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Growth Limiting Nutrient(s) and Their Effects on the Yield and Nutrient Uptake of Chickpea (Cicer arietinum L.) in Nitisols, Southern Ethiopia

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Abstract
Field experimentation on crops grown in soils with multiple nutrient limiting situations is a challenge in terms of cost and time. Soil testing programs are evolving towards more realistic decision support systems for nutrient recommendations. In order to identify the most limiting nutrient(s) and evaluate the effects of their applications on yield and nutrient uptake of chickpea (*Cicer arietinum* L.) on soil samples collected from Nitisols of wolaita zone. A systematic approach was used to determine the availability of nutrients in the test soils, the sorption capacities and greenhouse study was conducted to evaluate crop responses to nutrients' additions. The soil chemical analysis revealed that the amounts of total N, available P, S, Zn and Cu were deficient and less than three times the critical value in Nitisols. P, S, Zn and Cu were selected to conduct the sorption experiment. The results of the sorption experiment nutrient elements to be added to the optimum treatment were: P: 190, S: 37, Zn: 11 and Cu: 13 mg per kg soil. Soil showed a relatively high P, Zn and Cu sorption value. The highest relative biomass yield of 53% and grain yield of 32% were obtained from optimum treatment. Significant increase of N, P, S, Zn and Cu content in shoot and grain emphasized the superiority of optimum treatment. P, S, Zn and Cu in the soil are found to be highly limiting nutrients to support chickpea growth. Therefore, external supplies of P, S, Zn and Cu fertilizers could be recommended for improving production of chickpea in the study area.
Keywords: Systematic Approach, Optimum Treatment, Grain yield, Biological yield, Nutrient Content
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1. Introduction

In Ethiopia, poverty and malnutrition are attributable to the mismanagement of the soil, water and plant resources which are threatening the life and livelihood of millions. This challenge will continue to exist with time due to population increases and the needs become increasingly complex. Reversing this trend in Ethiopia lies in the enhancement of sustainable development of the agricultural sector, which is a function of several biotic and abiotic resources. Sustainable agricultural productivity, in turn, has partly its origin in plants that grow on soils to produce components that are directly consumed by human beings or livestock that significantly contribute to the satisfaction of human needs by producing quantity and quality products. The implication is that the overall productivity and sustainability of a given agricultural sector is a function of fertile and productive soils.

Soil fertility depletion is the root cause for declining per capita food production in the Sub-Saharan African countries in general and in Ethiopia in particular (Sanchez *et al*., 1997). In the past ten years, much attention in Sub-Saharan Africa (SSA) has been focused on the quantification and estimation of nutrients that enter and leave agricultural systems. The balance between these nutrient inputs and outputs shows whether the agricultural system is a net gainer or a net loser of soil fertility. Nitrogen (N), Phosphorus (P) and Potassium (K) balances for agricultural land use systems in Africa countries revealed a downward trend (FAO, 2003). Moreover, the densely populated and hilly countries in the Rift Valley area (Kenya, Ethiopia, Rwanda and Malawi) showed the most negative values because of a high ratio of cultivated land to total arable land, relatively higher crop yields and the high soil erosion rate. According to Eyassu (2004), in the major arable areas in Kindo Koysha District of Wolaita Zone, annual N balances were negative both in the lowland and highland areas, whereas P balance was highly negative in the highlands and relatively better in the lowland arable areas in Kindo Koysha district of Wolaita zone.

The major plant nutrients added to the soil include mainly N and P in the form of di-ammonium phosphate (DAP) and urea fertilizers. Limited attention has been given to other macronutrients and micronutrients in Ethiopia. However, balanced nutrition is an essential component of nutrient management and it plays a significant role in increasing crop production and its quality. As a result of continuous introduction of new crop varieties and with the application of only some macronutrients such as N and P is expected to cause imbalance in plant nutrients, the less important nutrients will be yield limiting factors. Despite the fact that optimum plant growth and crop productivity cannot be realized without adequate and balanced soil nutrient supplies, nutrient management in crops is frequently restricted to major fertilizer nutrients: N, P, and/or K.

