



## Full Length Research Article

### LIMING AND SELENIUM APPLICATION EFFECTS ON SELENIUM UPTAKE BY MAIZE (*ZEA MAYS*) AND SELENIUM CONTENTS IN MAIZE GRAIN

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#### ABSTRACT

Selenium, a ubiquitously occurring element in the environment, is classified as an essential element for human and animal health because, among others, it has antiviral and anticarcinogenic effects and plays a biological role as a component of the antioxidant enzyme glutathione peroxidase that catalyzes the decomposition of H<sub>2</sub>O<sub>2</sub> and consequently protects cells from oxidative damage. Dietary Se intake in Malawi is <20 µg person<sup>-1</sup> day<sup>-1</sup>, which is low compared to recommended 50-70 µg person<sup>-1</sup> day<sup>-1</sup> worldwide. Selenium bioavailability is affected largely by acidity effects. This study was aimed at determining liming effects on Se availability. Extractable Se significantly increased with increasing Se applications from 0.12 mg Se kg<sup>-1</sup> to 0.32 mg Se kg<sup>-1</sup>. Extractable Se content of the soil to which no Se had been added was 0.09 mg kg<sup>-1</sup> but increased with increasing liming effects to 0.15 mg kg<sup>-1</sup> at 5 t lime ha<sup>-1</sup>. Selenium uptake also increased with increasing lime application. About 95% of total variation in Se contents in maize grain was accounted for by liming effects while Se application accounted for about 98% of the total variation in maize grain Se contents. Lime and selenium effects on grain Se contents were statistically significant ( $P \leq 0.001$ ).

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#### INTRODUCTION

Selenium is a ubiquitously occurring element in the environment which belongs to group VIA of the periodic table and has chemical and physical properties that are intermediate between metals and non-metals (Table I). Selenium occurs in nature as six stable isotopes and can exist in multiple oxidation states including -2, 0, +4, and +6 (Combs, 2001; Fordyce 2005). Selenium is a metalloid, having physical and chemical properties that are intermediate between those of metals and non-metals. Se, like S, can exist in either an amorphous state or in one of three crystalline forms (Combs, *ibid.*). The chemical and physical properties of Se are very similar to those of S. The two elements have similar atomic sizes and outer-valence shell electronic configurations. Their bond energies, ionization potentials and electron affinities are practically the same (Combs, *ibid.*).

The availability of selenium in soil solution as selenate ( $\text{SeO}_4^{2-}$ ) or selenite ( $\text{SeO}_3^{2-}$ ) ions is dependent upon the activity of electron (pe) and hydrogen (pH) of the solution. The plant uptake of  $\text{SeO}_4^{2-}$  is similar to that of sulphate ( $\text{SO}_4^{2-}$ ). Both  $\text{SeO}_4^{2-}$  and  $\text{SO}_4^{2-}$  enter root epidermal cells across the plasma membrane through sulphate transporters against their electrochemical gradients, with uptake being driven by the co-transport of three protons for each ion (Terry *et al.*, 2000; Sors *et al.*, 2005).  $\text{SeO}_4^{2-}$  uptake from the soil is through high-affinity sulphate transporters in plants (Terry *et al.* 2000; Sors *et al.*, *ibid.*), whereas  $\text{SeO}_3^{2-}$  accumulates through passive diffusion and can be inhibited by phosphate (Terry *et al.*, *ibid.*; Sors *et al.*, *ibid.*).  $\text{SeO}_3^{2-}$  uptake in wheat is an active process, mediated by proton-coupled phosphate transporters (Li *et al.*, 2008). It has been observed that  $\text{SeO}_4^{2-}$  ions are rapidly absorbed and transported in plant xylem sap and that  $\text{SeO}_3^{2-}$  absorption is slower, but the Se is more rapidly metabolized to

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organoselenium compounds and transported into upper portions of the plant (Mayland *et al.* 1991). Selenium is classified as an essential element for human and animal health (Rayman, 2000) because of its physiological role as a component of the antioxidant enzyme glutathione peroxidase, an enzyme that catalyzes the decomposition of hydrogen peroxide ( $H_2O_2$ ), thus regulating intracellular redox state and protecting cells from oxidative damage (Rotruck *et al.*, 1973). Selenium has antiviral and anticarcinogenic effects (Spallholz, 2001; Rayman, 2000; Rayman, 2000; Rayman, 2002; Reilly, 2006; Combs, 2001; WHO/FAO, 2004). Selenium deficiency in humans and animals adversely affects thyroid hormone metabolism, which is detrimental to growth and development (Arthur and Beckett, 1994), and can cause homeostasis of hepatic haem, disruption in microsomal and mitochondrial electron transport, problems related to immune response, motility of spermatozoa, infertility, promotion of vitamin-E related diseases, impaired vision, and loss of protection against xenobiotics and exercise stresses liked muscle stiffness and tenderness and disinclination to move (Oldfield *et al.*, 1971; Shamberger, 1983; Underwood, 1956; WHO, 1987). Selenium deficiency has been observed to be a problem in areas where soil Se is particularly low, such areas as those in some parts of China, the USA as well as Australia, New Zealand and Finland (Oldfield, 2002).

