292	1.927	0.262	1.665	0.566	0.137	0.429	0.000	0.429
S98	Dambudas	Spring	Drin	0.348	0.063	0.285	0.082	0.204	1.324	0.364	0.960	0.000	0.960
S101	Kachura	Spring	Drin	0.643	0.000	0.643	0.065	0.578	0.313	0.056	0.257	0.000	0.257
S103	Kacura-Mrkt	Spring	Drin	0.070	0.000	0.070	0.050	0.020	0.117	0.038	0.079	0.000	0.079
S105	Gamba	Spring	Drin	1.455	0.098	1.357	0.060	1.297	0.380	0.098	0.282	0.000	0.282
S106	Ranga	Spring	Drin	1.146	0.000	1.146	0.002	1.144	0.351	0.079	0.272	0.000	0.272
S112	mantal	Spring	Drin	0.538	0.000	0.538	0.154	0.384	0.267	0.057	0.210	0.000	0.210
S108	Ranga	Well	Drin	0.893	0.000	0.893	0.775	0.117	0.031	0.031	0.000	0.000	0.000

Irrig = irrigation, Drin = drinking

Appendix 3: Geological Map of North Pakistan



Appendix 4: Average concentration of major and minor elements in soil samples

Sampling district	Sample code	I	Se	Na	Mg	P	K	Ca	Ti	Li	Be	Al
		($\mu g\ kg^{-1}$)		($mg\ kg^{-1}$)								
Gilgit	G01	796	168	17594	11510	754	13211	28712	1111	19.8	1.38	58439
	G02	784	165	18762	10891	862	10802	29985	1271	15.5	1.12	57111
	G03	391	289	17568	13827	878	9211	38439	764	10.8	0.589	52437
	G04	454	123	16655	12756	973	12522	30128	1477	18.9	1.25	55042
	G05	944	202	18778	16090	1397	7087	33886	2361	12.2	0.771	52955
Diamer	D06	813	109	17657	8577	1073	17499	26899	1095	23.8	1.81	51804
	D07	1080	199	14805	7525	1002	25071	14207	1372	47.2	3.63	46896
	D08	1030	143	14659	13755	826	12697	28908	1316	17.2	1.56	51327
	D09	1070	120	14007	22595	645	7117	39777	1279	9.1	0.705	54211
	D10	482	92.7	16605	11325	991	12581	31646	1252	16.8	1.41	54526
Hunza-N	N11	611	430	11943	11082	856	10132	51094	855	22.1	0.841	47434
	H12	877	329	8934	8332	1032	21309	26765	1191	38.9	2.11	48362
	N13	871	453	13242	18730	1258	14210	35866	1098	18.2	0.98	49525
	H14	393	333	14781	7147	987	26139	22694	1160	106.1	4.06	50688
	H15	596	265	16011	15632	2221	27865	28587	2077	66.6	2.39	57932
	N16	555	265	7835	18730	1381	16965	32149	1078	41.7	2.46	47822
Astor	A17	445	151	14884	8629	1215	26319	13670	1019	40.7	3.46	47949
	A18	492	135	14758	8723	1128	28362	11631	1100	64.5	3.63	51395
	A19	273	150	15480	8455	841	22252	13779	1014	25.5	3.00	47007
	A20	679	172	13510	8517	1209	23222	12069	1126	37.1	2.96	45191
	A21	558	109	18644	17247	951	7313	40276	1704	11.0	0.69	65333
Skardu	S22	742	178	18769	21985	1489	13676	43749	1513	12.8	1.08	66677
	S23	1180	219	14143	15024	1136	13893	28363	1189	26.1	1.38	54791
	S24	728	279	11905	16192	1006	15626	46840	1179	27.5	1.65	51310
	S25	387	216	13837	14314	795	16820	53681	996	25.7	1.64	52820
	S26	590	151	13364	13460	789	17895	27197	1001	27.7	1.56	52216