This is particularly true in low-input high-risk drought tolerant crops, like chickpea (*Cicer arietinum* L.), frequently grown under rainfed conditions in coarse-textured and/or low- fertility soils (Saxena, 1987). In chickpea grown under greenhouse conditions, application of 100 mg P kg^{-1} resulted in increased uptake of Zn, Cu, Fe and Mn but decreased the concentration of Ca and Mg in the plant tissues (Tufemkci *et al.,* 2005). In India, a number of studies have been conducted regarding effect of S on yield of leguminous crops and on average 22% increase in yield of legume crops (chickpea, pigeon pea, lentil, pea, urd bean, ground nut) has been recorded (Shrinivasarao *et al.,* 2004; Raina and Tanawade, 2005). While in European countries, 78% yield increase in pea (*Pisum sativum*) has been reported under pot experiments (Scherer *et al*., 2006).

Mineral nutrient deficiencies limit N fixation by the legume-*Rhizobium* symbiosis, resulting in low legume yields. Nutrient limitations to legume production result from deficiencies of not only major nutrients but also micronutrients (Bhuiyan *et al*., 1999). Micronutrients play important roles in increasing yield of pulses through their effects on the plant itself and on the N fixing symbiotic process. The deficiencies of these nutrients have been highly pronounced under multiple cropping systems due to excessive removal by crops.

Proper understanding of the nature and properties of the soils of Ethiopia and their management according to their potentials and constraints is imperative for maximization of crop production as close to the potential limits as possible (Abayneh and Brehanu, 2006). Assessment of soil fertility has considerable importance to determine the factors that limit yield and to take remedial measures.

Chickpea is a less labor-intensive crop and its production demands low external inputs compared to cereals. In Ethiopia, chickpea is widely grown across the country and serves as a multi-purpose crop (Shiferaw *et al*., 2007). First, it fixes atmospheric N in soils and thus improves soil fertility and saves fertilizer costs in subsequent crops. Second, it permits intensive and productive use of land, particularly in areas where land is scarce and the crop can be grown as a second crop using residual moisture. Third, it reduces malnutrition and improves human health especially for the poor who cannot afford livestock products. It is an excellent source of protein, fiber, complex carbohydrates, vitamins and minerals. Fourth, the growing demand in both the domestic and export markets provides a source of cash for smallholder producers. Fifth, it increases livestock productivity as the residue is rich in digestible crude protein content compared to cereals.

In order to make the best out of multiple benefits of chickpea, it is important to assess the soil fertility to improve the chickpea production in the study area. It is also important to determine nutrient imbalances through laboratory and greenhouse experiments to prevent nutrient deficiencies from impacting on crop growth and yields. Identifying the limiting soil nutrient and determining the optimal fertilizer requirement through laboratory analysis and greenhouse experiments are necessary prior to conducting field experiments (Portch and Hunter, 2002). The process allows the flexibility in this approach for repeating relatively inexpensive greenhouse experiments in case there is any need for further clarification of any detected nutrient disorders.

A study conducted in Taba sub-watershed showed that total N, available P and K are in the low range and Zn and Cu are also lower than three times the critical value in soil (Wondimu, 2012). In order to set priorities among the different plant nutrients for future considerations in soil fertility experiments and fertilizer trials, it is crucial to identify the most limiting nutrients and the response of chickpea to their application. Thus, this study was entailed to generate basic information of soil nutrient status and soil sorbing capacities and identify the most growth-limiting nutrients in major soil types of Gununo Sub-Watersheds and determine the response of chickpea to application of the deficient nutrients,

2. Materials and Methods

2.1 Description of the Study Sites

The study was conducted on soil samples collected from Gununo sub-watershed is located at about 430 km south of Addis Ababa and 23 km west of Sodo town. It is situated between 6051' and 6056' N latitude and 370 39' and 370 40' E longitude with altitude ranging between 1880 to 1960 m.a.s.l. The topography of the area is characterized by undulating slopes divided by v-shaped valleys of seasonal and intermittent streams, surrounded by steep slopes. The dominant soils in the study area are Eutric Nitisols, very deep (>30 m), acidic in nature, and very highly degraded, mostly because of soil erosion, and crop production is very difficult. These soils originated from kaolinitic minerals which are inherently low in nitrogen and phosphorus (Waigel, 1986).

The main crops grown in the sites are cereals such as teff *(Eragrostis tef),* maize *(Zea mays),* wheat *(Triticum aestivum)*, barley *(Hordeum vulgare)*, pulses such as faba bean (*Vicia faba),* field pea *(Pisum sativum)*, haricot bean *(Phaseolus vulgaris)*, chickpea *(Cicer arietinum)*, and root and tuber crops such as potato *(Solanum tuberosum)*, sweet potato *(Ipomea batatas)* and enset *(Ensete ventricosum)*.