Whereas the essentiality of Se for humans and animals has been established, its essentiality for plants has not yet been recognised. It is classified under the beneficial elements for plants (Pilon-Smits *et al.*, 2009) because low level Se applications have been observed, *inter alia*, to stimulate growth and dry-matter yield of lettuce (Xue *et al.* 2001); ryegrass (Hartikainen *et al.* 2000); potato (Turakainen *et al.* 2004); green tea (Hu *et al.* 2003); rice (Liu *et al.* 2004); and soybean (Djanaguiraman *et al.* 2005). Selenium varies widely from 0.01 to 2 mg kg<sup>-1</sup> in most soils, but most frequently ranges between 0.2 and 0.4 mg kg<sup>-1</sup> (McNeal and Balistriero, 1989). In seleniferous areas, however, it can be up to 1200 mg Se kg<sup>-1</sup> (White *et al.* 2007b). Some soils are low in selenium, ranging from 0.03 to 0.08 mg mg<sup>-1</sup> (NAS, 1976). Soils deficient in Se contain less than 0.5 mg Se mg<sup>-1</sup> of total Se (Mayland *et al.*, 1989). In aerobic soil solutions with pH ranging from pH 4 to 8,  $SeO_4^{2-}$  is the dominant Se species (Séby *et al.*, 2001).  $SeO_4^{2-}$  is highly soluble with low adsorption and precipitation capacities (Fordyce, 2007). Selenite (Se(IV)) is a weak acid ( $pK_{a_1} = 2.70$  and  $pK_{a_2} = 8.54$ ) and can exist as  $HSeO_3^-$  or  $SeO_3^{2-}$  in soil solutions (Séby *et al.*, 2001). In reduced soil environments,  $SeO_3^{2-}$  is the major Se species present in solutions and its effective concentration in solutions is mainly governed by sorption/desorption processes on solid surfaces such as metal hydroxyoxides. The various Se species are differentially taken up by plants. Plants may take up both inorganic and organic Se via active membrane transport while the role of simple diffusion is limited (Terry *et al.*, 2000; Li *et al.*, 2008). The active transport of Se appears to occur via shared transporter-proteins;  $SeO_4^{2-}$  may be actively internalized by sulphate transporters (Terry *et al.*, 2000; Ellis and Salt, 2003;

Zayed *et al.*, 1998) while  $SeO_3^{2-}$  is likely internalized via phosphate transporters (Terry *et al.*, 2000, Barrow *et al.*, 1989; Chilimba *et al.*, 2011; Gissel-Nielsen, 1971; Johnsson, 1991; De Temmerman *et al.*, 2014) and, for example in rice, Si-transporters (e.g in rice (Zhao *et al.*, 2010). In the presence of sulphate and phosphate, Se uptake is suppressed (Terry *et al.*, 2000; Hengl *et al.* 2014; Hopper and Parker, 1999; Schiavon *et al.*, 2015). Low S concentration enhances  $SeO_4^{2-}$  uptake while low P concentration suppresses uptake of selenite in soils (Li *et al.*, 2008) causing plants to increase the expression of sulphate and phosphate transporter genes in order to compensate for the lower availability of these nutrients. Both  $SeO_4^{2-}$  and  $SeO_3^{2-}$  are consequently taken up more readily when present in solution in the absence of sulphate and phosphate. Response to lower availability of nutrients has been reported to be more pronounced for  $SeO_4^{2-}$  than for  $SeO_3^{2-}$  uptake, which could be due to a different level of gene up-regulation in reaction to P or S starvation and/or to the lower affinity of the phosphate transporters for  $SeO_3^{2-}$  compared with that of sulphate transporters for  $SeO_4^{2-}$  (Li *et al.*, 2008).

Plants grown in alkaline and well aerated soils tend to accumulate high concentration of Se as compared to those in acid soils (Gupta *et al.*, 1975). In well-aerated, alkaline soils, Se occurs primarily as  $SeO_4^{2-}$  and is readily bioavailable. In acid soils,  $SeO_3^{2-}$  ions form complexes of very insoluble iron oxides and hydroxyoxides such as  $Fe_2(OH)_4SeO_3$ . The low solubility coupled with stronger adsorption makes  $SeO_3^{2-}$  less bioavailable than  $SeO_4^{2-}$ . Soils in Malawi are dominated by Oxisols and Ultisols which are predominately acidic and inherently low in fertility. Because soil acidity has detrimental effects on both plants and soil organisms (1989; Runge and Rode, 1991; Bloom, 2000), and the environment (Bolan *et al.*, 2003a, 2003b), farmers in Malawi are now commonly advised to apply liming materials to their soils. Liming effects generally increase soil Se availability and enhance the uptake of Se by plants (Adriano, 2001). Dietary Se intake levels mainly depend on the total concentration and bioavailability of Se in food sources. Selenium intake levels have, however, a relatively narrow range between deficiency and toxicity. It is currently estimated that intakes of 30 µg Se d<sup>-1</sup> (d<sup>-1</sup> = per day) are inadequate for humans, while Se intakes exceeding 900 µg d<sup>-1</sup> are potentially harmful (Fairweather-Tait *et al.*, 2011). Intake of 40 to 80 µg Se d<sup>-1</sup> are considered adequate for healthy adults but it has been observed that a higher level of 200 to 300 µg Se d<sup>-1</sup> may be more appropriate for the maintenance of health (Comb, 2001; Schrauzer and Surai, 2009). Since Se has a very narrow range between dietary deficiency and toxic levels, it is important to ensure that any soil management strategy that is promoted for use by farmers does not lead to excessive Se accumulations in food crops which adversely impact on food chain quality and human health. To prevent Se toxicity and to provide adequate Se in the human diet, it is important to establish the

extent to which Se uptake by plants growing on the acidic soils of Malawi is affected by liming effects. This study was therefore conducted to assess the effect of liming on the uptake of Se added to acidic soils.

