Soil physico-chemical characteristics and level of selenium in soil and staple crops from areas of contrasting human selenium status in the Amhara region, Ethiopia

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A research thesis submitted to Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Science in Food Science and Nutrition

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Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of the materials used for the thesis have been correctly acknowledged.

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ADDIS ABABA UNIVERSITY

CENTER FOR FOOD SCIENCE AND NUTRITION

The undersigned hereby certify that they have read and recommend to the Graduate Studies of college of Natural and Computational Science for the acceptance of a thesis entitled 'Soil physico-chemical characteristics and level of selenium in soil andstaple crops from areas of contrasting human selenium status in the Amhara region, Ethiopia' By Sahar Ali in partial fulfillment of the requirement for Master of degree in Food Science and Nutrition

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List of abbreviations and acronyms

AIDS	Acquired Immune Deficiency Syndrome
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
CRM	Certified Reference Material
DALYs	Disability Adjusted Life Years
DI	Deionized Water
FAO	Food and Agricultural Organization
GIS	Global Positioning System
GPXs	Glutathione Peroxidase
ICP-MS	Inductive Coupled Plasma -Mass Spectroscopy
IDI	Iodothironine Deiodinase
NIST	National Institute of Science and Technology
RDA	Relative Dietary Allowance
SSA	Sub-Sahran Africa
TrxRs	Thioredoxin Reductases
USDA	United States Agency for International Development
UV-VIS-NIR	Ultraviolet Visible Near Infra-Red

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Abstract

Back ground: Selenium (Se) is an important micronutrient for humans and livestock. It is an integral part of more than 25 seleno-proteins. It plays significant biological role through seleno-cystein compounds such as protection against free radical damage and thyroid hormone metabolism. More than half of the populations in the Amhara region (North and North-West Ethiopia) have Se inadequacy. However, the human Se distribution had spatial variation such that no children in east Amhara had Se inadequacy while majority (up to 90%) of the children from the west Amhara had Se inadequacy. Selenium concentration in the soil determines human Se nutrition particularly in those dependent on local food sources.

Method: In the present study, Se in crop and soil samples from areas of contrasting human Se concentration, in the Amhara region, Ethiopia were collected. Selenium in staple crops and soil samples was determined using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). In addition, soil physic-chemical characteristics (moisture, texture, organic matter content, pH, and minerals) were analyzed.

Results: There was wide variation in Se concentration within and among staple crops (median=47.7µg/kg for maize to 9653.5µg/kg for finger millet). In general, maize and finger millet had the least and highest Se concentration, respectively. About, 12% of crop samples (majority maize) had low or marginal Se concentration. T*eff* (median=0.43 vs 0.13; p=0.0003) and maize (0.27±0.1 vs 0.04±0.01; p=0.002) samples collected from east Amhara had significantly higher Se concentration than the corresponding samples from west Amhara.

Clay (55%) was the dominant textures in the present study soil samples. In addition, soil samples had diverse pH value (4.5-8.4). About 10% of soil samples had low or marginal Se concentration. In addition, 24.4% had low phosphate extractable Se. Phosphate extractable Se was correlated with soil organic matter (r=0.47; p<0.001), soil pH(r=0.54; p<0.001), and total Se (r=0.56; p<0.01). Soil samples from east Amhara had significantly lower concentration of total Se (0.4±0.1 vs 0.2±0.2 mg/kg; p=0.03), phosphate extractable Se (4.1±2.2 vs 6.7±3.4µg/kg; p<0.001), and organic matter than soil samples from west Amhara. On the other hand, soil samples from the western Amhara had significantly lower pH than soil samples from the east Amhara.

Selenium concentration in *teff* was significantly correlated with soil pH (r=0.45; p<0.01). In addition, there was significant correlation between maize Se concentration with soil aluminum (r= -0.64, p<0.01) and wheat Se concentration with soil calcium (r=0.7; p<0.01).

Conclusion: The crop samples in the present study exhibit distinct spatial Se concentration similar to the human Se distribution pattern reported previously. In contrast, total and phosphateextractable Se concentration in soil samples had opposite pattern to human Se. Soil total organic matter content and soil pH were the important factors responsible for the variation in Se concentration.

Key words: Selenium, Soil, Staple crops, Amhara, Ethiopia,

1. **Introduction**

1.1. Background and justification

Many African soils are deficient in one or more of micronutrients (Valuateet al, 2015).Insufficient micronutrient availability in soils in these regions not only cause low crop productivity but also poor nutritional quality of the crops. Diets in sub-Saharan Africa (SSA) are often low in diversity and dominated by starchy crops exposing populations for the risk of micronutrient deficiency. Micronutrient deficiency is wide spread in SSA and it is responsible for 1.5-12% of the total Disability Adjusted Life Years (DALYs) (Muthayyaet al, 2013) .

Selenium is an essential trace element for humans and livestock. More than 25 selenoproteins are found in the human body. Selenium through these selenoproteins performs diverse biological roles. For example, glutathione peroxidase (GPXs) and thioredoxin reductase (TrxR) families are potent antioxidants defending the body against oxidation, consequently inhibit development of cancer cells mainly by reduction of hydro-peroxides, H_2O_2 and thioredoxin substrate (Brown and Arthur, 2001). In addition, it is an integral part of iodothyroninedeiodinase (IDI) enzymes important for normal function of the thyroid gland and effective iodine utilization in the body (Arthur et al, 1992).

Selenium inadequacy is highly prevalent in Africa. It is estimated that almost half of the population have low Se intake (Melse-Boonstraet al, 2007).For example, Ivory Coast (Tiahou et al,2004;Arnauldetal,2001),Central Africa and Democratic Republic of Congo (Vanderpas et al, 2001),and Malawi (Hurst et al, 2013) are among the countries in Africa with high prevalence of Se deficiency. Available studies in Ethiopia also suggestedthe public health importance of Se inadequacy. It is reported that more than 50% of populations in the Amhara region, North Ethiopia have serum Se lower than the recommended amount (Gashu et al, 2016a; Amare et al, 2012; Kassu et al, 2008). However, the distribution has distinct spatial variation where only few or none of the population from the eastern part of the Amhara region had Se inadequacy while as manyas 90% of the population from the west part of the region had Se inadequacy (Gashu et al, 2018a).

Selenium status of populations is generally dictated by the amount of the element in soil where plant foods are grown and animals are reared.The distribution of Sein the world is highly variable. Selenium content in soil is between 0.01 and 2.0 mg/kg-1 (Neal, 1995; May land, 1994; Fordyce, 2007). Soils with concentrations below 0.1 mg Se/kgare described as Se deficient (Dillon and Dillon, 2003).Soil Se deficiency has been reported in a number of areas across the world, such as China, North America, New Zealand, Australia, Sweden and Finland (Gisele-Nielsen et al, 1984; Gupta and Gupta, 2000), and Malawi(Chilimba et al,2011).On the other hand, soil in some regions may contain adequate or high Se concentration but with limited availability for plant uptake (Fordyce et al, 2000).This is because; plant uptake of Se is influenced by several soil physico-chemical properties such aspH, organic matter content, mineral compositions and soil particle distribution (% of clay, silt and loam) (Stroud et al, 2010; Mikkelsen et al, 1989). The present study aims to determine soil physico-chemical charactertics influencing extractable Se concentration and Se concentration in staple crops from two areas of the Amhara region(West Gojjam and North and South Wollo), Northern Ethiopia with contrasting human serum Se status.

1.2. Statement of the problem

Selenium is an integral part of several proteins with crucial role in the human body. In general, it is important for protection of the body against free radicals, macromolecule synthesis such as thyroid hormones, development of the immune and muscle function, and male fertility(Rayman, 2000). Selenium deficiency however is widespread in the world. Globally, it is estimated that about one billion people have Se inadequacy (Haug et al, 2007). In general, soil Se concentration determines human Se nutrition status,especially in populations dependent on local food sources. Soil Se concentration is highly variable such that deficiency and toxicity can occur in short distances. Studies indicated that Se inadequacy is highly prevalent in the Amhara region of Ethiopia (Gashu et al, 2016a).The deficiency was implicated to affect cognitive function (Gashu et al, 2016b) and normal thyroid metabolism of children despite of iodine adequacy (Gashu et al, 2018b). However, the distribution has distinct geographical pattern that none of the children from the Eastern part of the region had Se inadequacy while their counterparts from west Amhara had low serum Se concentration (Gashu et al, 2018a). In that study, even though there was small but significant difference in dietary diversity in pattern similar to the serum Se concentration, the authors concluded that consumption of Se rich foods such as animal source foods by both groups (east vswest Amhara) was very low. Therefore, the variability could be attributed to dietary Se concentration (mainly staple crops) which in turn depends on soil Se concentration.

Selenium concentration in agricultural crops or animal foods depends on soil Se concentration. However, portion of Sereadily available for plant uptake (extractable Se)is the main determinant for crop Se concentration thus for human nutrition. However, this portion of soil Se is influenced by soil characteristics including pH, organic matter content, soil texture, concentration of competing minerals such as iron, aluminum, calcium, sulfur and phosphorous. Therefore, this study was designed to investigate Se concentration in staple crops and soil physic-chemical characteristics from two areas(west Gojjam and north and south Wollo) of the Amhara region with contrasting serum Se concentration. In addition, Se in staple crops exactly from the same field was analyzed to understand phyto-mobility of the element.

1.3. Objectives

1.3.1 General objective

- To determine soilphysico-chemical characteristics and level of Sein staple crops from two areas of contrastinghuman Sestatus in the Amhara region, Ethiopia.

1.3.2 Specific objectives

To determine soil physico-chemical characteristics from two areas of the Amhara region (west Gojjam and south and north Wollo), Ethiopia with contrasting human Se status.

- To determine level of Sein staple crops from two areas (west Gojjam and south and north Wollo) of contrasting human Se status in the Amhara region, Ethiopia.
- To identify soil physico-chemical characteristics affecting concentration of extractablesoil Sein the Amhara region, Ethiopia.

3. Literature review

3.1. Chemistry of Se

Selenium is the 34thelementin theperiodic table(period = 6, group = 4 and atomic mass = 32.066). It is classified as a metalloid, exhibiting intermediate properties between metals and non-metals. The element exists in organic forms associated with amino acids such as selenocysteine and selenomethionine. Naturally Seoccurs in different oxidation states varying between plus six and minus two; selenate (Se^{+6}), selenite (Se^{+4}), elemental Se and selenide (Se^{-2}) (Hartikainen, 2005). Compared to inorganic Se, organic Sehas greater bioavailability (Xia et al, 2005).

3.2. Biological role of Se

Selenium in the form of selenocysteine is a key component of the active site ofdiverse selenoproteins of crucial biological functions.In humans,25 selenoproteins have been discovered including GPXs, TrxR, IDIs, selenoprotein P, selenoprotein W,and seleno-phosphate synthetase. In general, Se as integral part of these seleno-proteins protects the body from damage by free radicals; catalyze activation and deactivation of thyroid hormones, and strengthening the immune system, and DNA repair. Thus it is implicated in protection against development of chronic diseases (cancer and diabetics)and immune response against viral diseases such as Acquired Immunity Deficiency Syndrome (AIDS). Selenium is also implicated inmusclefunction thus improve endurance, recovery and slowing the ageing process (Brown and Arthur, 2001).

Several researches reported a significant relationship between Se nutrition and protection against cancer mainly attributed to the antioxidant property of the element. In addition, it is suggested that Se may also play a role in alternation of cancer causing genes (Hu and Diamond, 2003). For example, in a study by Rejali et al, (2007) among additional risk factors for breast cancer such as usage of oral contraceptive pills, low serum Se level was associated with breast cancer.In addition, aninverse relationship between dietary Se concentration and the incidence of human breast cancer was reported (Lopez-Saeze et al, 2003).**S**elenoproteins are alsoimportant for initiation and regulation of the immune system by influencing leukocyte activity including adherence, migration, phagocytosis, and cytokine secretion. In addition, the element is important in regulation of cell signaling (Rundlöf and Arnér, 2004). It was reported that Se is important in slowing down the progression of HIV infection into AIDS (Rayman et al, 2000).