Appendix 4: Continued

Sampling district	Sample code	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	As
		(mg kg^{-1})									
Gilgit	G01	112	47.4	760	34788	14.71	24.92	33.9	67.50	15.21	3.97
	G02	111	54.1	693	33889	13.88	26.91	30.0	86.13	14.87	7.59
	G03	136	99.4	871	35989	16.13	30.17	85.5	64.06	12.01	2.01
	G04	124	83.4	682	37596	18.44	43.13	42.0	71.17	15.60	2.63
	G05	170	82.3	979	51427	23.59	58.67	31.5	80.19	14.14	1.95
Diamer	D06	98.2	38.7	622	32008	13.87	19.79	32.8	66.77	16.05	4.72
	D07	51.7	45.6	484	32329	10.00	18.37	14.3	77.98	18.96	11.10
	D08	127	85.1	768	38850	19.42	49.23	38.4	71.58	15.25	7.73
	D09	131	137	827	42228	31.35	84.75	56.6	64.25	13.29	3.39
	D10	137	42.5	723	39194	16.27	20.31	37.8	73.32	15.51	3.38
Hunza-N	N11	110	91.3	732	31776	16.02	69.31	59.3	65.30	11.82	30.0
	H12	86.5	76.3	483	30923	13.95	43.29	34.3	73.65	17.15	28.9
	N13	151	147	857	41457	22.93	52.04	97.0	86.27	13.19	18.0
	H14	78.8	57.4	439	27970	12.67	30.45	30.5	78.46	18.93	25.8
	H15	109	54.5	757	42822	18.01	26.36	32.2	113.86	19.01	5.24
	N16	75.0	96.5	469	27474	12.73	73.82	23.7	68.65	14.68	22.1
Astor	A17	61.0	53.3	453	26639	10.67	24.38	23.1	81.29	17.77	5.20
	A18	50.8	57.3	415	27925	10.09	22.65	17.7	91.36	19.50	6.76
	A19	68.8	46.2	445	24075	9.19	18.79	17.7	58.92	16.63	3.11
	A20	65.3	51.2	493	26831	11.51	27.91	25.1	89.49	17.23	17.0
	A21	186	33.2	1054	51480	20.83	16.75	43.8	84.02	15.42	4.20
Skardu	S22	182	53.7	922	47741	23.17	30.94	48.2	79.11	15.96	2.12
	S23	108	83.8	645	32465	15.28	43.50	30.7	81.08	15.36	6.92
	S24	104	112	682	33948	16.74	83.22	32.3	83.99	14.87	20.5
	S25	80.8	73.4	577	27405	12.47	50.24	27.4	69.67	14.41	17.7
	S26	79.9	84.1	598	28095	12.99	64.22	26.3	68.54	15.09	43.6

Appendix 4: Continued

Sampling district	Sample code	Rb	Sr	Mo	Ag	Cd	Cs	Ba	Tl	Pb	U
		(mg kg^{-1})									
Gilgit	G01	54.6	264	0.572	0.054	0.111	3.46	340	0.369	15.2	1.87
	G02	43.6	241	0.564	0.078	0.115	2.77	251	0.258	19.7	1.62
	G03	23.3	309	1.09	0.037	0.111	0.699	173	0.124	5.86	0.931
	G04	46.5	254	0.426	0.029	0.078	3.23	225	0.290	11.4	1.39
	G05	22.5	268	0.633	0.051	0.128	1.51	176	0.172	7.38	1.11
Diamer	D06	82.2	275	0.415	0.044	0.098	3.13	354	0.583	17.3	3.91
	D07	150	125	0.499	0.041	0.126	7.01	450	1.059	29.6	9.36
	D08	47.7	222	0.382	0.056	0.120	2.43	301	0.337	15.4	1.68
	D09	26.8	224	0.253	0.029	0.089	1.70	148	0.135	6.94	0.769
	D10	48.4	267	0.279	0.058	0.094	2.53	340	0.344	14.8	1.51
Hunza-N	N11	34.2	279	0.507	0.081	0.150	1.88	228	0.220	9.74	0.92
	H12	84.4	151	0.741	0.063	0.139	4.48	473	0.560	22.0	2.35
	N13	42.3	187	0.776	0.087	0.119	2.41	419	0.219	15.1	5.11
	H14	123	166	1.22	0.057	0.124	10.4	517	0.850	23.8	4.31
	H15	135	280	1.75	0.069	0.144	8.16	734	0.756	20.3	4.08
	N16	89.4	139	0.528	0.050	0.175	7.33	409	0.513	20.2	5.60
Astor	A17	134	122	0.422	0.037	0.110	5.59	427	0.912	29.0	3.34
	A18	182	113	0.394	0.052	0.103	10.1	454	1.203	31.5	5.43
	A19	104	142	0.421	0.021	0.064	3.55	401	0.696	25.0	3.29
	A20	127	119	0.485	0.082	0.172	5.06	444	0.856	26.9	4.31
	A21	24.8	277	0.483	0.034	0.116	1.76	242	0.142	8.7	0.738
Skardu	S22	46.7	507	0.459	0.047	0.098	1.65	386	0.215	9.6	0.833
	S23	70.0	229	0.352	0.087	0.159	4.65	281	0.402	17.5	1.68
	S24	74.8	223	0.435	0.113	0.224	5.45	351	0.466	22.6	1.59
	S25	80.3	267	0.711	0.079	0.170	5.56	404	0.499	22.4	1.87
	S26	86.8	191	0.532	0.077	0.152	6.46	336	0.531	21.5	1.65

Appendix 5: Average concentration of iodine in different plants types grown in different districts of Gilgit-Baltistan