## MATERIALS AND METHODS

### Description of the Study Site

The study was carried out on Mpherero Estate, one of the sites where the Clinton Development Initiative (CDI) was multiplying seed for Malawian farmers under the Alliance for Green Revolution in Africa (AGRA) project. Mpherero Estate is in Mchinji District (Latitude: 13° 46' 19" S Longitude: 33° 00' 03" E), about 150 km from Lilongwe University of Agriculture and Natural Resources, to the west of Malawi's Capital city, Lilongwe. The site topographically lies between 1200 and 1829 meters above sea level and has two distinct terrains: the hilly western part, comprising the Mchinji Mountain ranges with gentle slopes where almost all rivers found in the district originate from, and the rest of the district lying within a plain of mostly arable land that is generally drained by water ways and there are some *dambos* which are permanently or seasonally inundated wetlands characterized by grasses, rushes and sedges, contrasting with surrounding woodland such as Miombo (*Brachystegia*) woodland. Mchinji experiences annually mean temperatures ranging from 17 to 20°C. Lowest temperatures are experienced in June while high temperatures are experienced during the months of October and November. The district receives average rainfall of between 800 and 1230 mm per annum with the wet rainy season running from November to April.

### Cropping History

The site topographically lies between 1200 and 1829 meters above sea level and has two distinct terrains: the hilly western part, comprising the Mchinji Mountain ranges with gentle slopes where almost all rivers found in the district originate from, and the rest of the district lying within a plain of mostly arable land that is generally drained by water ways and there are some *dambos* which are permanently or seasonally inundated wetlands characterized by grasses, rushes and sedges, contrasting with surrounding woodland such as Miombo (*Brachystegia*) woodland. The experimental site is an area where the Anchor Farm Project, which is a Clinton Development Initiative (CDI)-operated commercial farm, was conducted. The CDI-sponsored project partners with thousands of neighbouring smallholder farmers and provides them with access to quality inputs for maize and soy production, as well as training and market access. Farmers in the project have access to improved soy seed and to training in advanced agronomic techniques. The Farm Manager of the commercial farm observed that crop yields were far below 2 t ha<sup>-1</sup> and that a plant locally known as "Nzobwe" (*Terminalia superb*), which is an indicator of acid soils (Bolan *et al.*, 1988), was thriving well on this site. After incorporating dolomitic limestone into the soil at a rate of 2 t ha<sup>-1</sup> and Super D (10.5 N + 24 P<sub>2</sub>O<sub>5</sub> + 20 K<sub>2</sub>O + (6 S and 0.15 B) at the rate of 300 kg ha<sup>-1</sup>), the Farm Manager observed that the crop yield increased significantly to an average of 2.9 t ha<sup>-1</sup>

of soybean seed from an average of 1.5 t ha<sup>-1</sup> that was obtained from the plots that had not been limed (Ngwira, 2011). It was, however, impossible to conclude on whether the 2 t ha<sup>-1</sup> of lime that was applied represented the maximum rate of dolomitic lime required to improve indices of chemical soil fertility to optimum yield of the soybean seed at the site.

### Soil sampling and incubation

Top (0–0.15 m) soil samples were randomly collected from 20 spots and mixed together to form a composite sample weighing 1,000 kg. The composite soil samples were air dried at room temperature (25°C ± 2°C) and sieved to pass through a 2 mm sieve mesh. The experiment was carried out in two phases. The first phase entailed mixing thoroughly 24 kg of the sieved soils with three levels (0, 5, 10 g Se ha<sup>-1</sup>) of sodium selenate and five levels (0, 2.5, 5.0, 7.5, 10 t ha<sup>-1</sup>) of dolomitic limestone, placing the mixture in 25-litre plastic pots, adding water to each of the pots to reach the matric suction of 10 kPa, and then incubating, at 30°C for 30 days under glasshouse conditions, the soils that were replicated three times and arranged in a randomized complete block design. Throughout the incubation period, moisture contents were maintained at field capacity. After 30 days of incubation, soil samples were taken and analysed. The second phase of the experiment entailed cropping the incubated soils to maize (*Zea mays*) in a glasshouse under room temperature.