3.3. Epidemiology of Senutrition

Selenium status varies widely in different parts of theworld mainly due to concentration of Se in the soil where food crops are grown and animals are reared. Selenium was known for its toxicity until recently when its role in thyroid metabolism was discovered, hence, only limited studies documenting Se status of population are available. In addition, the element is found at very low concentration thus requires sophisticated instruments with very low detection limit which could be another plausible justification for availability of small number of studies determining Se nutrition status of populations in the world. However, it is estimated that about one billion people are Se deficient. Parts of China(Tan et al, 2002), New Zealand(Thomson,2004), United Kingdom (Broadly et al, 2006), the Russia Federation, Serbia, and Belarus (Ermakov and Jovanović, 2010), Malawi (Chilimba et al, 2011), North west Ethiopia (Gashu et al, 2016a; Kassu et al, 2008; Amare et al, 2012), and Democratic Republic of Congo (Vanderpas et al, 2001) are notably Se deficient. On the other hand, soils in some areas of China (Tan et al, 2002; Qin et al, 2013) and the USA (Banuelos et al, 2002) are seleniferous (Banuelos et al, 2002).

In New Zealand, Se deficiency was associated with white muscle disease in lambs,calves and goats, and infertility of ewes and ill-thrift of sheep (Gupta and Gupta, 2000). In addition, the deficiency was also attributed to growth retardation and a decrease in milk production of cattle's(Enjalbert et al, 2006).Moreover, due to the innate low concentration of Se in soils, Se deficiency was recognized as a public health problem in some European countries. Finland was one of the countries known for Se deficiency howeverbased on the current evidence, the national utilization of Se blended fertilizers increased Se concentration in human foods thus significantly increased human health (Alfthan et al, 2015). UK soils also contain low Se concentration, there is evidence that Se deficiency become a public health problem after importation of bread-makingwheat from the US was seized (Lyons et al,2003).

In North western part of Ethiopia, more than half of the population are Se deficient (Gashu et al, 2016a). The deficiency was also associated to less cognitive performance (Gashu et al, 2016b) and impaired thyroid metabolism in children despite ofadequate iodine nutrition (Gashu et al, 2018b). Similarly, in Gonder town (Northern Ethiopia),Amare et al, (2012) and Kassu et al, (2008) reported public health significance of Se deficiency in the area(62% of school children and 22% of pregnant women). In addition, national food consumption patterns studies and soil and staple crop surveys in Malawi revealed suboptimal intake of dietary Se (Chilimba et al, 2011). This suboptimal intake was associated to lower concentration of available Se in the soil (Hurst et al, 2013). Similarly, biomarker data showed that more than half of the Malawian population is Se deficient. On the other hand, there is less Se deficiency in populations residing by Lake Malawi mainly attributable to consumption of fish (personal communication FlixPeri). This study confirms available findings that dietsfrom aquatic sources are good sources of Se. This is because, Se on the surface of the soil are subjected to erosion and is washed away to water bodies (Lyons et al, 2007).

The human body can tolerate quite high levels of Se without adverse effects on health. However, at high doses (>900 µg/day), Se can elicit toxic effects called selenosis, with symptoms including gastrointestinal upset, hair loss, nausea, irritability, fatigue and mild nerve damage (MacFarquhar et al, 2010). While high intake levels of Se are usually difficult to ingest from food sources alone, selenosis has been observed in Enshi District, Hubei Provinces of China where high local soil Se levels were associated with excessive dietary Se intake (Huang et al, 2013).

Selenium deficiency in humans becomes evident at intakes below 30µg/d. Keshan disease characterized by muscle and bone deformity, even though overlapped with the symptoms of the disease due to Coxa virus infection, was documented in Se deficient regions of China where the soil has the lowest Se content in the world. The incidence of the disease has been reduced markedly since the implementation of Se supplementation to populations in the affected areas.(Moreno-Reyes et al,2001). Similarly, in Ivory Coast and D. R. Congo formerly Zaire, Se coupled with iodine deficiency was reported to causing cretinism in children (mental and physical growth retardation) (Tiahou et al, 2004; Arnauld et al, 2001; Vanderpas et al, 2001).

3.4. Common strategies to address Se nutrition

Although several researches indicate the significance of Se for normal human physiological activity or the deficiency causes grave consequences, strategic interventions to overcome this deficiency compared to other minerals are still limited. Similar to ways of addressing inadequacies of other micronutrients; food diversification, fortification, and supplementation strategies are employed to improve Se nutrition. However, fortification is the common one.Basically, there are two ways of fortification strategies to increase Se intake by humans. The first strategy entails the direct increase of Se intake by livestock either by direct supplementation or throughtheir feed.The second strategy involve agronomic activities such as breeding of Se accumulating plants or addition of Se to commercial fertilizers to increase Se concentration in staple crops in areas of Se deficiency (Ros et al, 2016).

3.4.1.Supplementation

Selenium supplements (in the form of capsules, powder or syrup) are one of the strategies to increase Se intake in humans. It is considered to supply an optimal amount of specific micronutrients in their absorbable form and helps in fixing micronutrient deficiencies in short term. Selenium supplements are found in different chemical forms, selenomethione being the dominant one. In addition, sodium selenite and sodium selenate are also common. They are mostly provided with proteins and amino-acids and 200 µg/d is considered safe and sufficient for an adult person (70 kg) (Schrauzer, 2001).

Several reports indicated that Se supplements are effective in boosting body Se concentration and the respective activity of Sedependent proteins. A study in Se deficient areas of China reported that a daily Se supplementation of66 µg /d for 20 weeks had significantly increased plasma Se concentration in the intervention groups but not in the control subjects. In addition, the studyfurther reported that an optimal activity of GPX enzyme was achieved at 37 µg Se/d as selenomethionine and 66 µg/d as selenite suggesting the influence of the chemical nature of the supplement on absorption. However, the optimal activity of selenoprotein P was not achieved at this concentration of either forms of Se (Xia et al, 2005). This is because, selenoprotein P requires relatively higher concentration of Se compared to the dose sufficient to boost GPX activity. In addition to increasing blood Se status and activity of Se dependent enzymes, Se supplements are indicated to prevent incidence of chronic disease. For example, a review of long term Se supplementation trial indicated the preventive role of Se supplementation on the incidence of risk of prostate cancer (Gromadzińska et al, 2008).

However, researches have pointed out that the use of supplements is uncertain in terms of efficiency due to the Se forms added in commercial supplements (Pietinen et al, 2010). In addition, Aldosary et al, (2012) and Morris and Crane (2013) reported several cases of Se intoxication in people due to the intake of these supplements. Supplements and industrially fortified foods mostly contain inorganic Se forms (selenate and/or selenite). It is well known that the Se speciation significantly affects the potential benefits of this element, being the organic Se forms (selenocysteine and/or selenimethionine) the most effective bioavailable (Thomson, 2004; Fairweather-Tait et al, 2010). Studies have reported that inorganic Se forms have lower bioavailability in the human organism and also present higher risk of toxicity by excessive intake(Finley, 2006; Thomson, 2004)

3.4.2. Selenium biofortification of staple crops

Bio fortification is defined as a process to increase the nutritional values of crops in terms of essential nutrients for human nutrition, such as vitamins, iron (Fe), zinc (Zn), iodine (I), and (Se) (Garcia-Casal et al, 2017). This may involveconventional and molecular plant breeding approaches to choose cultivars or species that are efficient to accumulate Se. In addition, agronomic biofortification refers to the use of Se-containing fertilizers (applied to soil and/or by foliar spray) to increase contents of Se in staple plant foods. Seleniumbio fortificationcan increase the amount of Se in the diet and prevent the risks of excessive Se intake which mineral supplements can induce (Bouis, 2003). In addition to addressing Se needs to the body of consumers, Se plays important roles in plants. For example, in fruits, Se may modulate the ripening process, probably through its antioxidant and anti-senescence properties with beneficial effects in terms of post-harvest commercial life. (Pezzarossa et al, 2012).

A good example of using fertilizers containing Se to increase the Se intake in foods has been currently observed in Finland. The low available Se contents in the Finnish soils result in an insufficient amount of Se intake by the humans. Hence, the country mandated addition of Se to commercial fertilizers during the early 1980's, since then the occurrence of Se deficiency and associated health problems in the population has improved greatly (Alfthan et al, 2015).

The ability of plants to accumulate Se (without causing toxicity) varies among species and even among cultivars of the same species. Most agricultural plants are classified as Se non-accumulators because they do not exhibit more than 100 μg g-1 of Se in their dry mass when grown in seleniferous soils (Terry et al, 2000). On the other hand, a selected group of plants (e.g., species of the genera Astragalus, Xylorrhiza, Stanleyea, Allium, and Brassica) may contain thousands of micrograms of Se by grams of dry mass when grown in Se-enriched soils, being these crops classified in Se accumulators and hyper accumulators (Terry et al, 2000; Pilon-Smits and Quinn, 2010; Fairweather-Tait et al, 2011).

Breeding or genetic manipulation of crops with enhanced Se uptake has been proposed as an effective way of increasing human Se intake through plants (Lyons et al, 2003; Broadley et al, 2006). Wheat plants have been reported to accumulate Se to levels safe for human and animal consumption at concentrations lower than 3 μg Se/g of soil though a significant reduction in dry matter yield was observed for plants grown on soils with soil Se concentrations greater than 4 μg/g (Rani et al, 2005).

Micronutrient sprinkles that contain a variety of vitamins and minerals are added to ready to eat staple diets during home fortification which is a novel approach to increasing micronutrient intake and most micronutrient powders also contain Se. However, flour fortification of Se is not common.

3.4.3. Dietary diversification

Micronutrient deficiency is a global problem. However, populations in low income countries are the most affected. This is because the diets of populations in poor resource settings characterized by monotonous type of diet comprised mainly of starchy staples. In addition, intake of animal; source foods which contain relatively high concentration of minerals in their most absorbable form is limited putting these population at special risk of hidden hunger (Gibson and Hotz, 2001). Food diversification is an effective and sustainable approach to increasing micronutrient intake by improving the intake of many foods. These include adoption and development of aqua-culture, home gardening, urban agriculture, and poultry and dairy farm. However, this approach requires the implementation of different programs that increase access and availability of micronutrient rich foods. In addition, it may require change in the existing dietary intake pattern of the population which is difficult to attain (Allen et al, 2006).

3.5. Factors affecting Se plant uptake

The worldwide distribution of Se in nature varies widely depending mainly on nature of the soil or the parent material and geographical areas(Duntas, 2010; Zhao et al. 2005). Selenium enters the food chain through plants, which take it up from the soil. Unlike other micronutrients where there absorption andconsequent metabolism is influenced by many variables, level of Se in the soil determine its concentration in foodand influencesSenutrition status of populations particularly thosewith narrow food choice.Absorption of Se in humans is efficient and thus, deficiency mainly occurs in areas where dietary intakes are inadequate due to soil factors(Navarro-Alarcon and Cabrera-Vique, 2008; Tan et al,2002; Brownand Arthur, 2001).

However, plant availability and the subsequent entry of Seinto the food chain is influenced by its oxidation state and several physical and chemical soil properties such as pH, chemical and mineralogical composition, organic matter, soil texture (soil clay, silt and sand fractions), and presence of competing ions(Wang and Chen, 2003; Hopper and Parker, 1999).Thus, knowledge of the total soil Se concentration is not sufficient to evaluate Se availability (Seby et al, 1997).

3.5.1.Soil pH

Oxidation state of Se consequently mobility of the element into plants from the soil depends strongly on the pH of the soil. Selenite (SeO_3^{2-}) is the dominant Se species under lower pH, where it is strongly adsorbed by iron and aluminum oxides and hydroxides, resulting in limited plant availability and a low potential for leaching. Moreover, elemental Se, selenides and selenium sulphide salts tend to exist in reducing, acidic and organic rich environments, which due to their low oxidation potential and solubility, these species are not bioavailable to plants (Fordyce, 2005). Therefore, Se deficiency is mainly expected in crops grown on acidic soils. On the other hand, selenate (SeO_4^{2-}) is the dominant species under high redox soil conditions(alkaline or calcareous soils). Under high pH, selenate is weakly adsorbed by electrostatic forces of attraction thus much more mobile resulting in high plant availability and potential for leaching (Eich-Greatorex et al, 2007).An experiment designed to determine which species of Se (0.5 or 5.0 µm Se as selenate, selenite) are most rapidly taken up by Durum wheat (*Triticum turgidum*) and spring canola (*Brassica napus*) that were grown hydroponically, shows that canola accumulated more Se than wheat. In addition, organic forms of Se were taken up at a greater rate than inorganic forms and selenate than selenite was taken rapidly suggesting the influence of Se speciation, Se forms and plant species on Se up take (Kikkert and Berkelaar, 2013a).

Similarly, a comparison of Se accumulation in durum wheat (*Triticumturgidum* L.) and spring canola (*Brassica napus* L.) sown in potted soil amended with 0, 0.1, 1.0, or 5.0 mg kg^{-1} Se as $SeO_4{}^{2-}$ or $SeO_3{}^{2-}$ showed that $SeO_3{}^{2-}$ exposed plants accumulated the least Se (Kikkert and Berkelaar, 2013b).