2.2 Soil Sampling and Sample Preparation

Thirty random samples (0-20 cm depth) were collected from dominant homogenous arable lands following random sampling technique and a composite was made. The bulk soil samples used for pot were crushed with a wood pestle and mortar to pass through a 2 mm sieve and separated experiment for physico-chemical analyses. The

portions of the samples for determinations of organic carbon and total nitrogen were further ground to pass through a 0.5 mm sieve.

2.3 Soil Analysis

Particle size analysis was carried out by the modified sedimentation hydrometer procedure (Bouyoucos, 1962). Soil pH was measured in the supernatant suspension of a 1:2.5 soil: water mixture by using pH meters. Organic carbon content of the soil was determined following the wet digestion method (Walkley and Black, 1934). Total N was analyzed by the Kjeldahl procedure (Bremner and Mulvaney, 1982). The available phosphorus content of the soil was analyzed using 0.5*M* sodium bicarbonate extractant at pH 8.5 and blue color development by ascorbic acid method (Olsen and Sommers, 1982).

Exchangeable basic cations and the cation exchange capacity (CEC) of the soils were determined using the 1*M* ammonium acetate (pH 7) method according to the percolation procedure (Van Reeuwijk, 1993). The exchangeable cations, Ca and Mg, in the leachate were determined by Atomic Absorption Spectrophotometer, whereas K and Na were measured by flamephotometer. For CEC determination, the excess ammonium was washed with 95% ethanol, the adsorbed ammonium was exchanged for Na by leaching with 100 ml of 10% NaCl solution. The amount of ammonium was determined by titrating against 0.1 *M* NaOH after adding four drops of methyl red indicator.

Sulphate was determined by potassium dihydrogen phosphate solution as extractant (Johnson and Fixen, 1990). Available micronutrients (Fe, Mn, Zn, and Cu) contents of the soils were determined by DTPA method (Lindsay and Norvell, 1978). The concentration of water-soluble boron was determined by hot water extraction (Sippola and Ervio, 1977).

2.4 Sorption Studies

2.4.1 Preparation of sorption treatment solutions

A series of 5 sorption treatment solutions were prepared by using stock solution from each nutrient source (Table 1).

Table 1: Concentrations of some essential nutrients used in sorption treatment solutions.

Sorption treatment solution No.	Concentration of elements in sorption treatment solutions (mg L^{-1})			
	P	S	Zn	Cu
1	20	10	1	1
2	40	20	2	2
3	80	40	4	4
4	160	80	8	8
5	320	160	16	16

2.4.2 Sorption of P, S, Zn and Cu

The quantitative description of soil nutrient sorption is important for the prediction of nutrients fertilizer requirements for optimum plant growth (Fox and Kamprath, 1970). Sorption of P, S, Zn and Cu by soils plays a crucial role among all nutrients. The sorption study for P, S, Zn and Cu were done in bottles using 2 checks and 5 treatments in duplicates. Five gm of soil was weighed into 12 bottles. Bottle No.1 was used as the check; where only 5 ml of distilled water was added. Five ml of sorption treatment solutions, 1 to 5 were added to bottles No 2 to No 6, respectively (Table 1). After the addition of sorption treatment solutions, the bottles were gently shaken to ensure complete mixing of the solution with the soil and allowed to incubate for 72 hours (Hunter, 1980). The bottles were placed in a dust-free place and allowed to air dry. After the samples were air dried, they were extracted and analyzed for available P, S, Zn and Cu following standard procedures as indicated in soil analysis.

A sorption curve was constructed for each element, plotting the amount of element extracted against the amount of element added. The amount of element added was equal to the concentrations of the sorption treatment solutions (Table 1). These sorption curves were used to determine the amount of elements to be added in the treatments of greenhouse experiment.