### Characterisation of physical and chemical properties of the soils

After the sieved soil samples were incubated for 30 days, aliquots of the soils were taken and analysed to assess liming effects on soil chemical properties after the incubation period. The soils were analysed for pH in a 1:2.5 soil to water slurry using a pH electrode as outlined by described by Blakemore *et al.*, (1987), particle size distribution using the Bouyoucos hydrometer method (Day, 1965) as described by Anderson and Ingram (1993). The organic carbon contents of the soils were determined by the potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) oxidation method of Walkley and Black (1934) as described by Anderson and Ingram (1993). Organic matter was estimated by multiplying the total soil organic carbon with 1.724. Total nitrogen was determined using the Kjeldhal wet oxidation process as described by Anderson and Ingram (1993), while available phosphorus was extracted by the Mehlich 3 method (Mehlich, 1984) and P in the clear extracts was determined colorimetrically by the ascorbic acid method (Murphy and Riley, 1962) on a Perkin Elmer Lambda 25 UV/VIS spectrometer. The extract obtained by the Mehlich 3 method (Mehlich, *ibid.*) was used for the determination of K by flame emission photometry at a wavelength of 766.5 nm, and of both Ca and Mg using Atomic Absorbance Spectrophotometer model 6200 at a wave length of 422.7 and 285.2 nm respectively (Anderson and Ingram 1993).

### Determination of extractable soil selenium

Aliquots of the soils, 30 days after they had been incubated, and then after the soils were cropped to maize, were taken for extractable Se determinations. The extracting solution used in this study was prepared by mixing 1.97 g of DTPA (diethylene

triamine pentaacetic acid), 1.47 g of CaCl<sub>2</sub>·2H<sub>2</sub>O and 14.2 g of Triethylamine (TEA) as described by Jump and Sabey (1989). An aliquot of 40 millilitres of extracting solution was added to 5 grams of air dried, 2-mm sieved soil in a plastic shaking bottle and covered tightly to avoid leakages. The bottles were then shaken for two hours on a reciprocal shaker. After shaking, the suspension was filtered into clean plastic bottles. The filtrates were then packed for selenium concentration determination using Atomic Absorption Spectrometer.

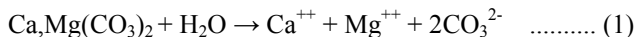
**Statistical analysis**

Data collected from the experiment were subjected to statistical analysis using Genstat statistical software 16<sup>th</sup> edition. The differences between and within treatments were analysed using ANOVA at 0.05 probability level and fisher’s LSD was used to separate means. The results were also subjected to regression analysis.

**RESULTS AND DISCUSSION**

**Liming effects on soil properties after 30 days of incubation**

The results presented in Tables 1 and 2 and Figure 1 show that the effects of lime, applied at the rate of 10 t ha<sup>-1</sup>, on soil pH increased to pH<sub>H<sub>2</sub>O</sub> 7.64 at maize harvest from pH 5.3 which was the soil reaction after the 30-day incubation period (Table 2). This suggests that the liming material continued to neutralise exchangeable acidity (easily replaceable H<sup>+</sup> and Al<sup>3+</sup> ions) after the H<sup>+</sup> and Al<sup>3+</sup> ions had been replaced on the soil colloidal surface by Ca<sup>2+</sup> and Mg<sup>2+</sup> as explained earlier in Section 2.10 of this thesis. The reaction of dolomitic limestone involved may be illustrated the process.



**Table 1. Effects of liming on some soil chemical properties after 30-day incubation period**

Lime rate	Organic matter	N	pH <sub>KCl</sub>	pH <sub>H<sub>2</sub>O</sub>	P	Ca	Mg	K
	g kg <sup>-1</sup>				(mg kg <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>		
0.0	1.92	0.10	4.84d	5.26d	52.5b	1.7c	0.4c	0.25
2.5	1.88	0.09	5.87c	6.13c	52.5b	2.3cb	0.6bc	0.21
5.0	1.86	0.09	5.98c	6.23c	57.4ba	2.6b	0.7b	0.22
7.5	1.93	0.10	6.18b	6.61b	58.1a	2.8ab	0.8b	0.20
10	1.73	0.09	6.37a	6.78a	57.5ba	3.4a	1.1a	0.19
F pr.	0.36ns	0.41ns	<0.001**	<0.001**	0.007*	<0.001**	<0.001**	0.083ns
LSD	0.21	0.01	0.16	0.12	4.0	0.6	0.2	0.04
CV(%)	1.2	1.2	2.9	2.0	7.5	2.4	3.3	2.18

\*\*=P ≤ 0.01, \*= P ≤ 0.05, ns= not significant; means with different subscripts along a column are significantly different

Selenium (Se) deficiency (less than 0.125 mg kg<sup>-1</sup>) occurs when this element is not readily available for plant uptake (Fordyce, 2007). Soils with a Se concentration greater than 0.5 mg kg<sup>-1</sup> have been found to produce vegetation absorbing Se at levels toxic for animal consumption (Dhillon et al., 1992). An exception to this was observed in Hawaii with up to 20 mg kg<sup>-1</sup> total Se but without any effect on vegetation and livestock

because the element was held in iron and aluminium complexes in the humid lateritic soils of that region (Oldfield, 1999).