3.5.2.Organic Matter

Soil organic matter reduces Se availability by binding and retaining Se in the soil. The influences of organic matter on Se bioavailability are twofold: the binding of Se by soil organic matter and the decomposition of Se containing organic matter, releasing Se either in bioavailable or non-bioavailable form.A reduction in plant Se accumulation with increased organic C in soils was reported by Schmidtet al, (2011). The main mechanism is due to fixation by organometallic complexes. Experimental set-up by Johnson (1991) revealed that an increase in organic matter content decreased the Se concentration in wheat grains from 1350to 160 µg/kg.Another evidence on the antagonistic effect of soil organic matter content on Se availability is the fact that amendment of soils with manures reduces plant Se availability (Park et al, 2011). In addition, organic matters are applied into soil of high Se concentration as toxicity remediation strategy (Dhillon et al, 2010). However, in addition to percent of organic matter content in the soil, total Se concentration and soil pH along with soil organic matter influences Se plant uptake. For example, retention of Se with organic matter content is more pronounced in low pH soil and soils that are naturally low in total Se (Floor et al, 2011). Therefore, due to the strong immobilization soil organic matter content Se deficiency could occur in areas of high organic matter but with adequate total Se concentration (Li et al, 2017).

3.5.3. Soil texture

The percentage of clay, silt and sand fraction in soils is another determinant factor for Se plant mobilization. For example, astudy on the relationship between soil Se and plant Se uptake by Zhao et al, (2005) indicated that presence of silt and clay grains are among factors (in addition to $CaCO_3$ andorganic matter) influencing the concentration of plant available Se. Gissel-Nielsen, (1971) also reported reduction of Se uptake by plants as a function of increasing soil clay content. Similarly, on a pot experiment Johnsson, (1991) showed an increase in the proportion of clay soil caused a decrease in Se absorption by wheat grain. The inverse association of Se plant availability and soil clay content was explained on the bases of two reasons a) Se can’t replace smaller ions in the clay lattice due to its ionic radius (1.4 A^o) and b) It can be easily adsorbed in to negative charges of clays to make a complex (Bar-Yosef and Meek, 1987). In connection, Fordyce et al, (2000) reported selenium deficiency as contributory factor for the prevalence of goiter in Sri Lanka populations living in villages with the highest total soil Seattributed to the inhibitory role of soil clays.

3.5.4. Other competing minerals

Oxides of iron, aluminum, and manganese can bind and strongly fix soil Se, thus, reducing its phytoavailability (Li et al, 2017). In addition, minerals such as sulfur due to similarity in their chemical structure share transporting proteins in plants thus could exhibit competition at absorptive site. For example, a Se uptake study in wheat supplied with selenate and selenite reported that selenate and selenite uptake were enhanced in sulphur-starved and phosphorus-starved plants, respectively (Li et al, 2008). Phosphate has a dual role in Se uptake by plants such that it can render the process by co-precipitation of Seor facilitate plant Se uptake by the desorption of bound Se from mineral complexes in the soil attributed to its high affinity to trivalent ions. Example, Vuoriet al, (1989) reported a negative correlation between Se sorption with level of soil phosphate was negatively correlated. Owing chemical similarity (Zhao et al,2005), Se and Sulfur (S) use common membrane transporters at absorption sites of plant roots and hence, degree of antagonistic interactionwith a competing phosphate ion is another factor influencing Se phytoavailability (Haug et al, 2007; Hopper and Parker, 1999).

3.5.5. Plant Species

Plants vary considerably in their physiological response to availableSe in soil medium Appreciable differences in the uptake and transport of various Se compounds in plants have been reported by Yu and Gu, (2008). In contaminated soils cultivating Se accumulating plants has been suggested for lowering the Se levels (Banuelos, 2001). Brassica species have been deployed in phytoremediation of Se in soils. These were reported to have a potential for reducing up to 40% of total soil Se under greenhouse conditions and up to 20% under field conditions after one growing season (Banuelos et al, 2000). In seleniferous regions, plants belonging to the cruciferae family absorb much greater amounts of Se compared to those belonging to the graminae family (Dhillon and Dhillon, 1997). In addition, as indicated above in this chapter, a comparison of Se accumulation by durum wheat (*Triticum turgidum*) and spring canola (*Brassica napus*) shows that canola accumulated more Se (Kikkert and Berkelaar, 2013a).Selenium accumulation could also vary in food crops but different genotypically (Lyons et al, 2005).

4. Materials and Methods

4.1. Study area

The study was conducted in three administrative zones (West Gojjam, North and South Wollo) of the Amhara region, Ethiopia situated within 9° and 13′45N and 36° and 40′30E. This is because a previous study by Gashu et al, (2016a) reported the presence of Se deficiency as public health significance. The region is characterized by spatial human Se variation such that few or none of the people from the eastern part of the region has Se inadequacy while majority (up to 90%) of the people from the western part of the region had Se inadequacy (Gashu et al, 2018a).

4.2. Sample Collection

4.2.1. Crop sample collection

Major staple crops in the area have been identified in consultation with the regional agricultural bureau before sample collection. In addition, a reconnesunse visit to the region was made to estimate the maturity of crops in the field and plan for sample collection time. Samples have been collected approximately every 4-7 km and from farm lands and 0.5 to 2 km from the main road(Russell et al, 2011), (**Figure 1**). In case of crops in standing position, samples were collected following the ‘W’ pattern across the field. On the other hand, for crops already harvested and stacked, five samples were collected from the center (waist)of the stack and homogenized. The center of the sampling farm was geo-referenced for mapping purpose. Crop samples were trashed manually to avoid contamination. The samples were then kept in a clean paper bag and transported to Center for Food Science and Nutrition, Addis Ababa University for preparation.

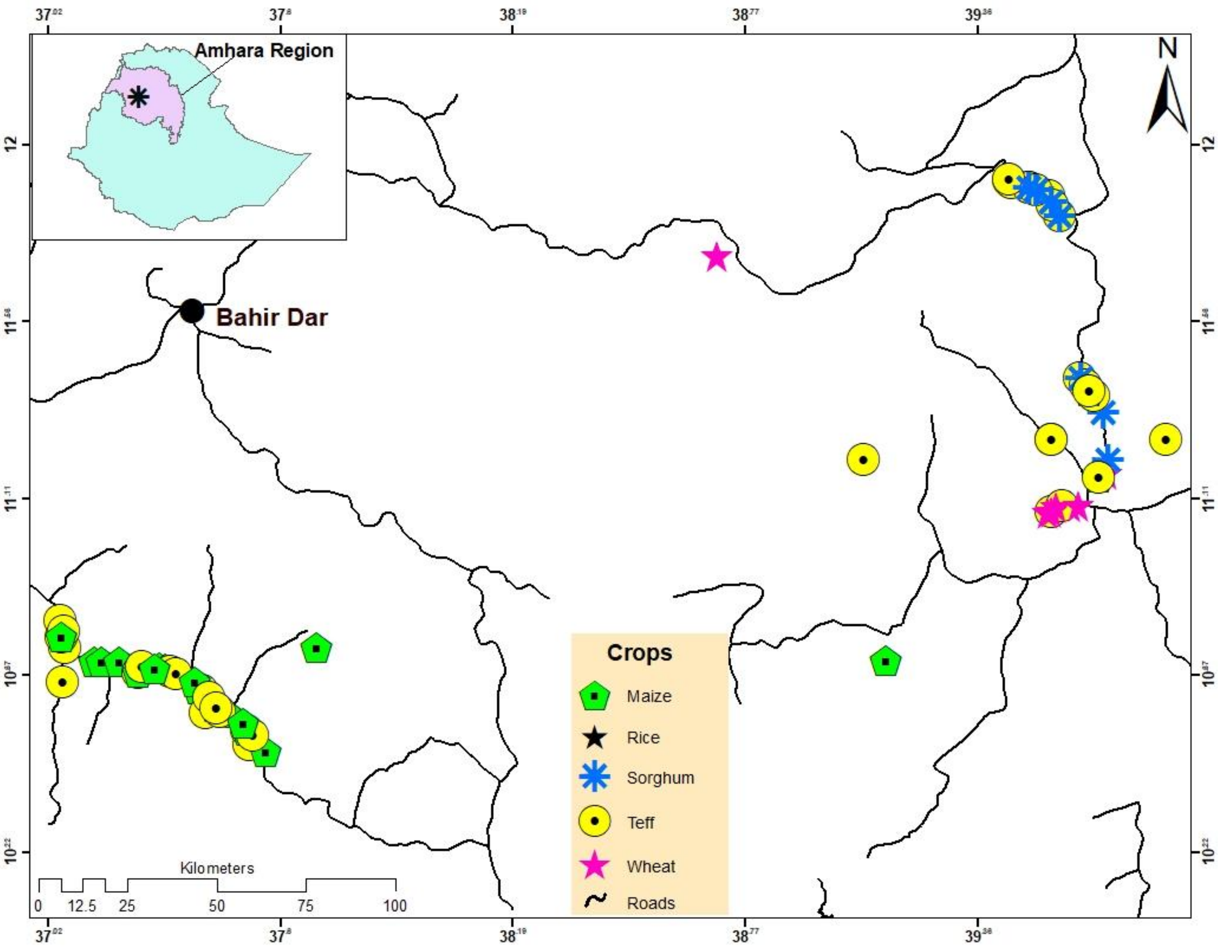

Figure 1. Crop collection sites (Developed using Arc GIS)

4.2.2. Soil sample collection

Agricultural soil samples of 20 cm depth were collected by exactly from the same farm at the sampling spot as the crop samples by using a metallic augers following 'W' pattern

Boone et al,(1999) (**Figure 2**). The soil samples then was homogenized and kept in paper bags that allow one way air movement to dry the moisture until ready for analysis and transported to Addis Ababa University, Center for Food science and Nutrition.

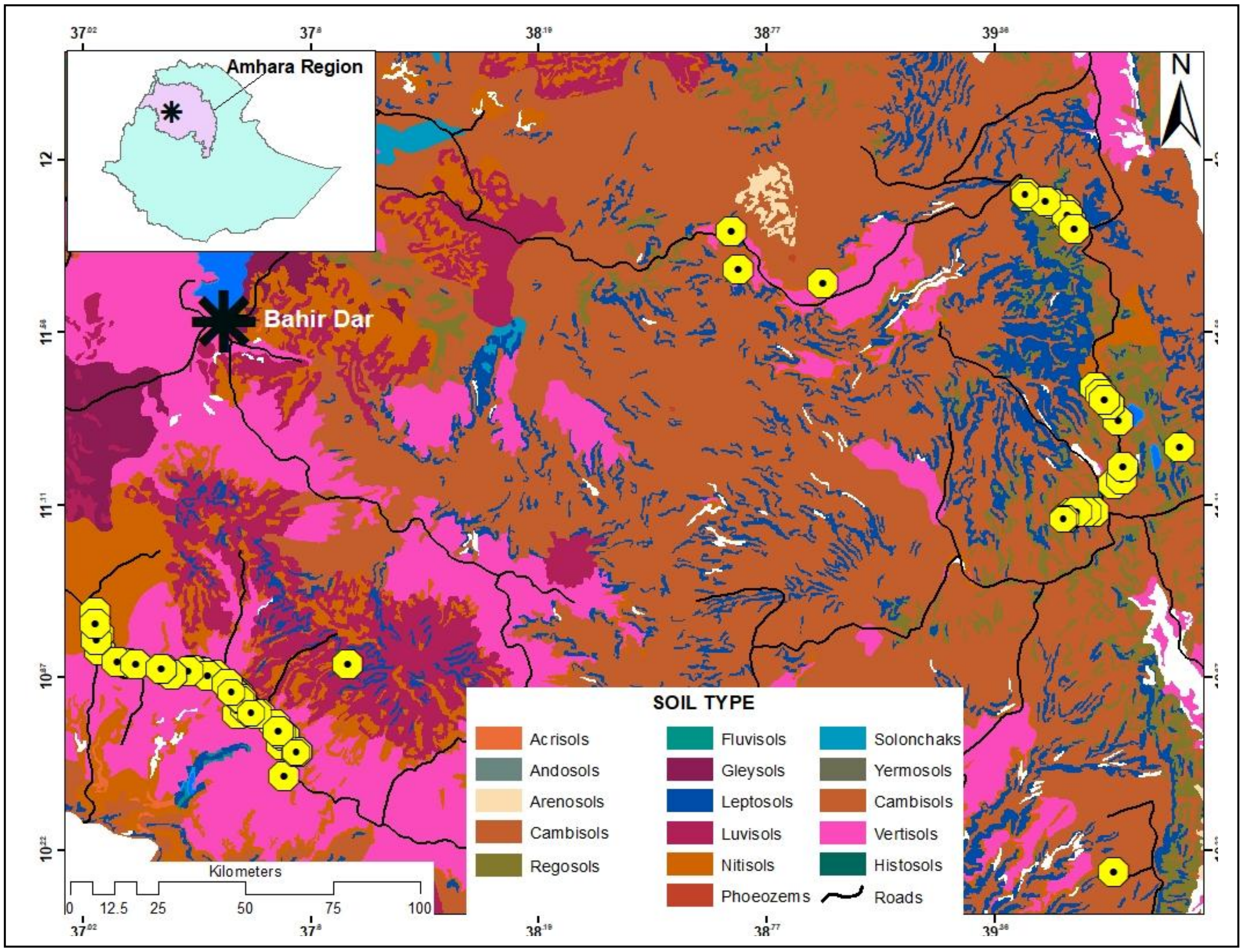

Figure 2. Soil sampling sites at the background of soil profile(developed by Arc GIS)

4.3. Sample preparation

4.3.1. Crop sample preparation

Air dried grain samples were milled in a stainless steel electronic miller (no significant difference in Se concentration of samples prepared in mortal and pestle) following the removal of foreign materials and husks. About 0.5g of flour samples was digested in a closed system (high performance microwave digestion system-ETHOS) with the addition of 6 ml of trace metal grade 65% HNO_3 (Fisher scientific) and 2ml of H_20_2 (Fisher Scientific) according to the manufacturer's recommendation under high pressure and temperature at Ethiopian Leather Development Institute. The digest was transferred in to clean tubes following rising with Milli Q water.