2.3 Greenhouse Studies

2.3.1 Determination of optimum levels of elements

Greenhouse experiment using chickpea as the test crop was conducted based on both soil analysis and sorption results. The amounts of nutrient elements extracted from the original soil sample were below 3 times the critical levels of the elements and the chemical fertilizers used were: Urea, TSP, Na_2SO_4, $ZnCl_2$, and $CuCl_2$ as sources of N, P, S, Zn and Cu, respectively. Then the amounts of these elements necessary to bring the levels to 3 times the critical levels were added. The critical levels considered for the nutrients were; P: 14, S: 14, Zn: 2 and Cu: 1 mg kg^{-1} soil (Hunter, 1980)

Table 2: Treatment descriptions and the amounts of elements added to the soils

Treatment No	Description	Amount of element added (mg kg^{-1} soil)
	Check	None
2	Opt.	N: 18, P: 190, S: 37, Zn: 11, Cu: 13.
3	Opt. (- N)	(-18)
4	Opt. (- P)	(-190)
5	Opt. (- S)	(-37)
6	Opt. (- Zn)	(-11)
7	Opt. (- Cu)	(-13)

Opt. = Optimum Treatment, Opt. - = Optimum Treatment without the indicated element

2.3.2 Plant data collection and sampling

The experiment was conducted in a greenhouse at Hawassa University. Three kg of soil was placed in plastic pot. The soil from each pot was spread in a thin layer on a paper sheet, measured amounts of stock nutrient were applied and the soil was mixed thoroughly before returning it to the pot. The treatments were arranged in a completely randomized design (CRD) with three replications.

Chickpea was used as test crop and six seeds were sown in each pot and thinned to 4 plants per pot at 21 days after emergence. The soils in all pots were maintained at approximately field capacity during the experimental period by watering with distilled water. Following each watering, leachates on the saucers were reapplied to the pots.

Nodulation assessment was undertaken at flowering stage (50%) by carefully uprooting two plants randomly from each pot and the plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to drain water from their surface. Nodules were counted and their average was taken as number of nodule per plant. Thereafter, the nodules were oven-dried at 70 ^{0}C for 48 hours and nodule dry weights were determined.

At physiological maturity (90 days), plant height was recorded and the above ground parts of the plants were harvested and fresh weights were measured. Contaminants were removed from the above ground parts by first dipping samples in 0.001 *M* HCl, and then washing with tap water and finally rinsed with distilled water. The above ground parts were dried in an oven at 70 ^{0} C to constant weight and shoot dry weights were measured. The dry plant material was finely ground using stainless steel grinder to pass through 1 mm sieve and preserved for analysis.

2.4 Plant Analysis

Nitrogen in the plant material was analyzed by modified Kjeldahl wet oxidation procedure that involves digestion of the sample to convert organic N to NH_4^+-N and determination of NH_4^+-N in the digest (Nelson and Sommers, 1973). One gram of plant material (dried at 450^0 C for 4 hours) was calcinated in a muffle furnace by placing into porcelain crucibles. The calcinated plant material was dissolved in 20 ml of 20% nitric acid and filtered for the determination of nutrient elements by filling the flask to 100 ml mark with distilled water. Ca, Mg, Mn, Fe, Zn and Cu in the filtrate were determined by Atomic Absorption Spectrophotometer (AAS), whereas K was measured by flame photometer. P determination was carried out in the digest aliquot obtained through calcinations. The P in the solution was determined colorimetrically by using molybdate and metavanadate for color development (Wolf, 1982). Sulfur was determined by magnesium nitrate dry ashing method as described by Wolf (1982). Boron in plant samples was measured colorimetrically using Azomethine-H by dry ashing with CaO (Sippola and Ervio, 1977).

2.5 Statistical Analysis

Statistical analysis was carried out on number of nodules, nodule dry weight, plant height, biological yield, grain yield, nutrient concentration and uptake using statistical analysis software (SAS, 1997 version 9.0). Mean separation was done using least significant difference (LSD) at 0.05 P level and correlation analysis was also conducted following Pearson correlation coefficients.

3. Results and Discussion

3.1 Major Physico-chemical characteristics of the experimental soils

The composite soil sample which was collected from the Gununo area before sorption study and greenhouse planting was analyzed for some of physical and chemical properties: texture, pH, EC, OC, Total N, CEC, available P, exchangeable bases and micronutrients (Table 1). Analytical results indicated that the soil texture of Gununo Nitisols was clay loam. Soil texture is a fundamental physical property of a soil and it is closely related to the water-holding capacity of the soils, since loams and clays hold more water than that of sandy soils (Brady, 2000). Thus, the soil of the study area has good water holding capacity, which creates a good growing media for chickpea.