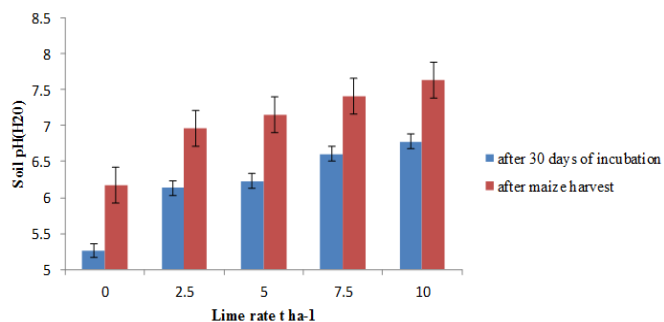
**Table 2. Effects of liming on soil pH at harvest relative to the pH after the 30-day incubation**

Lime rate	pH <sub>H<sub>2</sub>O</sub>		±
	D <sub>30</sub>	A <sub>H</sub>	
0.0	5.3	6.2	0.9
2.5	6.1	6.8	0.7
5.0	6.2	7.3	1.1
7.5	6.6	7.5	0.9
10.0	6.8	7.5	0.7

where:

D<sub>30</sub> denotes after 30-day incubation

A<sub>H</sub> denotes at harvest



**Figure 1. Relationship between lime rate and soil pH**

The bioavailability of Se is a function of the effective concentration of Se in the soil solution. The effective concentration of Se in soil solution is dependent upon soil properties including soil pH, electron activity (pe), the speciation of the element, the presence of competitive ions in soil solution, organic matter, and soil texture. The effect of cations such as Mg<sup>2+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> on the effective concentration of any of the Se species in soil solution is not yet well known but an increase in hydroxyl ions (Equation 2)

in soil solution with increasing rates of lime application can lead to not only the precipitation of aluminium ions but also excess OH<sup>-</sup> ions exchanging with selenate (SeO<sub>4</sub><sup>2-</sup>) and selenite (SeO<sub>3</sub><sup>2-</sup>) adsorbed on the soil colloidal surfaces, thus rendering the Se species bioavailable. Such a ligand-exchange reaction has been suggested (Peack and Sparks, 2001). Anions

such as phosphate and sulphate affect the bioavailability of Se through their competition with Se for adsorption on the surface of soil colloids. In this study, extractable (available) P increased with increasing rate of lime application and the difference between the P value extracted from the unlimed soil and the P extracted after 7.5 t ha<sup>-1</sup> dolomitic limestone was incorporated into the soil was statistically significant ( $P \leq 0.05$ ). The increasing rates of dolomitic limestone considered in this study accounted for about 79% of the total variation in extractable P (Fig. 2). The increase in P bioavailability due to liming effects is in accord with past findings (Sime, 2001). Phosphate ions in the soil solution readily adsorb on the sorptive surfaces of soil colloidal particles thus decreasing the sorption of Se on the soil colloidal surface and increasing Se bioavailability. The present data show that after a 30-day incubation period, extractable Se in the soil to which Se had not been added was low (0.087 mg Se kg<sup>-1</sup>) but increased with increasing liming effects to 0.151 mg Se kg<sup>-1</sup> after 5 t lime ha<sup>-1</sup> was added (Fig. 3).

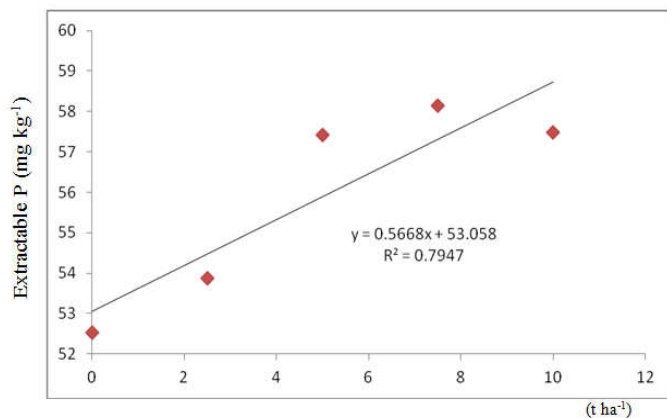


Fig. 2. Liming effects on extractable P

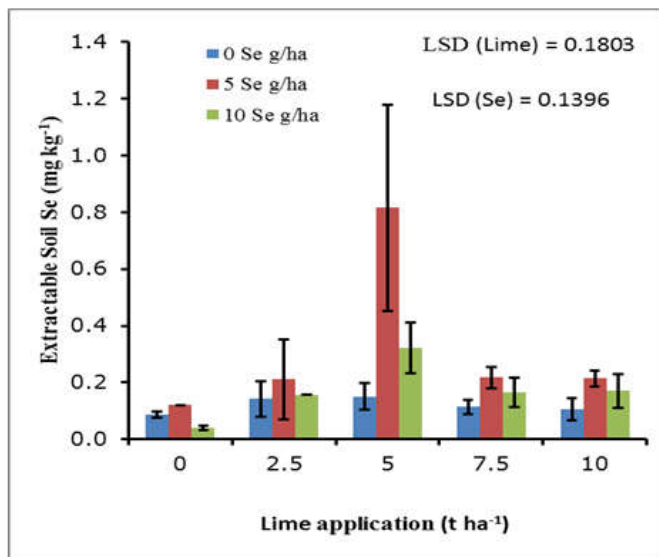


Fig. 3. Liming effects on extractable Se

Since available Se less than 0.125 mg kg<sup>-1</sup> is considered deficient (Fordyce, 2007), the results of this study suggest that during the first 30 days of a cropping period, the status of available Se in a soil deficient in this element may be improved due to the mineralisation of organically bound Se.