4.3.2. Soil sample preparation

Plant fragments, stones and other foreign materials were handpicked and soil samples have been dried in an electric oven at 45 min overnight. The dried soil samples were disaggregated in a mortar and pestle to pass through a 2 mm nylon sieve mesh (Zhao, 2005). The prepared soil samples were used for the analysis of moisture, pH, total organic matter, soil texture, and multi-elemental analysis including total and available Se (Cabrera et al, 1999).

For total Se analyses, about 0.5 g (dry weight) of soil sample was fully digested in 70% HF, 35%HCl and 65% HNO_3 using a closed vessel high performance microwave digestion system (ETHOS) following the manufacturer recommendation at the Ethiopian Leather Development Institute (Addis Ababa). Digested samples were diluted to 50 ml using Milli Q water and transferred in to clean and sterilized sample bottles until analysis.

For extractable soil Se, an aliquot of 0.1 M KH_2PO_4 of pH=4.8 was added to soil samples (10g ca) and shaken for 1 hr on an electronic shaker. The solution was filtered in to clean 50 ml plastic tubes and marked up with distilled deionized water (Zhao, 2005).

4.4. Crop and soil samples analysis

4.4.1. Crop and soil multi-elemental analysis

Naturally, agricultural soils and crop samples contain very low concentration of Se which requires sophisticated instruments with low detection limit such as ICP-MS for quantification. For this purpose, both digested and extracted sample solutions of the present study were sent to University of Nottingham, UK for elemental analysis. The concentration of Se in the digest was quantified by ICP-MS using gallium as internal standard (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). Samples were introduced from an auto-sampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L-1), Rh (10 µg L-1) and Ir (5 µg L-1) in 2% trace analysis grade (Fisher Scientific, UK) HNO3. External multi-element calibration standards (Claritas-PPT grade

CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) includingAg, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, U, V and Zn, in the range 0 – 100 µg L-1 (0, 20, 40, 100 µg L-1) were used. A bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, France) was used to create Ca, Mg, Na and K standards in the range 0-30 mg L-1. In addition, KH2PO4, K2SO4 and H3BO3solutionswere used to calibrate the machine during determination of P, BO and S. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required. However, the present study is limited to presenting results of only selected minerals of the studied samples.

4.4.2. Soil moisture

The moisture content of soils was determined by using the method of International Center for Agricultural Research in the dry areas. About 10 g of soil (< 2-mm) was dried in an oven at 105 °C overnight. The moisture content was calculated as:

$$soil\ moisture = \frac{wet\ \mathrm{soil(g)} - \mathrm{dry\ soil(g)}}{dry\ soil(g)}$$

$$dry\ soil(g) = \frac{1}{1 + \frac{\theta}{100}} \times wet\ soil$$

$$moisture\ factor = \frac{wet\ \mathrm{soil(g)}}{dry\ soil(g)}\ or\ \frac{100 + \%\theta}{100}$$

4.4.3.Soil texture

Well mixed suspensionof soil samples were prepared in the hydrometer jar with paddle, thenthe paddlewas withdrawnwith a great careleaving the suspension undistributed.After 4 hours the hydrometerwasinserted and the reading was taken. Percentage of each soil fractions was determined according to the following formula:

Percentage clay in soil:

$$\%\,Clay(w/w) = (R_c - R_b) \times \frac{100}{oven\ dry\ soil\ (g/g)}$$

Percentage silt in soil:

$$\%\,Silt\ (w/w) = [\%\,silt + clay\ (w/w)] - [\%\,clay\ (w/w)]$$

Percentage sand in soil

$$\%\,Sand\ (w/w) = sand\ weight \times \frac{100}{Oven\ dry\ soil\ (g)}$$

After taking the readings required for clay and silt, the suspension waspouredthrough a 50-µm sieve. The sieve was washed with water and the sand was transferred in to a 50 ml beaker of known weight. The sand was allowed to settle in the beaker, and excess water was decanted.The beaker with the sand was dried overnight at 105^{0}C. After cooling the sand containing beaker in a desiccator, the content (sand+ beaker) was weighed .Soil texture was analyzed by using Bouyoucos hydrometer method. Once the percentage of sand, silt, and clay was determined, the soil has been assigned a textural class using the USDA textural triangle (**Figure 3**).

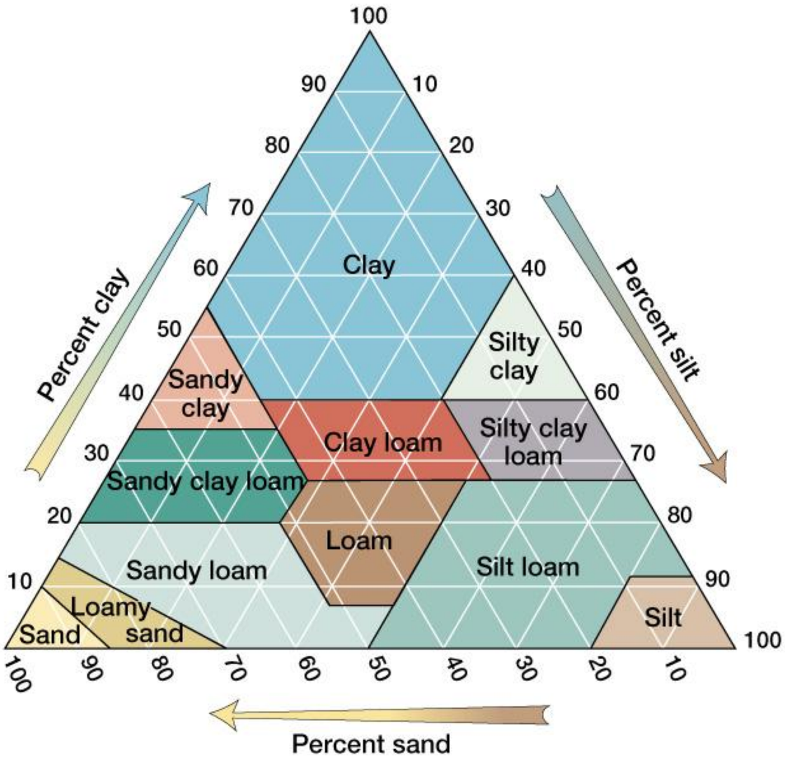

Figure 3. Textural triangle for determination of soil textural class

4.4.4. Total organic matter

Organic carbon of the soil was estimated by Walkley and Black's Method (Walkley and Black , 1934). About 1 g of oven-dried soil was weighed into a 500ml beaker and 10 mlof1Npotassium dichromate solution was added using a pipette. In addition, about 20 mlofconcentrated H_2SO_4 was added using a dispenser, mixed and left to stand for 30 minutes. After adding 200 mldeionized water, 10 mlof concentrated H_3PO_4 was added using a dispenser then the mixture was allowed to cool. After adding 10 to 15 drops of diphenylamine indicator, a Teflon-coated magnetic stirring bar was used to stir the content. Finally, the sample was titrated with 0.5 Mferrous ammonium sulfate solution until the color changes from violet-blue to green. Two blanks containing all reagents but no soils were prepared and treated in exactly the same way as the soil suspensions.The following equations were used to calculate soil organic content.

$$\mathrm{M} = \frac{10}{\mathrm{Vblank}}$$

$$\text{oxidizable organic carbon } (\%) = \frac{[\mathrm{Vblank} - \mathrm{Vsample}] \times 0.3 \times \mathrm{M}}{\mathrm{Wt}}$$

$$\text{total organic carbon } (\%) = 1.334 \times \text{oxidizable organic carbon } (\%)$$

$$\text{organic matter } (\%) = 1.724 \times \text{total organic carbon } (\%)$$

Where:

M = Molarity of $(NH_4)_2SO4.FeSO_4.6H_2O$ solution (about 0.5 M)

V blank = Volume of (NH_4) $2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (ml)

Vsample = Volume of (NH_4) $2SO_4.FeSO_4.6H_2O$ solution required to titrate the sample (ml)

Wt = Weight of air-dry soil (g)

4.4.5. Soil pH

The pH of soil has been estimated by using glass electrode pH meter.pH was determined with a calibrated pH meter on suspension of soil in deionized water (1:5, w/v, 10g: 50ml) (Chilimbaet al, 2003).The reading has been taken after 30 seconds. After every samples analysis, the electrode was removed from the suspension and rinsed thoroughly with deionized water in a separate beakerand carefully dried with tissue paper.

4.5. Quality control

Distilled water for soil organic matter analysis or MilliQ water for crop and soil digestion and mineral analysis was used. Blank digest containing all except crop samples or soil samples were subjected for similar treatment and analysis procedures to control for other sources of minerals except the analytical samples of interest. In addition, blank samples were also prepared for soil organic matter analysis. The pH meter was calibrated with standard buffer solutions before use. A marine sediment certified reference material (CRM), PAC-2 for trace minerals (contains certified reference value for Se) from National Research Council Canada was analyzed to check the analytical performance. Moreover, a Certified Reference Material (SRM 1567b –wheat flour) from National Institute of Standards and Technology (NIST), USA was used for quality control purpose of crop selenium analysis. Trace or no vanadium and tellurium minerals are expected in plants thus the presence of any appreciable concentration of these minerals in crops is a sign of contributions from extrinsic sources or contamination. For this purpose, vanadium and tellurium were determined in the crop samples.

4.6. Statistical Analysis

Statistical analysis of data was performed using SPSS for Windows (v18). Descriptive statistics were used to present results. Normal distribution of data was checked with the Kolmogorov–Smirnov test. Student's t-test was used to compare data or One way Analysis of Variance (ANOVA) in case of three or more variables. Comparison of non- normally distributed data was carried out using non-parametric test (Mann-Whitney U test).Pearson correlation was used to study correlation between variables.. A probability level of $p < 0.05$ was considered statistically significant.

5. Results

Staple crops (teff, maize, sorghum, wheat, rice and finger millet) were collected from west Gojjam, North and South Wollo zones of the Amhara region. A summary of Se concentration of crop samples is shown in table 1.The experimental value of the CRM total Se concentration was 0.95±0.03 mg/kg and the recovery percentage was 103% and the precision was 3.1% (verified value=0.92±0.03 mg/kg). In addition, the recovery percentage of Se concentration for wheat flour CRM was 104.5% (verified value=1.1±0.2 mg/kg; analytical value 1.15±0.3mg/kg). Vanadium and tellurium minerals are not important for plant physiology, hence, the presence of any noticeable concentration of these or either of the minerals could be considered as a sign of contamination from extrinsic sources. In the present study, no significant correlation between Se concentration in any of the staple crops with vanadium or tellurium was found (p>0.05).