The soil pH of the experimental soil is slightly acidic with pH values of 5.9 and thus within the range of optimum soil pH for crop production (Havlin et al., 1999). According to Havlin et al, (1999) soils are classified depending on their total nitrogen content (%), as very low (<0.1), low (0.1-0.15) medium (0.15-0.25), and high (>0.25). Thus, the experimental soil has low (0.15 %) nitrogen content. According to Landon (1991) the classifies for the organic carbon content of soils are: low (< 4), medium (4 - 10), high (> 10). The Experimental soil organic matter content is 1.26 % and this is considered to be low. The results are in accordance with the findings of Wakene and Heluf (2003), and Wondwosen (2008) who reported that intensive and continuous cultivation forced oxidation of organic carbon and thus resulted in reduction of Total N.

The cation exchange capacity (CEC) of the soils are medium in the soil, According to Landon (1991), CEC values are rated < 5 as very low, 5 - 15 as low; 15 - 25 as medium, 25 - 40 as high and > 40 as very high. According to Brady and Weil (2002), CEC depends on the nature and amount of colloidal particles. The exchange complex of the soils is dominated by Ca followed by Mg, K and Na (Table 3). According to Havlin et al. (1999), the dominance of Ca followed by Mg, K, and Na in the exchange site of soils is favorable for crop production. This result is in agreement with the findings of Wondwosen (2008) on Alfisols and Ultisols in Kindo Koye and Delbo area, Southern Ethiopia and Wakene (2001) on Alfisols around Bako area, Ethiopia.

Available phosphorus content was 3.5 mg per kg soil and this is considered to be low. The range of Olsen P ratings are very low (<3), low (4-7) medium (8-11), and high (>12) according to Havlin et al. (1999). According to Tekalign and Haque, (1991), Olsen soil tests for plant available P was recommended for all types of Ethiopian soils. The low availability of P in the soil may be due to the inherent P deficiency of the soils and the P fixation with Fe and Al, as these soil was slightly acidic in reaction (Table 3). Therefore, the soils is deficient in available P and require application of P fertilizer for optimum crop production.

The available sulfur content was 9.5 mg/kg soil. According to Havlin et al. (1999), the available S contents of the soils could be considered to be adequate for most crop production, although the value in the soil is below three times the critical value (42 mg S per kg soil). Adsorbed sulphate is an important fraction in highly weathered soils in regions of high rainfall containing large amounts of Al/Fe oxides. Adsorbed sulphate in highly weathered soils can contribute significantly to the S needs of plants because it is usually readily available (Havlin et al., 1999).

Table 3: Physico-chemical properties and available nutrients status of experimental soil

Soil property	Value
Soil Taxonomy	Nitisols
pH (1:2.5)	5.9
Organic carbon (%)	1.26
Total N (%)	0.15
Sand (%)	31
Silt (%)	36
Clay (%)	33
Textural Class	Clay Loam
Na [cmol(+) kg^{-1} soil]	0.12
K [cmol(+) kg^{-1} soil]	1.39
Ca [cmol(+) kg^{-1} soil]	9.06
Mg [cmol(+) kg^{-1} soil]	2.22
CEC [cmol(+) kg^{-1} soil]	23.49
K:Mg	0.63
Ca:Mg	4.1
Av. P (mg kg^{-1} soil)	3.35
Av. K (mg kg^{-1} soil)	545
Av. S (mg kg^{-1} soil)	9.5
Av. Fe (mg kg^{-1} soil)	27.2
Av. Mn (mg kg^{-1} soil)	7.7
Av. Zn (mg kg^{-1} soil)	1.3
Av. Cu (mg kg^{-1} soil)	0.11
Av. B (mg kg^{-1} soil)	1.10

The concentration of available micronutrients in the soil from highest to lowest concentration is Fe > Mn> Zn > Cu. The amount of Fe, Mn, Zn and Cu in mg kg^{-1} in the soil is 27.2, 7.7, 1.3 and 0.11, respectively, (Table 3). The micronutrient content of soils is influenced by several factors among which soil organic matter content, soil reaction and clay content are the major ones (Fisseha, 1992). According to critical values of available micronutrients set by Havlin *et al.* (1999) are 4.5, 1.0, 1.0 and 0.6 mg kg^{-1} for Fe, Mn, Zn, and Cu, respectively. The amount of Mn and Fe in the soil is above the critical limits and crops may not have a deficiency of these elements. However, the amount of Zn and Cu in the soil is below the critical value. Zinc and Copper are one of the yield limiting nutrients in experimental soil.