Plant type	Gilgit			Diamer			Hunza-N			Astor			Skardu		
	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range
		($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)	
Leafy vegetables															
Lettuce	-	-	-	-	-	-	-	-	-	-	-	-	1	107	-
Spinach	5	62.6	30.6-102	6	75.5	39.4-109	4	52.2	28.3-96.5	4	58.7	37.9-105	2	43.9	28.7-59
Amaranth leaves	3	41.6	23.8-63.8	4	28.0	11.6-53.2	2	22.3	15.9-28.6	5	16.9	6.17-30.8	-	-	-
Kohlrabi	2	17.1	16.4-17.7	4	17.9	13.6-22	2	12.7	12.7-12.8	2	19.1	17.5-20.8	3	18.0	13.2-27.4
Cabbage	1	4.8	-	-	-	-	2	7.6	6.53-8.75	-	-	-	4	4.6	3.05-6.07
Grains															
Barley	-	-	-	-	-	-	2	33.7	30.8-36.5	-	-	-	1	34.5	-
Maize	5	23.1	19.7-28.1	5	28.0	24.2-32.1	6	32.1	25.9-49.8	5	18.8	12.7-26.0	4	16.5	9.16-20.1
Wheat	5	35.9	29.5-44.2	5	13.5	5.24-27.9	6	8.24	1.18-13.1	4	14.6	3.44-28.6	5	22.5	14.7-41.2
Buckwheat	-	-	-	-	-	-	2	10.0	9.97-10.1	-	-	-	-	-	-
Crop vegetables															
Okra	3	31.3	18.9-37.9	5	40.85	19.6-104	2	16.1	15-17.1	-	-	-	-	-	-
Tomato	5	44.1	12-142	4	15.1	5-21.8	5	14.0	7.04-16.7	4	12.5	6.37-23.2	3	14.3	11.4-17.8
Pumpkin	-	-	-	-	-	-	2	7.07	4.07-10.1	3	19.4	11.2-24.7	1	21.0	-
Chilli	3	25.3	22-30.7	3	17.4	15.2-19.9	2	8.55	8.46-8.64	-	-	-	3	8.04	7.43-8.65
Cucumber	1	20.5	-	1	16.4	-	1	5.21	-	1	11.3	-	1	19.30	-
Aubergine	2	21.2	13.1-29.3	2	6.79	4.75-8.83	-	-	-	1	6.37	-	2	5.97	5.93-6.02
Capsicum	2	7.2	-	1	8.69	-	-	-	-	-	-	-	-	-	-
Beans	2	8.71	6-11.4	-	-	-	5	4.98	1.42-10.5	3	4.35	2.2-8.55	1	6.93	-
Green pea	-	-	-	1	0.79	-	-	-	-	-	-	-	-	-	-

Appendix 5: Continued

Plant type	Gilgit			Diamer			Hunza-N			Astor			Skardu		
	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range
	($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)		
Tubers															
Radish	-	-	-	1	16.0	-	-	-	-	-	-	-	-	-	-
Carrot	1	24.2	-	-	-	-	1	6.84	-	-	-	-	2	9.60	6.67-12.5
Onion	4	7.18	4.54-9.83	1	66.4	-	3	4.87	2.94-6.64	-	-	-	4	5.93	4.54-7.88
Potato	2	11.7	10.1-13.2	-	-	-	3	7.54	5.28-11.5	5	6.10	5.41-6.69	1	8.97	-
Turnip	1	5.43	-	-	-	-	-	-	-	-	-	-	1	8.19	-
Nuts															
Almond	1	10.7	-	-	-	-	-	-	-	-	-	-	-	-	-
Walnut	1	2.52	-	-	-	-	-	-	-	-	-	-	-	-	-
Fruits															
Grapes	-	-	-	-	-	-	-	-	-	-	-	-	1	32.4	-
Bair	-	-	-	1	12.0	-	-	-	-	-	-	-	-	-	-
Mulberry	1	10.5	-	-	-	-	-	-	-	-	-	-	2	11.8	9.80-13.7
Fig	2	9.22	5.05-13.4	-	-	-	1	6.00	-	-	-	-	-	-	-
Pear	4	7.56	4.07-9.78	-	-	-	4	5.69	4.81-6.39	1	5.01	-	2	7.49	6.58-8.41
Apricot	3	5.71	4.65-6.5	2	7.59	3.35-11.8	6	4.89	3.62-6.07	3	6.50	6.01-6.97	5	5.26	4.22-6.26
Peach	-	-	-	1	6.43	-	1	3.86	-	1	7.62	-	1	3.89	-
Apple	5	8.28	6.43-12.4	3	3.88	2.11-4.97	4	3.70	1.26-8.18	2	4.81	3.89-5.72	4	4.84	2.32-9.66
Persimmon	1	3.47	-	1	2.79	-	1	1.74	-	-	-	-	-	-	-