Table 1: A summary of Se concentration (µg/kg) in staple crops from the Amhara region, Ethiopia (2018)

Crop type	n	Minimum	Maximum	Median [IQR 25, 75]
Teff	38	56.2	2772.0	218.0 [132.6,457.6
Maize	22	13.8	650.7	47.7 [31.4, 60.5]
Wheat	11	36.5	1000.0	124.1 [45.7,274.1]
Sorghum	9	50.3	1496.3	310.0 [88.3,706.3]

Selenium concentration in rice (n=1) and finger millet (n=1) samples were 57.9 µg/kg and 9653.5 µg/kg, respectively. According to the threshold point to define crop Se status by Tan et al. (2002), 6.2% (n=5) of crop samples (all maize) were Se deficient (<25µg/kg). In addition, another 6.2% (n=5) samples (3 maize and 2 wheat samples) had Se concentration in the marginal threshold (25-40µg/kg).Comparison of Se concentration among staple crops (except rice and finger millet) shows that maize has inferior Se concentration (p< 0.001) (**Figure4**). On the other hand, even though, only one sample was available for mineral analysis that do not allow statistical comparison, finger millet has the highest Se concentration (more than tenfold of the Seconcentration in other crops). A comparison of Se concentration in staple crops grown in clay soil but from the same area shows that wheat accumulates significantly higher Se concentration than maize (p<0.001), sorghum (p=0.005) and *teff*(p=0.01).

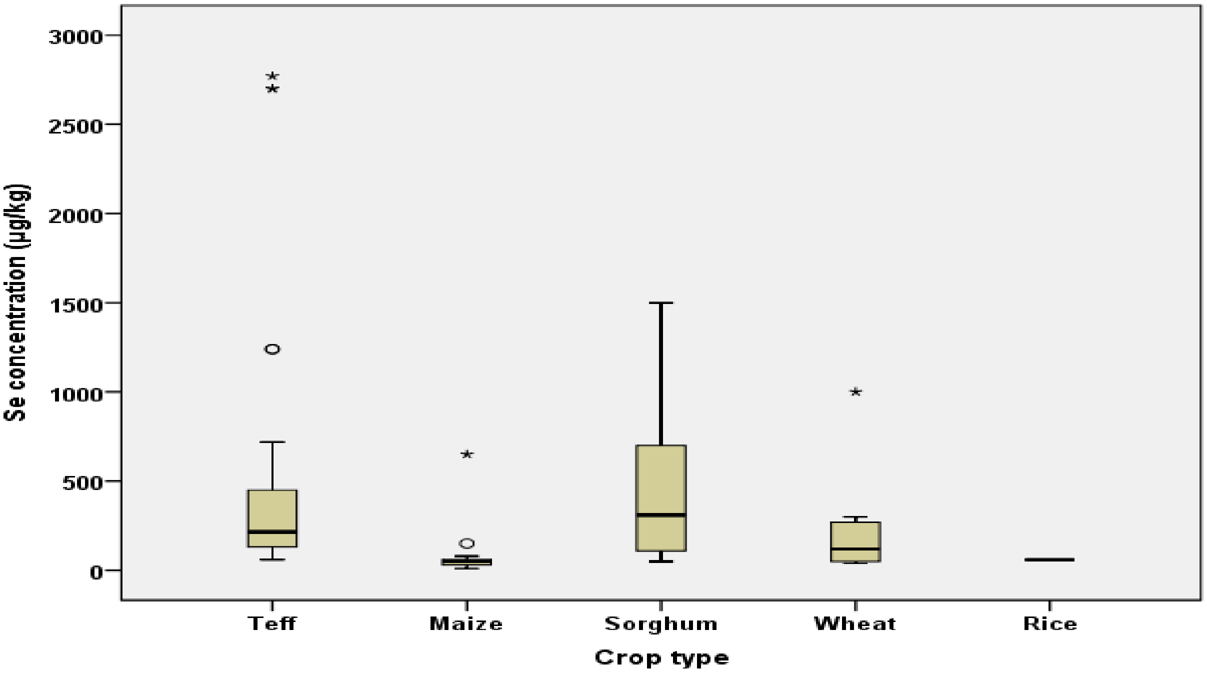

Figure 4: Se concentration in staple crops from the Amhara region, Ethiopia

Selenium concentration in *teff* samples collected from the eastern Amhara region (n=20) (north and south Wollo zones) was significantly higher than Se concentration in *teff* samples from the western Amhara(n=18) (0.43[IQR 0.24, 1.11] vs 0.13 [IQR 0.09,0.17]; p=0.0003) (**Figure 5**). Similarly, Se concentration in maize sample from the eastern Amhara was significantly greater than maize samples from western Amhara (0.27±0.1 vs 0.04±0.01; p=0.002).In general, crop Se concentration has no significant correlation with soil physico-chemical properties such as total soil Se, phosphate extractable Se, soil pH, organic matter concentration, or soil texture. However, Se concentration in *teff* was significantly correlated with soil pH (r=0.45; p<0.01). In addition, there was significant correlations between maize Se concentration with (r=-0.64, p<0.01) and wheat Se concentration with soil calcium(r=0.7; p<0.01).

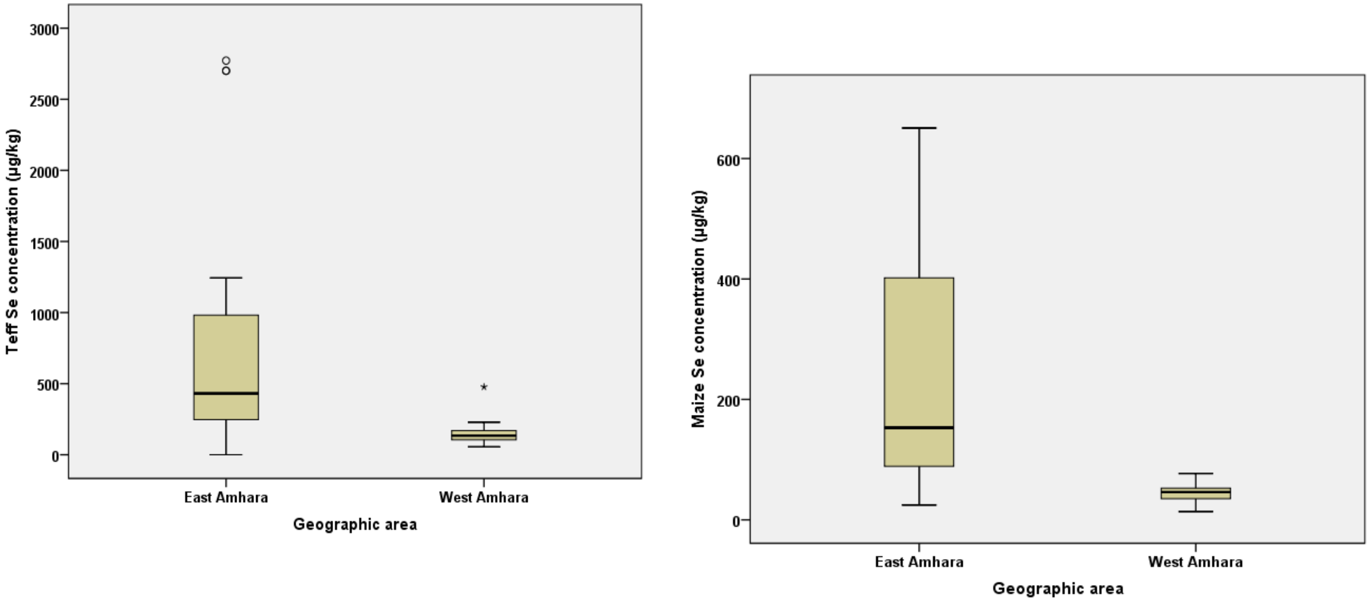

Figure 5: Comparison of Se concentration in staple crops from Ethiopia by geographical region.

Table 2: Summary of physico-chemical charactertics of soil samples (n=82) from the Amhara region, Ethiopia (2018)

Variable	Minimum	Maximum	Mean (SD)	Median [IQR 25,75]
Soil organic matter (%)	0.6	6.6	2.85(1.37)	
soil pH	4.5	8.4	6.57(1.14)	
Clay content (%)	15.0	72.0	43.2 (15.7)	
Silt content (%)	0.0	60.0	33.3 (11.3)	
Sand content (%)	8.0	68.0	24.2(11.4)	
Total Se (mg/kg)	0.03	1.1		0.4 [0.3,0.5]
Available Se (µg/kg)	0.28	18.0		5.0[3.1,6.9]
Zn (mg/kg)	74.3	194.6	127.3(20.4)	
Fe (mg/kg)	42818.2	129061.5		83205.2[75314.1,93253.7]
Al (mg/kg)	1789.53	39972.2		14858.8[11981.9,16495.4]
Ca (mg/kg)	1111.0	58798.7		4235.5[2122.8,22277.3]
K (mg/kg)	1464.8	15729.5		6354.9[4886.9,7715.5]
P (mg/kg)	589.5	5552.14		432.7[132.4,901.8]
Na (mg/kg)	603.2	21933.3		2337.1[1089.8,4242.1]

Clay (55%) or clay-loam (23.8) were the dominant textures in the present study soil samples. In addition, the present study soil samples had a diverse pH value in the range of 4.5-8.4. According to the threshold point to define soil Se status by Tan et al. (2002), 7.3% (n=6) soil samples were Se deficient (<125µg/kg) and 3.7% (n=3) had marginal Se concentration (125-175µg/kg). In addition, 24.4% (n=20) had low phosphate extractable Se (<3µg/kg).Phosphate extractable Se concentration was positively correlated with soil organic matter content ($r=0.47$; $p<0.001$), soil pH($r=0.54$; $p<0.001$), potassium ($r=0.26$; $p<0.05$), and total Se ($r=0.56$; $p<0.01$) (**Figure 6**). However, there was no significant correlation with other soil minerals such as Na, P, Ca, Al, Fe and Zn ($p>0.05$).

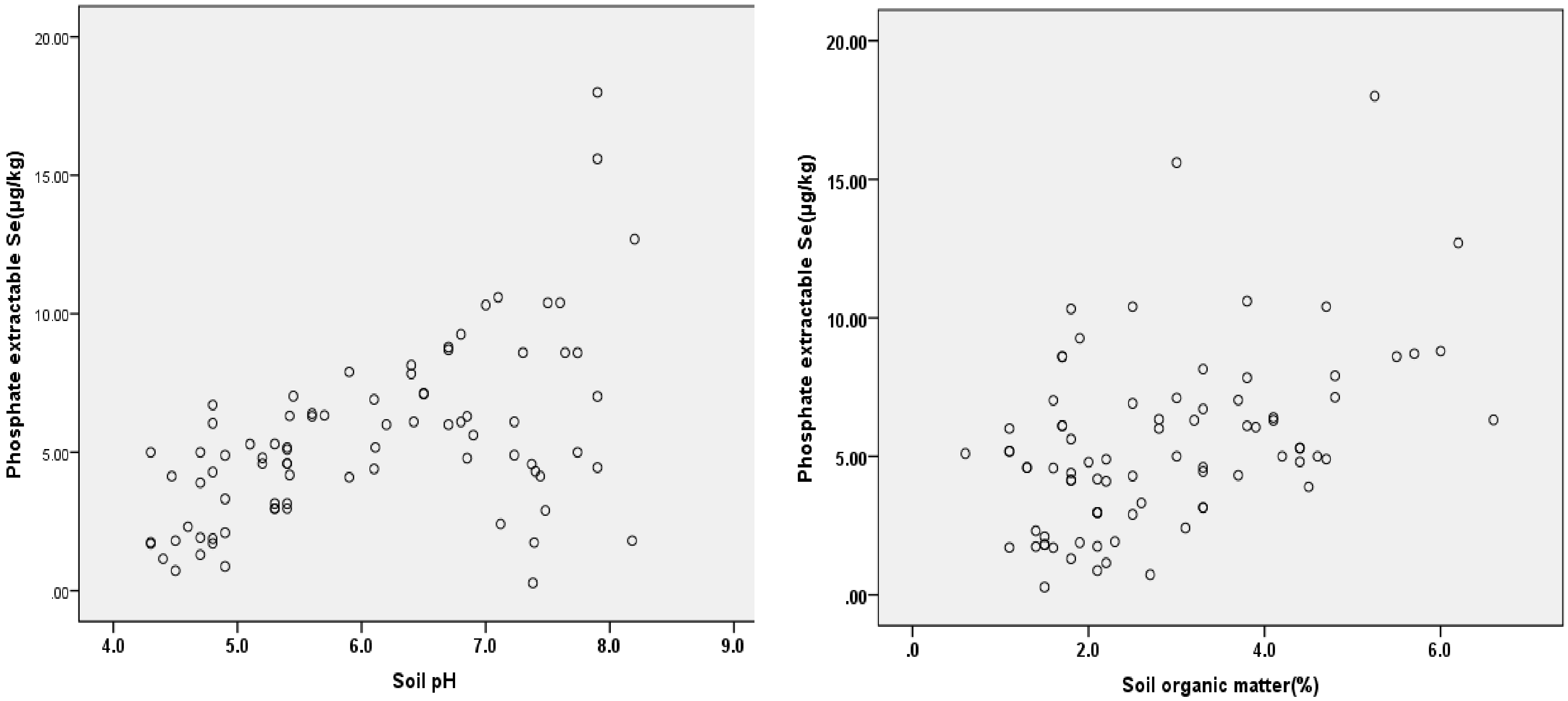

Figure 6: Relationship between Soil Se concentration and soil charactertics

Phosphate extractable and total Se concentrations (**Figure 7**) and organic matter content in soil samples from the west Amhara were significantly higher than soil samples from the eastern Amhara. On the other hand, soil samples from the western Amhara had significantly lower pH than soil samples from the eastern Amhara. Soil samples from East Amhara (n=43) had significantly lower concentration of total Se (0.4±0.1 vs 0.2±0.2 mg/kg; p=0.03) and phosphate extractable Se (4.1±2.2 vs 6.7±3.4µg/kg; p<0.001) than soil samples from the western Amhara (n=39) (**Table3**).