3.2 Sorption Characteristics of the experimental soils

Sorption curves for P, S, Zn and Cu were constructed for the determination of amounts of elements to be added to obtain extractable amounts equal to three times the critical value (the optimum level). The value for each element was obtained from the graph and converted to the respective fertilizer equivalents (Figure 1-4).

The amounts of P, S, Zn and Cu retention, as percent of applied nutrients, in the soil were 62-84, 3-67, 53-65 and 81-87%, respectively. The sorption results indicated that P, Zn and Cu were highly fixed by the soil. This could be due to the weathering stage of the soils. Based on the sorption curve the nutrient elements to be added to the optimum treatment were: P: 190, S: 37, Zn: 11 and Cu: 13 mg kg^{-1} soil.

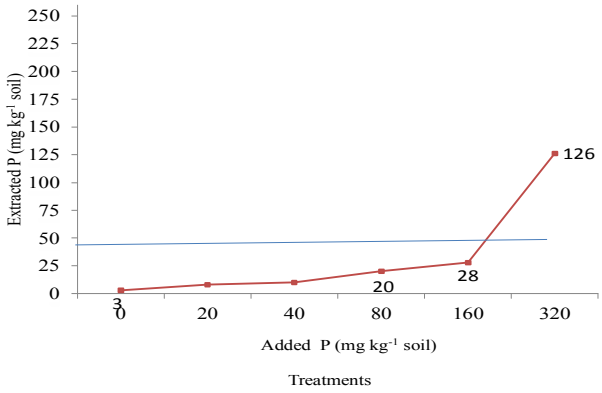

Figure 1: Phosphorus sorption of the experimental soils

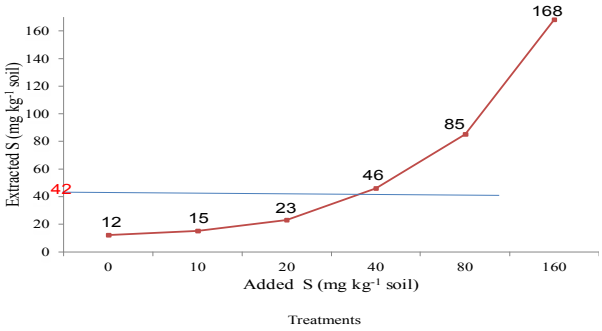

Figure 2: Sulfur sorption of the experimental soils

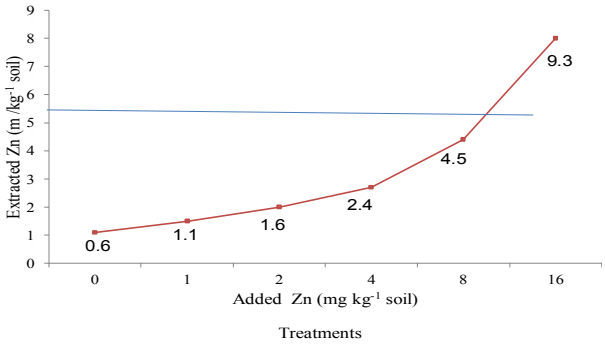

Figure 3: Zinc sorption of the experimental soils

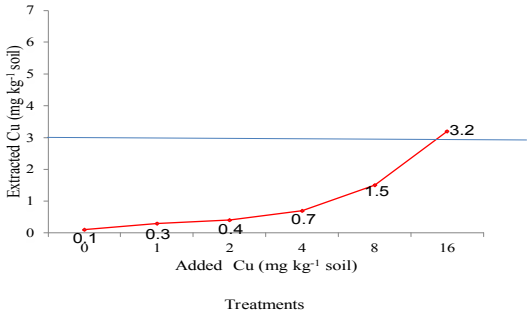

Figure 4 Copper sorption of the experimental soils

The clay mineralogy, presence of oxides and hydroxides of Fe and Al seem to be the dominant factors affecting P, Zn and Cu sorption. The mechanism of phosphate zinc and copper adsorption is considered to be mainly through replacement of hydroxyl ions on crystal lattice, and hydrated iron and aluminum by phosphate, zinc and copper ions. High sorption of phosphate by amorphous materials, and oxides and hydroxides of iron and aluminum have been reported by several investigators (Fitter, 1974 and Sahlemedhin and Ahmed, 1983). The P adsorption capacity by clay minerals depends, among other factors, on the proportion of surface area occupied by edge faces. Accordingly, kaolinite absorbs more P per unit surface area than 2:1 clay minerals (Havlin *et al*., 1999).