This is consistent with the observation of Zawislanski and Zavarin (1996) who have noted that a large portion of total Se in soils can be in the organic fraction, and have concluded that the release of low molecular-weight Se compounds during organic matter decomposition may eventually be a source of Se for plant uptake. The importance of organic-Se mineralisation in Se uptake by plants is explicit from the observation that about 50 per cent of Se in some soils is held in organic compounds (Fordyce, 2005). Selenomethionine and selenocysteine are the known organic Se forms.

Selenomethionine has been extracted from soils and has been found to be two to four times more bioavailable to plants than inorganic selenite while selenocysteine is less bioavailable than selenomethionine (Jacobs, 1989; Mayland, 1994; Neal, 1995). The mineralisation of selenium from seleniferous organic material has, however, been observed to be slow and a large proportion of Se (>80%) has been observed to remain unutilized by the following crops (Ajwa *et al.*, 1998; Dhillon *et al.*, 2007). The extent to which the increase in the Se status of the soil following the 30-day incubation observed in the present study may be bioavailable will depend on soil factors such as pH and redox potential as well as the presence of competitive ions such as  $\text{SO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  and sorptive colloidal surfaces. Dissolved polysaccharides such as starch, amylopectin, water-soluble lignin, cellulose acetate, chitosan and carboxymethylcellulose in soils can immobilise Se by forming stable compounds with selenate (Ferri and Sangiorgio, 1999). The chemical form of Se in soil is largely governed by the soil pH and pe. Se solubility and, therefore, mobility increases with increasing redox potential.  $\text{SeO}_3^{2-}$  is the dominant Se species under moderate soil redox potential, where it is strongly sorbed by iron and aluminium oxides and hydroxides, resulting in limited bioavailability and a low potential for leaching (Elrashidi *et al.*, 1987). In the areas where diets do not contain sufficient Se to meet needs of humans and animals, there is need to promote use of Se-enriched fertilizers. The use of Se-enriched multi-element fertilizer has, for example, been mandatory in Finland since 1984 because of the need to raise Se content in crops and in Finland's Se intake (Varo, 1993). It has, however, been observed that the recovery of applied Se is usually 20-50% (Broadley *et al.*, 2010) and this emphasises the need to ensure that any strategies aimed at promoting agronomic biofortification of crops or enhancing Se bioavailability by changing soil reaction through liming should optimise Se uptake and should not lead to too high Se intake and Se excess in the environment.

#### Effects of lime and selenium applications on Se uptake

Previous investigations have shown higher soil organic matter content to result in higher grain Se concentration (Eich-Greatorex, 2007). An increase of organic matter from 49 to 69 g kg<sup>-1</sup> in a clay soil has also been observed to result in an increased plant Se uptake (Johnsson, 1991). The present study showed that Se taken up by maize plants grown on the soil that did not receive sodium selenate increased with increasing rate of dolomitic limestone addition but that the uptake decreased after the liming material was applied at the rate of 5 t ha<sup>-1</sup> (Figure 4). This reinforces the observation made earlier that the

observed increase in soil  $\text{pH}_{\text{H}_2\text{O}}$  from 5.3 to 6.2 might have created soil conditions that were conducive to increased microbial activity that resulted in mineralisation of organically bound Se. Fungi and bacteria are the two principal organic matter decomposer groups in soil. High acidity inhibits the growth of soil bacteria in favour of more resistant fungi. In contrast, liming which increases the soil pH usually improves the bacteria growth. The increase of soil pH by liming has been observed to be favourable for the microbial growth, causing microbial biomass to increase after two years following application of liming materials compared to unlimed control (Bezdicek *et al.* 2003).

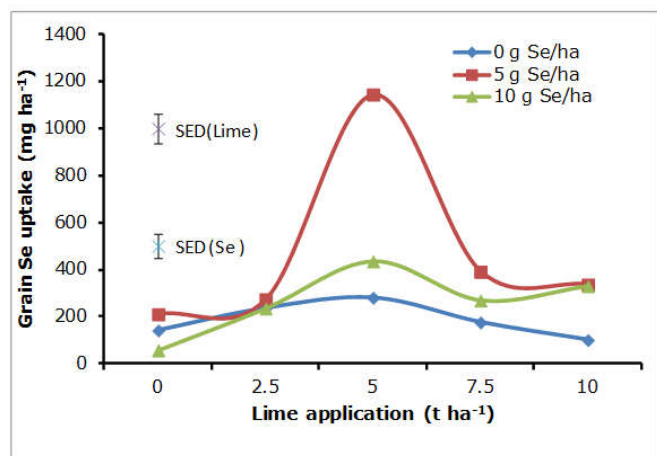


Figure 4. Effect of liming and Se application on grain Se uptake ( $\text{mg ha}^{-1}$ )