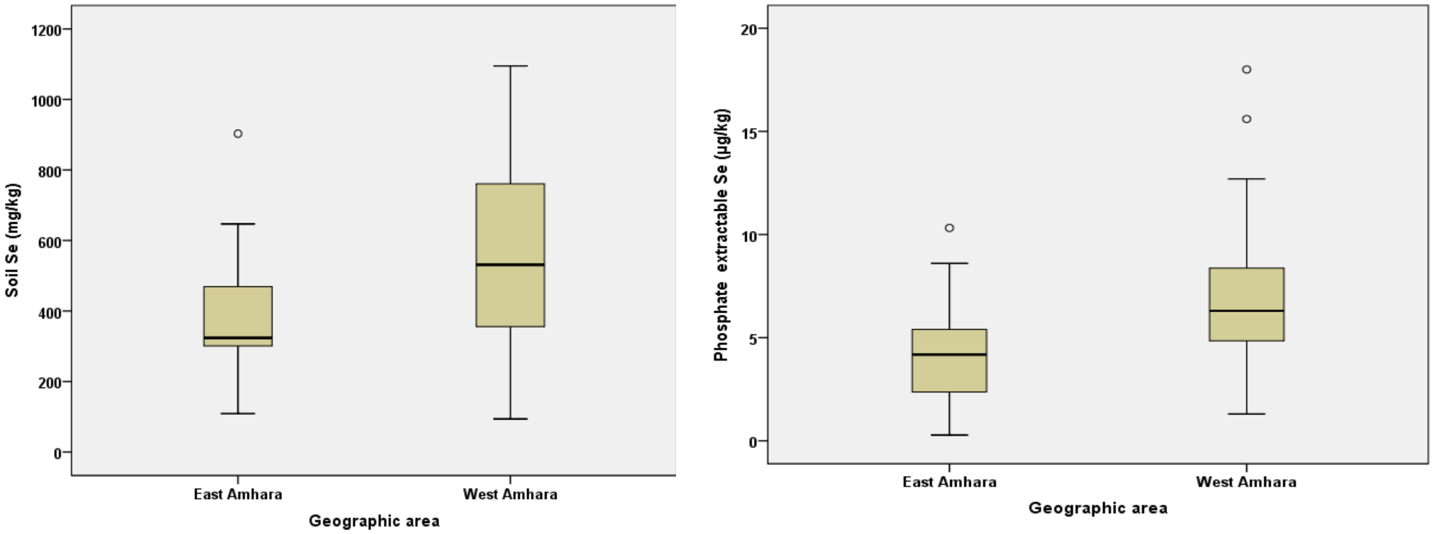

Figure 7: Comparison of soil Se concentration in soil from east and west Amhara, Ethiopia(2018)

Table 3: Comparison of soil chemical character tics in East and West Amhara, Ethiopia (2018)

	East (*n=39*)	West(*n=43*)	P-value
Phosphate extractable Se (µg/kg)	4.1±2.2	6.7±3.4	p<0.001
Soil total Se	0.4±0.2	0.51±0.2	p=0.03
pH	7.4±0.6	5.5±0.7	p<0.001
Organic matter	2.1±0.7	3.6±1.4	p<0.001

There was no significant difference in total Se concentration (mg/kg) (0.43±0.21 for loam; 0.51±0.24 for clay, and 0.37±0.21 for clay-loam; p=0.08) and phosphate extractable Se(µg/kg) (loam 5.2±2.3, clay5.5±3.6, and clay-loam 5.0±2.9) among the different soil classes.

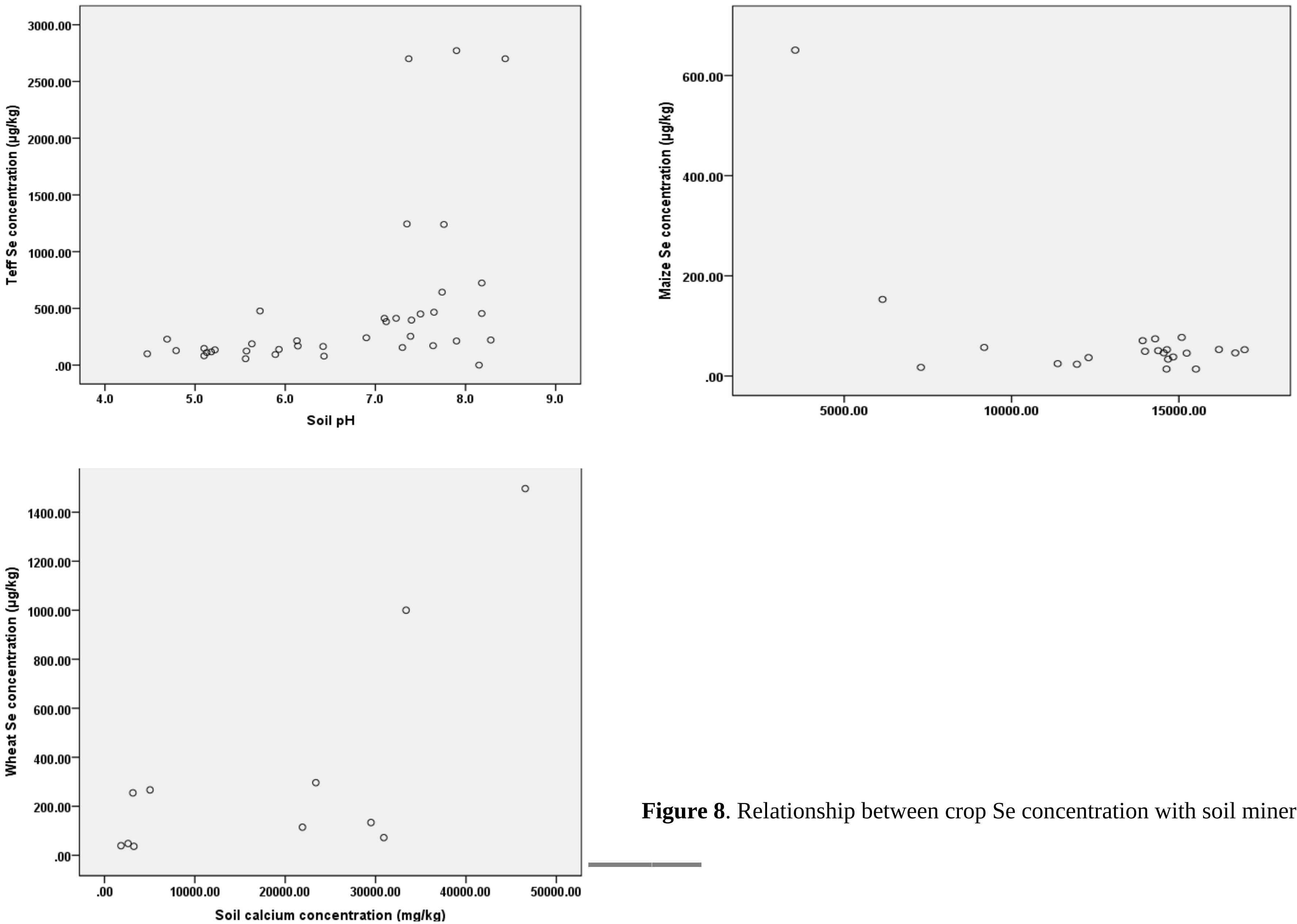

Figure 8. Relationship between crop Se concentration with soil minerals.

6. Discussion

Selenium is an important element for humans and livestock. In humans it is implicated in the protection against free radicals, thyroid hormone metabolism, fertility, and muscle movement. A previous study by Gashu et al. (2016) reported a public health significance of Se deficiency in Amhara region. The deficiency was associated with poor cognitive function (Gashu et al, 2016a) and impaired thyroid metabolism despite of iodine repletion (Gashu et al, 2018b). However, Se nutrition status in humans reveals spatial variation such that more than 90% of the communities residing in the West Amhara had Se inadequacy while people from the East Amhara had little or no Se inadequacy. The present study, determined Se concentration in crops and soil from areas of distinct Se human nutrition in the Amhara region (West Gojjam, North and South Wollo). In addition, soil physic-chemical characteristics that are potentially associated with phytoavailable Se was determined. Selenium concentration in the staple crops varied between 13.8 µg/kg for maize to 9650 µg/kg for finger millet. According to the cut-off point for Se deficiency in crops by Tan et al, (2002), 12.4% of crops had low amount of Se concentration. Similar to the serum Se concentration pattern in Amhara region as reported by Gashu et al, (2018a), *teff* and wheat crops from East Amhara had significantly greater Se concentration compared to samples from West Amhara. However, soil total and phosphate extractable Se concentration in the soil samples from the west Amhara were significantly higher than in samples from East Amhara. Soil pH and organic matter content were positively associated with phosphate extractable Se.

Several strategies including food diversification, food fortification, agronomic biofortification, supplementation and undertaking nutrition related public health measures each with one or more limitations are recommended to control the prevailed micronutrient deficiency in the world. In terms of addressing Se nutrition, eating the minimum acceptable dietary diversity may not be achieved in short term particularly in poor resource settings. In addition, food fortification requires tight monitoring at the production and distribution level as the range of toxicity and deficiency is very narrow. Even though, agronomic biofortification is effective, an additional cost due to micronutrient blend to the tradition or existing chemical fertilizers could discourage farmers. On the other hand, selection of food crops and genotypes with superior ability to accumulate micronutrients is important (Genc et al, 2005). For example, a study by Lyons et al, (2005) shows non-significant difference in grain Se concentration among bread or durum wheat, triticale or barley varieties. However, another wheat variety (diploid wheat, *Aegilopstauschii*) and rye had 42% and 35% higher Se concentration compared to other cereal varieties suggesting the importance of genotype and crop types. In the present study, Seconcentration in the staple crops varied between 13.8 µg/kg for maize to 9650 µg/kg for finger millet. About 12.4% of the staple crops were Se deficient or had marginal Se concentration. However, the majority of Se deficient samples were maize suggesting maize could be crop of less importance to consider for adequate Se nutrition. On the other hand, even though, only one sample was analyzed, finger millet had more than tenfold of Se concentration of other crops (maize, rice, *teff*, sorghum, wheat). In fact, finger millet had superior concentration of other micronutrients such as Ca, Fe, Zn,(data not shown).

The worldwide distribution of Se in nature varies widely (0.005 to 8000parts per million) depending mainly on nature of the soil or the parent material and geographical areas (Duntas, 2010; Zhao et al. 2005). Unlike other micronutrients where there absorption and consequent metabolism is influenced by many variables, level of Se in the soil determine its concentration in food and influences Se nutrition status of populations particularly those with narrow food choice (Navarro-Alarcon and Cabrera-Vique, 2008; Tan et al, 2002; Brown and Arthur, 2001). In the present study, teff and maize grain samples from North and South Wollo (East Amhara) had significantly higher Se concentration compared to teff and maize samples collected from West Gojjam (West Amhara). This result is consistent with the report by Gashu et al, (2018a) based on serum samples that children from Eastern Amhara had higher serum Se concentration (thus few or Se inadequacy) while majority of children from the western Amhara had Se inadequacy. Such contrasting difference in human Se concentration was also reported in Enshi district of China (Tan et al, 2002).