Appendix 6: Average concentration of selenium in different plants types grown in different districts of Gilgit-Baltistan

Plant type	Gilgit			Diamer			Hunza-N			Astor			Skardu		
	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range
		($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)	
Leafy vegetables															
Lettuce	-	-	-	-	-	-	-	-	-	-	-	-	1	31.1	
Spinach	5	54.0	12.3-131	6	70.9	4.16-309	4	148	16.1-536	4	64.7	11.5-135	2	39.6	5.73-73.5
Amaranth leaves	3	27.6	19.6-39.1	4	35.3	19.1-48.6	2	129	39.8-218	5	39.6	13.9-67.4	-	-	-
Kohlrabi	2	32.1	21.0-43.2	4	92.2	18.3-149	2	697	588-807	2	20.2	4.68-35.8	3	88.3	2.64-193
Cabbage	1	118	-	-	-	-	2	515	10.5-1021	-	-	-	4	65.7	1.17-198
Grains															
Barley	-	-	-	-	-	-	2	34.9	25.1-44.7	-	-	-	1	9.4	-
Maize	5	22.8	7.82-37.3	5	8.0	1.38-18.0	6	31.1	3.56-55.2	5	3.8	3.14-5.71	4	28.8	3.50-50.3
Wheat	5	19.7	2.95-50.6	5	6.1	2.04-10.6	6	50.2	7.49-132	4	34.2		5	31.2	
Buckwheat	-	-	-	-	-	-	2	16.2		-	-	-	-	-	-
Crop vegetables															
Okra	3	2.9	2.65-3.43	5	10.3	0.938-29.7	2	99.3	7.27-191	-	-	-	-	-	-
Tomato	5	21.6	5.33-48.4	4	16.8	4.28-30.0	5	48.3	8.20-78.0	4	25.5	8.78-68.7	3	26.6	12.9-53.7
Pumpkin	-	-	-	-	-	-	2	28.8	21.2-36.3	3	10.2	3.11-24.2	1	37.6	-
Chilli	3	9.6	4.47-19.9	3	9.90	0.904-23.0	2	24.4	19.8-29.1	-	-	-	3	4.08	0.782-6.39
Cucumber	1	10.3	-	1	17.1	-	1	8.10	-	1	8.70	-	1	54.9	-
Aubergine	2	6.1	4.16-8.03	2	7.54	7.19-7.90	-	-	-	1	10.5	-	2	11.3	10.4-12.2
Capsicum	2	4.07	3.04-5.11	1	2.23	-	-	-	-	-	-	-	-	-	-
Beans	2	569	1.43-1137	-	-	-	5	21.3	3.49-62.6	3	6.47	5.73-6.87	1	10.0	-
Green pea	-	-	-	1	19.4	-	-	-	-	-	-	-	-	-	-

Appendix 6: Continued

Plant type	Gilgit			Diamer			Hunza-N			Astor			Skardu		
	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range
	($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)			($\mu g\ kg^{-1}$)		
Tubers															
Radish	-	-	-	1	24.0	-	-	-	-	-	-	-	-	-	-
Carrot	1	38.2	-	-	-	-	1	3.87	-	-	-	-	2	4.08	1.49-6.67
Onion	4	8.06	4.68-17.1	1	5.0	-	3	31.4	3.45-45.7	-	-	-	4	3.10	2.25-4.26
Potato	2	10.8	6.53-14.8	-	-	-	3	14.4	206-37.5	5	8.11	1.89-20.8	1	4.64	-
Turnip	1	29.2	-	-	-	-	-	-	-	-	-	-	1	30.1	-
Nuts															
Almond	1	4.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Walnut	1	52.9	-	-	-	-	-	-	-	-	-	-	-	-	-
Fruits															
Grapes	-	-	-	-	-	-	-	-	-	-	-	-	1	14.9	-
Bair	-	-	-	1	14.0	-	-	-	-	-	-	-	-	-	-
Mulberry	1	4.70	-	-	-	-	-	-	-	-	-	-	2	6.1	5.76-6.50
Fig	2	7.87	4.21-11.5	-	-	-	1	8.65	-	-	-	-	-	-	-
Pear	4	3.90	0.741-11.7	-	-	-	4	4.33	0.990-9.84	1	2.00	-	2	2.49	0.850-4.14
Apricot	3	4.07	2.27-5.78	2	6.51	4.29-8.75	6	3.24	2.27-4.50	3	3.41	2.55-4.62	5	5.21	1.09-13.1
Peach	-	-	-	1	0.80	-	1	1.72	-	1	1.74	-	1	1.72	-
Apple	5	3.27	2.17-4.27	3	7.32	1.56-10.3	4	3.35	1.39-4.53	2	1.69	1.34-2.04	4	4.69	1.15-8.04
Persimmon	1	3.17	-	1	1.45	-	1	1.43	-	-	-	-	-	-	-