Selenite ( $\text{SeO}_3^{2-}$ ) adsorption by clays has been observed to reach a maximum between pH 3 and 5 and then to decrease with increasing pH while its adsorption by  $\text{Fe}_2\text{O}_3$  is observed to be almost constant between 3 and 8 (Gissel-Nielsen, 1971; Lauchli, 1993; Masscheleyn, 1991a). Whereas  $\text{SeO}_3^{2-}$  is specifically sorbed,  $\text{SeO}_4^{2-}$  is coulombically (electrostatically) attracted to positively charged surfaces of colloidal particles where they are not specifically sorbed on ligand-exchange sites.  $\text{SeO}_4^{2-}$  is therefore labile. As the soil pH increases, the hydroxyl ion ( $\text{OH}^-$ ) is able to replace  $\text{SeO}_3^{2-}$  on the adsorption sites thus rendering the latter available for uptake. It has, however, been suggested that between pH 4 and 8 Se solubility is governed by adsorption due to the fact that the hydroxyl ions ( $\text{OH}^-$ ) are more effective in modifying the selenium ion's adsorption capacity than in competing with selenium for common adsorption sites (Bar-Yosef and Meek, 1987). Selenium uptake also significantly increased with increasing Se application but stated to decrease after the liming material was applied at the rate of 5  $\text{t ha}^{-1}$  (Fig. 4). Liming acid soils, inherently low in selenium, often increases availability and uptake of selenium by plants (Gupta *et al.*, 1982). The effects of both lime and selenium applications were statistically significant ( $P \leq 0.01$ ) on Se taken up by maize grain. The interaction effects between lime and Se application on Se uptake, however, were not statistically significant (Fig. 4). The observed response may be attributed

to the reduced adsorption capacity of clays and Fe oxides caused by the increased pH (Neal *et al.*, 1987) and exchange of hydroxyl ions for  $\text{SeO}_3^{2-}$ . The present study showed that selenium uptake by maize plant was increased from 17.1 to 186.2  $\text{mg ha}^{-1}$  with an increase in selenium application. Liming, however, did not have any significant effect on selenium uptake by maize plant.

#### Effect of lime and selenium application on maize grain Se content

In the present study, lime applications to the soil that had not received sodium selenate resulted in an increase in Se contents of maize grain from 0.006  $\text{mg Se kg}^{-1}$ , which was the Se content of the maize grain produced on the soil to which no lime was applied, to 0.015  $\text{mg Se kg}^{-1}$ , the Se content of the maize grain produced on the soil to which the liming material was applied at the rate of 2.5  $\text{t ha}^{-1}$  (Figs. 5(a) and 5(b)).

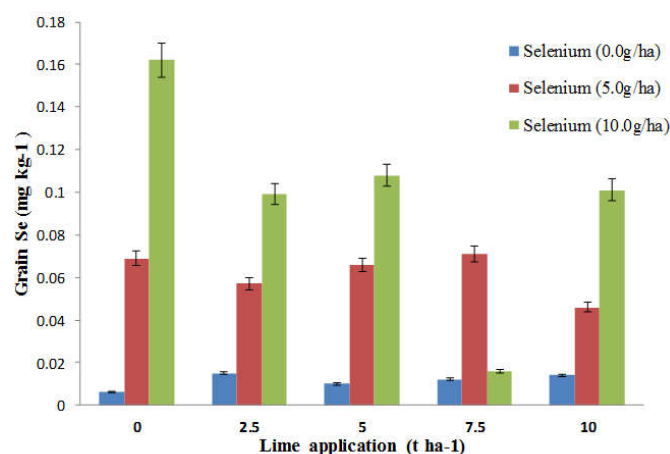


Figure 5(a). Effect of liming and selenium application on maize grain Se

Application of both dolomitic lime and sodium selenate had a statistically significant effect on grain selenium contents ( $P \leq 0.001$ ). Applications of dolomitic limestone and sodium selenate were conducive to an increase in Se contents of maize grains, each accounting for about 95% and 98% of the total variations in Se contents in maize grain respectively (Figs. 6 and 7). There was a significant interaction ( $P < 0.05$ ) between liming and selenium application rates in influencing selenium content of the maize grain. Selenium application increased maize grain selenium content from 0.054  $\text{mg Se kg}^{-1}$  to 0.079  $\text{mg Se kg}^{-1}$  with no liming. An increase in lime rate had a negative effect on maize grain Se content. Increasing lime rate caused a reduction in grain Se content. The highest Se content of 0.079  $\text{mg Se kg}^{-1}$  was found in maize grain produced on the soil to which no lime was applied. At lime rate of 2.5  $\text{t ha}^{-1}$ , mean grain Se value was 0.057  $\text{mg kg}^{-1}$  which increased to 0.061  $\text{mg kg}^{-1}$  following a lime application rate of 5  $\text{t ha}^{-1}$ . As expected, the lowest mean Se value of 0.012  $\text{mg kg}^{-1}$  was found in maize grains produced from the soil to which no selenium was applied. At selenium application rate of 5  $\text{g ha}^{-1}$ , Se mean value was 0.062  $\text{mg kg}^{-1}$ .