Selenium plant availability and its subsequent entry in to the food chain is influenced by its oxidation state and several physical and chemical soil properties such as pH, chemical and mineralogical composition, organic matter, soil texture (soil clay, silt and sand fractions), and presence of competing ions(Wang and Chen, 2003; Hopper and Parker, 1999). And thus, knowledge of the total soil Se concentration is not sufficient to evaluate Se availability (Seby et al, 1997). Example, Selenite (S^{+4}) can be tightly bound to positively charged soil binding sites and is less bioavailable for plant uptake than the corresponding oxidized form Selenate (S^{+6}) (Eich-Greatorex et al, 2007; Haug et al, 2007). Gissel-Nielsen, (1971) reported reduction of selenium uptake by plants as a function of increasing soil clay content. Similarly, on a pot experiment Johnsson, (1991) showed an increase in the proportion of clay soil caused a decrease in Se absorption by wheat grain. The inverse association of Se plant availability and soil clay content was explained on the bases of two reasons a) Se can't replace smaller ions in the clay lattice due to its ionic radius (1.4 A^o) and b). It can be easily adsorbed in to negative charges of clays to make a complex (Bar-Yosef and Meek, 1987). In connection, Fordyce et al. (2000) reported selenium deficiency as contributory factor for the prevalence of goiter in Sri Lanka populations living in villages with the highest total soil selenium attributed to the inhibitory role of soil clays. In the present study, 55% of the soil samples were characterized by clay particle distribution. However, in general, there was no significant correlation between clay soil and phosphate extractable Se or crop Se concentration. An earlier pot experiment to investigate the influence of soil pH and clay content on Se absorption by ryegrass grown in Se deficient soil by Gissel-Nielsen, (1971) shows that increased in clay content in slightly acidic soil suppressed Se absorption. On the other hand, at pH greater than 7, an increase in clay content, increased Se absorption suggesting the presence of positive and negative interactions between soil pH and clay content on Se absorption.

Soil pH affects Se availability mainly by determining it species (selenite-SeO_3^{2-} and selenate – SeO_4^{2-} are the dominant ones). At a lower pH, Se is found in a reduced form (selenite) while selenate is the dominant species at neutral or high pH. Selenite in the soil is less water soluble because it forms complexes with soil particle thus is less readily available for plant uptake (Chilimba et al. 2011). Similarly in the present study, soil pH was positively correlated with extractable Se. Several studies reported the effect of soil pH on available Se and crop Se concentrations. In a pot experiment of sandy soils at two pH levels (pH 5 and 7) to which 0.5 mg Se/l was added to the soil, at lower pH the uptake of Se was decreased by 72% for wheat and 77% for rape seed (Johnsson, 1991). However, the relationship between soil pH and extractable or crop Se is mediated by soil type and organic matter content. For example, in a pot experiment with a peat soil, a loam soil and a peat/loam soil mixture at a range of pH values between 5 and 7; below pH 6, Se uptake from added Se fertilizer was higher in the soil types with high organic matter content than in the loam. On the other hand, at a soil pH above 6, Se uptake was higher in the loam than in the peat soil (Eich-Greatorex et al. 2007) suggesting the significant influence of soil characteristics other than pH on the concentration of available Se.

Phosphate has a dual role in Se uptake by plants such that it can render the process by co-precipitation of Se or facilitate plant Se uptake by the desorption of bound Se from mineral complexes in the soil. Example, Vuori et al. (1989) reported a negative correlation between Se sorption with level of soil phosphate. Owing chemical similarity (Zhao et al. 2005), Se and Sulfur (S) use common membrane transporters at absorption sites of plant roots and hence, degree of antagonistic interaction with competing sulfur is another factor influencing Se phytoavailability (Haug et al. 2007; Hopper and Parker, 1999). A hydroponic experiment investigated that selenate uptake of wheat grown under sulfur deprived conditions was significantly enhanced (Li et al. 2008).In the present study however, no significant correlation was found between extractable Se and sulfur or phosphorous. This could be that the interaction could be pronounced as a function of dose of the specific elements in the soil and that the two competing minerals may not be found at a critical concentration in the soil to affect extractable Se concentration (Goh and Lim, 2004).

Aluminum and iron oxides adsorbs Se and make complexes inhibiting its availability to plants (Nakamaru and Sekine, 2008). Perhaps, for the same reason in the present study, soil Al was negatively correlated with maize Se concentration. On the other hand, soil calcium could increase available Se concentration by affecting the pH of the soil thus speciation of Se in to the form favoring absorption in to plants. In the present study, soil calcium concentration was positively correlated with Se level in wheat grain.

7. Conclusion

In general, staple crops in the present study had wide range of Se concentration (36.5 µg/kg to 9653.5µg/kg). Maize crop had the least Se concentration while finger millet had the highest or in some cases more than tenfold of Se concentration than other study crops. In addition, 12.4% of crop samples (majority of maize or few wheat samples) had deficient or marginal Se concentration). Similar to Se distribution pattern in humans as reported in Gashu et al. (2018) *teff* and maize samples collected from the East part of Amhara had significantly higher Se concentration than *teff*and maize samples from West Amhara.

The soil samples had variable organic matter content (0.6%-6.6%) and pH value (4.5-8.4). Majority of soil samples had clay (55%) or clay-loam (23.8) texture. About one in ten soil samples had deficient or marginal total Se. In addition, a quarter of samples had low or marginal extractable Se concentration. There was no significant difference in soil Se (total or available) concentration among different soil classes (clay, clay-loam or loam).

Surprisingly, in contrast to crop Se concentration or the Se distribution pattern in human samples, soil samples from West Amhara had significantly high concentration of both total and available Se compared to samples from the East Amhara. Similarly, available Se to total Se concentration ratio was higher in the soil samples from the west Amhara. Yet, soil samples from the east Amhara had significantly higher pH content than samples from the western Amhara. In addition, soil pH was significantly positively correlated with extractable Se, suggesting the potential role of pH influencing crops (*teff*and maize) from East Amhara to accumulate high Se concentration despite the soil had significantly lower Se (both total and the available form) concentration. Se concentration in crops were significantly associated with some soil properties, Se concentration in *teff* with soil pH (r=0.45; p<0.01), maize Se with soil Al concentration (r= -0.64, p<0.01) and wheat Se with soil Ca concentration (r=0.7; p<0.01) suggesting that Se concentration in different crops could be influenced different soil elements differently.

8. Recommendations

Maize contain the least Se concentration and among the studied samples only the maize samples could be labeled as deficient with respect to Se concentration which could expose populations dependent on such staple crops to Se deficiency. On the other hand, finger millet (even though only one sample was collected from same area as the maize samples) is not only a super accumulator of Se and other important minerals (data not shown). However, production and consumption finger millet is not common thus, promotion is important.

Even though, finger millet has the highest mineral concentration (including Se), this study was limited to analysis of one finger millet sample thus a study aiming analysis of more finger millet sample is worth considering.

Even though, soil samples from west Amhara had significantly higher total and available Se concentration; the crop samples on the other hand contain significantly lower Se concentration. Perhaps, soil pH could explain the variation thus soil amendment to adjust the soil pH could be important to increase Se mobility in to crops.

Even though, agronomic bio-fortification or addition of micronutrients to commercial fertilizers is adopted as a successful strategy to improve grain micronutrient concentration consequently human nutrition, in the west Amhara were the soil contain higher Se (both total and available Se) but the crops contain low Se, application of Se-fertilizer blend may not be effective unless the pH is corrected. On the other hand, application strategies avoiding the influence of and interaction with soil characteristics foliar application could be effective. However, this hypothesis warrants further investigation.

9. Limitation of the study

Most or all of wheat and sorghum samples were collected from East Amhara thus comparison was not possible. In addition, the present study was limited to analysis of finger millet thus inferences in the present study regarding the crop's Se concentration should be interpreted cautiously.

Even though both crop and soil samples were collected pair wise from same spot and collection sites were geo-referenced, the sampling frame was not conditioned to cover or represent agricultural farms in the sampling administrative districts.

Dietary studies show that legumes are also the dominant food groups consumed by the population next to cereals in the Amhara region, however, Se determination in legumes was not considered in the present study.

10. **References**

Aldosary, B.M., Sutter, M.E., Schwartz, M. and Morgan, B.W., 2012. Case series of selenium toxicity from a nutritional supplement. *Clinical Toxicology*, *50*(1), pp.57-64.

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen, E.R., Root, T., Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila, P. and Aro, A., 2015. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology*, *31*, pp.142-147.

Allen, L.H., De Benoist, B., Dary, O., Hurrell, R. and World Health Organization. Guidelines on food fortification with micronutrients. In :Lindsay Allen, Bruno de Benolst, Omar Dary and Richard Hurrell (ed). World Health Organization and Food and Agriculture Organization of the United Nation, 2006.

Amare, B., Moges, B., Fantahun, B., Tafess, K., Woldeyohannes, D., Yismaw, G., Ayane, T., Yabutani, T., Mulu, A., Ota, F. and Kassu, A., 2012. Micronutrient levels and nutritional status of school children living in Northwest Ethiopia. *Nutrition journal*, *11*, 108.

Arnaud, J., Malvy, D., Richard, M.J., Faure, H. and Chaventré, A., 2001. Selenium status in an iodine deficient population of the West Ivory coast. *Journal of physiological anthropology and applied human science*, 20, 81-84.

Arthur, J.R., Nicol, F. and Beckett, G.J., 1992. The role of selenium in thyroid hormone metabolism and effects of selenium deficiency on thyroid hormone and iodine metabolism. *Biological Trace Element Research*, 33, 37-42.

Banuelos, G.S., 2001. The green technology of selenium phytoremediation. *biofactors*, 31, 142-147

Banuelos, G.S., Lin, Z.Q., Wu, L. and Terry, N., 2002. Phytoremediation of selenium-contaminated soils and waters: fundamentals and future prospects. *Reviews on Environmental Health*, 17, 291-306.

Bañuelos, G.S., Zambrzuski, S. and Mackey, B., 2000. Phytoextraction of selenium from soils irrigated with selenium-laden effluent. *Plant and Soil*, 224,251-258

Bar-Yosef, B. and Meek, D., 1987. Selenium sorption by kaolinite and montmorillonite. *Soil Science*, 144,11-19.

Boone, R.D., Grigal, D.F., Sollins, P., Ahrens, R.J. and Armstrong, D.E., 1999.Soil sampling, preparation, archiving, and quality control.Standard soil methods for long-term ecological research. *Oxford University Press, New York*, 3-28

Bouis, H.E., 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost. *Proceedings of the Nutrition Society*, 62, 403-411

Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S.E., Hawkesford, M.J., McGrath, S.P., Zhao, F.J., Breward, N. and Harriman, M., 2006. Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society*, 65, 169-181.

Brown, K.M. and Arthur, J.R., 2001. Selenium, selenoproteins and human health: a review. *Public Health Nutrition*, 4, 593-599.

Chilimba, A.D., Young, S.D., Black, C.R., Rogerson, K.B., Ander, E.L., Watts, M.J., Lammel, J. and Broadley, M.R., 2011. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports*, 1, 72.

Dhillon, K.S. and Dhillon, S.K., 1997. Distribution of seleniferous soils in north-west India and associated toxicity problems in the soil-plant-animal-human continuum. *Land Contamination & Reclamation*, *5*, 313-322.Dhillon, K.S. and Dhillon, S.K., 2003. Distribution and management of seleniferous soils. *Advances in Agronomy*, 79,119-184.

Dhillon, K.S., Dhillon, S.K. and Dogra, R., 2010. Selenium accumulation by forage and grain crops and volatilization from seleniferous soils amended with different organic materials. *Chemosphere,* 78,548-556.

Duntas, L.H., 2010. Selenium and the thyroid: a close-knit connection. *The Journal of Clinical Endocrinology & Metabolism,* 95,5180-5188.

Eich-Greatorex, S., Sogn, T.A., Øgaard, A.F. and Aasen, I., 2007. Plant availability of inorganic and organic selenium fertiliser as influenced by soil organic matter content and pH. *Nutrient Cycling in Agroecosystems*, 79,221-231.

Enjalbert, F., Lebreton, P. and Salat, O., 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. *Journal of Animal Physiology and Animal Nutrition,* 90, 459-466.

Ermakov, V. and Jovanović, L., 2010. Selenium deficiency as a consequence of human activity and its correction. *Journal of Geochemical Exploration,* 107,193-199.

Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E. and Hurst, R., 2011. Selenium in human health and disease. *Antioxidants &Redox Signaling*, 14, 1337-1383.

Finley, J.W., 2006. Bioavailability of selenium from foods. *Nutrition Reviews*, 64, 146-151.

Floor, G.H., Calabrese, S., Román-Ross, G. and Aiuppa, A., 2011. Selenium mobilization in soils due to volcanic derived acid rain: an example from Mt Etna volcano, Sicily. 81, 829-834.

Fordyce, F., 2007. Selenium geochemistry and health. AMBIO: *Journal of the Human Environment, 36,94-97.*

Fordyce, F.M., Johnson, C.C., Navaratna, U.R., Appleton, J.D. and Dissanayake, C.B., 2000. Selenium and iodine in soil, rice and drinking water in relation to endemic goitre in Sri Lanka. *Science of the Total Environment*, 263, 127-141.

Garcia-Casal, M.N., Peña-Rosas, J.P. and Giyose, B., 2017. Staple crops biofortified with increased vitamins and minerals: considerations for a public health strategy. *Annals of the New York Academy of Sciences*, 1390, 3-13.

Gashu, D., Marquis, G.S., Bougma, K. and Stoecker, B.J., 2018. Selenium inadequacy hampers thyroid response of young children after iodine repletion. *Journal of Trace Elements in Medicine and Biology*, 50, 291-295.

Gashu, D., Marquis, G.S., Bougma, K. and Stoecker, B.J., 2018a. Spatial Variation of Human Selenium in Ethiopia. *Biological Trace Element Research*, 1-7.

Gashu, D., Marquis, G.S., Bougma, K. and Stoecker, B.J., 2018b. Selenium inadequacy hampers thyroid response of young children after iodine repletion. *Journal of Trace Elements in Medicine and Biology*, 50,291-295.

Gashu, D., Stoecker, B.J., Adish, A., Haki, G.D., Bougma, K. and Marquis, G.S., 2016. Ethiopian pre-school children consuming a predominantly unrefined plant-based diet have low prevalence of iron-deficiency anaemia. *Public Health Hutrition*, 19,1834-1841.

Gashu, D., Stoecker, B.J., Adish, A., Haki, G.D., Bougma, K., Aboud, F.E. and Marquis, G.S., 2016a. Association of serum selenium with thyroxin in severely iodine-deficient young children from the Amhara region of Ethiopia. *European Journal of Clinical Nutrition*, 70, 929.

Gashu, D., Stoecker, B.J., Bougma, K., Adish, A., Haki, G.D. and Marquis, G.S., 2016b. Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition Journal*, 15,38.

Genc, Y., Humphries, J.M., Lyons, G.H. and Graham, R.D., 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Journal of Trace Elements in Medicine and Biology*, 18, 319-324.

Gibson, R.S. and Hotz, C., 2001. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition*, 85, 159-166.

Gissel-Nielsen, G., 1971. Influence of pH and texture of the soil on plant uptake of added selenium. *Journal of Agricultural and Food Chemistry*, 19, 1165-1167.

Goh, K.H. and Lim, T.T., 2004. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. *Chemosphere,* 55,849-859.

Gromadzińska, J., Reszka, E., Bruzelius, K., Wąsowicz, W. and Åkesson, B., 2008. Selenium and cancer: biomarkers of selenium status and molecular action of selenium supplements. *European Journal of Nutrition*, 47, 29-50.

Gupta, U.C. and Gupta, S.C., 2000. Selenium in soils and crops, its deficiencies in livestock and humans: implications for management. *Communications in Soil Science and Plant Analysis*, 31,1791-1807.

Haug, A., Graham, R.D., Christophersen, O.A. and Lyons, G.H., 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microbial Ecology in Health and Disease*, 19, 209-228.

Hopper, J.L. and Parker, D.R., 1999. Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulfate. *Plant and Soil*, 210, 199-207.

Hu, Y.J. and Diamond, A.M., 2003. Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. *Cancer Research*, 63,120

Huang, Y., Wang, Q., Gao, J., Lin, Z., Banuelos, G.S., Yuan, L. and Yin, X., 2013. Daily dietary selenium intake in a high selenium area of Enshi, China. N*utrients*, 5, 700-710

Hurst, R., Siyame, E.W., Young, S.D., Chilimba, A.D., Joy, E.J., Black, C.R., Ander, E.L., Watts, M.J., Chilima, B., Gondwe, J. and Kang'ombe, D., 2013. Soil-type influences human selenium status and underlies widespread selenium deficiency risks in Malawi. *Scientific Reports*, 3, 1425.

Johnsson, L., 1991. Selenium uptake by plants as a function of soil type, organic matter content and pH. *Plant and Soil*, 133, 57-64.

Kassu, A., Yabutani, T., Mulu, A., Tessema, B. and Ota, F., 2008. Serum zinc, copper, selenium, calcium, and magnesium levels in pregnant and non-pregnant women in Gondar, Northwest Ethiopia. *Biological Trace Element Resear*ch, 122, 97-106.

Kikkert, J. and Berkelaar, E., 2013a. Plant uptake and translocation of inorganic and organic forms of selenium. Archives of environmental contamination and toxicology, 65,458-465.

Kikkert, J., Hale, B. and Berkelaar, E., 2013b. Selenium accumulation in durum wheat and spring canola as a function of amending soils with selenite, selenate and or sulphate. *Plant and Soil*, 372,629-641.

Li, H.F., McGrath, S.P. and Zhao, F.J., 2008. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytologist*, 178, 92-102.

Li, Z., Liang, D., Peng, Q., Cui, Z., Huang, J. and Lin, Z., 2017. Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: a review. *Geoderma*, 295, 69-79.

Lopez-Saez, J.B., Senra-Varela, A. and Pousa-Estevez, L., 2003. Selenium in breast cancer. *Oncology*, 64, 227-231.

Lyons, G., Ortiz-Monasterio, I., Stangoulis, J. and Graham, R., 2005. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding?. *Plant and Soil*, 269, 369-380.

Lyons, M.P., Papazyan, T.T. and Surai, P.F., 2007. Selenium in food chain and animal nutrition: Lessons from nature. *Asian Journal of . Animal Science, 20*, 1135-1155.

MacFarquhar, J.K., Broussard, D.L., Melstrom, P., Hutchinson, R., Wolkin, A., Martin, C., Burk, R.F., Dunn, J.R., Green, A.L., Hammond, R. and Schaffner, W., 2010. Acute selenium toxicity associated with a dietary supplement. *Archives of Internal Medicine*, 170, 256-261.

Melse-Boonstra, A., Hogenkamp, P. and Lungu, O.I., 2007. Mitigating HIV/AIDS in Sub-Saharan Africa through selenium in food. Farmer Publication, Lusaka. *Golden Valley Agricultural Research Tust*(GART), 11.

Mikkelsen, R.L., Page, A. Jacobs L.W. 1989. Factors affecting selenium accumulation by agricultural crops. In: Jacobs L, W. (ed.) Selenium in agriculture and the environment. Soil Science Society of America and American Society of Agronomy, Madison, USA.

Morris, J.S. and Crane, S.B., 2013. Selenium toxicity from a misformulated dietary supplement, adverse health effects, and the temporal response in the nail biologic monitor. *Nutrients, 5*1024-1057.

Muthayya, S., Rah, J.H., Sugimoto, J.D., Roos, F.F., Kraemer, K. and Black, R.E., 2013. The global hidden hunger indices and maps: an advocacy tool for action. *PLoS One*, 8,e67860.

Nakamaru, Y.M. and Sekine, K., 2008. Sorption behavior of selenium and antimony in soils as a function of phosphate ion concentration. *Soil Science & Plant Nutrition*, 54, 332-341.

Navarro-Alarcon, M. and Cabrera-Vique, C., 2008. Selenium in food and the human body: a review. *Science of the Total Environment*, 40,115-141.

Neal, R.H. and Alloway, B.J., 1995. Selenium, in Heavy metals in soils. BJ Alloway, 237-260.
Ohlendorf, H.M., Hoffman, D.J., Saiki, M.K. and Aldrich, T.W., 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drain water. *Science of the Total Environment*, 52, 49-63.

Park, J.H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N. and Chung, J.W., 2011. Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Journal of Hazardous Materials*, 185,549-574.

Pezzarossa, B., Remorini, D., Gentile, M.L. and Massai, R., 2012. Effects of foliar and fruit addition of sodium selenate on selenium accumulation and fruit quality. *Journal of the Science of Food and Agriculture*, 92, 781-786.

Pietinen, P., Paturi, M., Reinivuo, H., Tapanainen, H. and Valsta, L.M., 2010. FINDIET 2007 Survey: energy and nutrient intakes. *Public Health Hutrition*, 13, 920-924.

Qin, H.B., Zhu, J.M., Liang, L., Wang, M.S. and Su, H., 2013. The bioavailability of selenium and risk assessment for human selenium poisoning in high-Se areas, China. *Environment International*, 52, 66-74.

Rayman, M.P., 2000. The importance of selenium to human health. The lancet, 356, 233-241.

Rejali, L., Jaafar, M.H. and Ismail, N.H., 2007. Serum selenium level and other risk factors for breast cancer among patients in a Malaysian hospital. *Environmental Health andPreventiveMedicine*, 12,105.

Ros, G.H., Van Rotterdam, A.M.D., Bussink, D.W. and Bindraban, P.S., 2016. Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach. *Plant and Soil*, 404, 99-112.

Russell, M.A., Walling, D.E. and Hodgkinson, R.A., 2001. Suspended sediment sources in two small lowland agricultural catchments in the UK.*Journal of hydrology, 252,* 1-24

Rundlöf, A.K. and Arnér, E.S., 2004. Regulation of the mammalian selenoproteinthioredoxin reductase 1 in relation to cellular phenotype, growth, and signaling events. *Antioxidants and Redox Signaling*, 6, 41-52.

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A. and Nannipieri, P., 2011. Persistence of soil organic matter as an ecosystem property. *Nature*, 478,49.

Schrauzer, G.N., 2001. Nutritional selenium supplements: product types, quality, and safety. *Journal of the American College of Nutrition*, 20, 1-4.

Séby, F., Gautier, M.P., Lespes, G. and Astruc, M., 1997. Selenium speciation in soils after alkaline extraction. *Science of the Total Environment*, 207, 81-90.

Stroud, J.L., Broadley, M.R., Foot, I., Fairweather-Tait, S.J., Hart, D.J., Hurst, R., Knott, P., Mowat, H., Norman, K., Scott, P. and Tucker, M., 2010. Soil factors affecting selenium concentration in wheat grain and the fate and speciation of Se fertilisers applied to soil. *Plant and Soil*, 332, 19-30.

Tan, J.A., Zhu, W., Wang, W., Li, R., Hou, S., Wang, D. and Yang, L., 2002. Selenium in soil and endemic diseases in China. *Science of the Total Environment*, 284, 227-235.

Terry, N., Zayed, A.M., De Souza, M.P. and Tarun, A.S., 2000. Selenium in higher plants. *Annual review of Plant Biology*, 51, 401-432.

Thomson, C.D., 2004. Selenium and iodine intakes and status in New Zealand and Australia. *British Journal of Nutrition*, 91,661-672.

Tiahou, G., Maire, B., Dupuy, A., Delage, M., Vernet, M.H., Mathieu-Daude, J.C., Michel, F., Sess, E.D. and Cristol, J.P., 2004. Lack of oxidative stress in a selenium deficient area in Ivory Coast. *European Journal of Nutrition*, 43,367-374.

Vanderpas, J.B., Contempré, B., Duale, N.L., Goossens, W., Bebe, N.G.O., Thorpe, R., Ntambue, K., Dumont, J., Thilly, C.H. and Diplock, A.T., 1990. Iodine and selenium deficiency associated with cretinism in northern Zaire. *The American Journal of Clinical Nutrition*, 52,1087-1093.

Vuori, E., Vääriskoski, J., Hartikainen, H., Vakkilainen, P., Kumpulainen, J. and Niinivaara, K., 1989. Sorption of selenate by Finnish agricultural soils. *Agriculture, Ecosystems &Environment*, 25,.111-118.

Walkley, A. and Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37,9-38.

Wang, M.C. and Chen, H.M., 2003. Forms and distribution of selenium at different depths and among particle size fractions of three Taiwan soils. *Chemosphere*, 52,585-593.

Xia, Y., Hill, K.E., Byrne, D.W., Xu, J. and Burk, R.F., 2005. Effectiveness of selenium supplements in a low-selenium area of China–. *The American Journal of Clinical Nutrition*, 81, 829-834.

Yu, X.Z. and Gu, J.D., 2008. Differences in uptake and translocation of selenate and selenite by the weeping willow and hybrid willow. *Environmental Science and Pollution Research*, 15,499-508.

Zhao, C., Ren, J., Xue, C. and Lin, E., 2005. Study on the relationship between soil selenium and plant selenium uptake. *Plant and Soil*, 277,197-206.
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