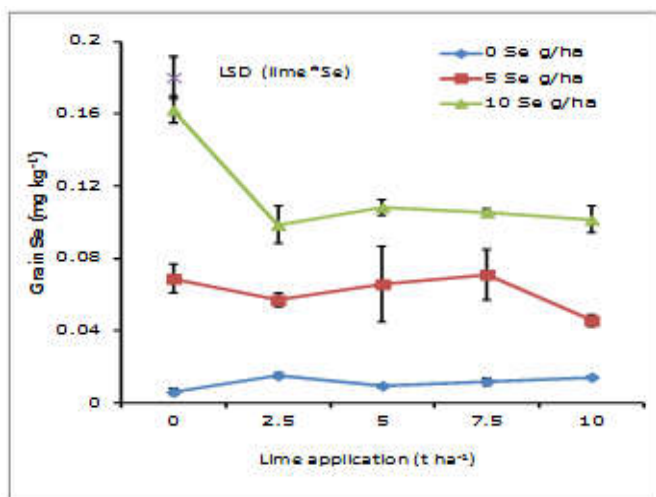


Figure 5 (b). Effect of lime and selenium application on maize grain Se content (mg kg<sup>-1</sup>)

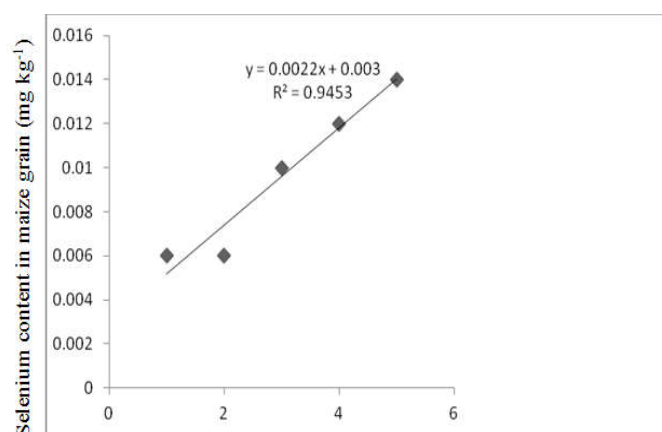


Figure 6. Lime application (t ha<sup>-1</sup>) on Se grain contents (mg kg<sup>-1</sup>)

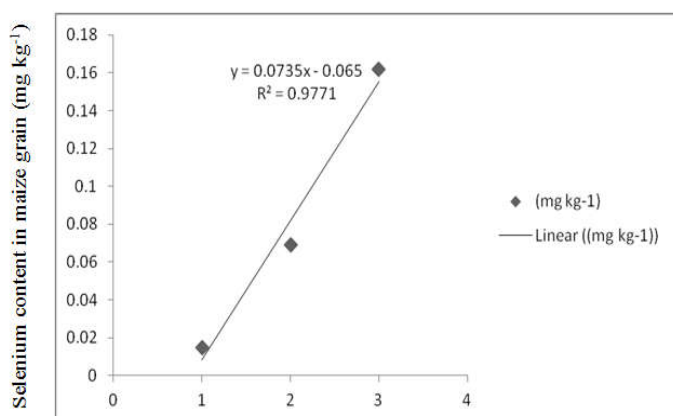


Figure 7. Selenium application (g ha<sup>-1</sup>) on Se grain contents (mg kg<sup>-1</sup>)

The highest Se content of 0.115 mg kg<sup>-1</sup> was found in maize grain produced on the soil to which lime was applied at the highest Se application rate of 10 g ha<sup>-1</sup>. The results are consistent with reports of increased Se content of most UK wheat grain at application of 10 g Se ha<sup>-1</sup> (Broadley *et al.* 2010). The Se content in maize grain increased with increasing

application of sodium selenate (Fig. 5a, b). Similar findings were also observed in Finland, where Se content of bread baked with wheat increased from 0.03 to 0.35 mg kg<sup>-1</sup> (Euroola, 2005; Sager M. and Hoesch, 2006). Chilimba *et al.* (2011) has also observed that an addition of selenium to soils leads to a significant increase in grain selenium.

## Conclusions

It has been shown that the soils used in this study were inherently Se-deficient. Maintaining moisture contents of the soils at field capacity for 30 days resulted in an increase in available Se with increasing liming effects, reaching a maximum of available Se after dolomitic limestone was applied at the rate of 5 t ha<sup>-1</sup>. This suggested that crops growing on Se-deficient, acidic soils having adequate moisture contents may benefit from the availability of organically bound Se when the soils are limed but that the extent to which optimum benefits are obtained from the liming effects is dependent on the amount of liming materials applied. Both Se uptake and Se contents of maize grain increased with the rate of sodium selenate application, reaching their maxima after dolomitic lime was applied at the rate of 5 t ha<sup>-1</sup>. The highest Se uptake was obtained from the soil that received dolomitic limestone at the rate of 5 t ha<sup>-1</sup> and to which 10 g Se ha<sup>-1</sup> was added. An application of 5 g Se ha<sup>-1</sup> to the soil that received dolomitic limestone at the rate of 5 t ha<sup>-1</sup> resulted in the Se contents in the maize grain to be the highest. Both the availability of organically bound Se and the statistically significant increase in Se contents in the maize grain suggest the need for strategies aimed at enhancing Se contents in grains to take into account the potential contribution of organically bound Se. There is also need for the execution of collaborative investigations by soil scientists, agronomists, plant breeders and molecular biologists aimed at establishing the limiting factors of Se uptake, accumulation and biotransformation in plants and selecting cultivars for their higher accumulation of Se and the forms of organic Se that are distributed in the edible parts of a given crop and have impact on daily dietary Se intake.

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