The sorption result showed that S was fixed by the soil in medium amount. This may be due to S adsorbed in oxides and hydroxides of iron and aluminum and clay minerals. According to Mengel and Kirkby (1987), sulphate like phosphate is adsorbed in sesquioxides and clay minerals, although the binding strength for sulphate is not as strong as that for phosphate.

3.3 Pot Experiment

3.3.1 Treatment effects on number of nodules per plant and nodule dry weight

Fertilizer treatments had a highly significant ($P \leq 0.01$) effect on number of nodules per plant (NNPP) and nodule dry weight (NDW). Higher NNPP, 10.3, was obtained from optimum treatments as compared to others. Similarly, the highest NDW was recorded in the optimum treatment in the soil (Table 4). This might be due to the adequate supply of nutrients, which possibly promoted the formation of lateral and fibrous root growth (Havlin *et al*., 2007). The increased root growth creates higher surface area (infection site) for the bacteria to form relatively higher number of nodules. Majid *et al*. (2009) also found increasing nodules per plant with increased phosphorus application.

Omission of N fertilizer did not differ from the optimum treatment in terms of NNPP. Low levels of N fertilizer supply as starter-N increased nodulation and total amount of nitrogen derived from N_2 fixation (George *et al.,* 1992). On the other hand, phosphorus-omitted treatment showed lower NNPP and NDW compared to all others. This might be due to the influence of P on root growth, photosynthesis, translocation of sugars, and other such functions which directly or indirectly influence N-fixation by legume plants.

Phosphorus fertilization enhanced number of nodules and nodule dry mass, as well as greater N_2-fixation ability per plant (Sessitsch *et al.*, 2002). Shu-Jie *et al*. (2007) also reported that application of P increased N concentrations in grain and root, and total N uptake. The omission of Zn showed lower NDW as compared to optimum treatment (Table 4), indicating that low levels of these nutrients affect root nodulation and the ability to fix enough atmospheric N to meet the N_2 needs of the plants. Misra *et al*. (2002) reported an increase of 55% in root nodulation and 26% in N content of nodules with application of 20 mg Zn kg^{-1} soil.

Number of nodules per plant was positively and highly significantly ($P \leq 0.001$) correlated with nodule dry weight, uptake and concentrations of P, and also significantly ($P \leq 0.01$) with dry matter. Similarly nodule dry weight was also positively and very highly ($P \leq 0.001$) correlated with number of nodules, dry matter, uptake and concentration of P in both soils (Appendix Table 1 and 2).

3.3.2 Plant height and dry matter yield

Plant height was significantly ($P \leq 0.05$) influenced by fertilizer treatments in the soil. The maximum mean plant heights were obtained from the optimum nutrient supply S- and Cu-omitted treatments in the soil. (Tables 4). Significantly ($P \leq 0.05$) lower plant heights were recorded in control and P-omitted treatments as compared to all the others, indicating that plant height was mainly affected by omission of P. These could be attributed to the pivotal role that P plays in early root growth and the formation of lateral fibrous and healthy roots.

Table 4: Number of nodules, nodule dry weight, plant height and biomass yield of chickpea as influenced by treatments

	At flowering (50 days)		At maturity stage (90 days)		
Treatment	**NN PP**	**NDW (g plant^{-1})**	**Plant height (cm)**	**DMY (g pot^{-1})**	**Grain yield (g/pot)**
Control	9^{c}	0.012^{c}	29.8^{c}	2.21^{d}	1.78^{e}
Optimum	27^{a}	0.071^{a}	40.4^{a}	4.73^{a}	2.62^{a}
Opt.-N	24^{a}	0.035^{b}	35.2^{abc}	4.32^{ab}	2.50^{ab}
Opt.-P	10^{bc}	0.021^{c}	32.2^{bc}	2.26^{d}	2.04^{de}
Opt.-S	22^{a}	0.074^{a}	38.4^{a}	3.78^{c}	2.15^{cd}
Opt.-Zn	20^{a}	0.053^{ab}	37.4^{ab}	4.18^{bc}	2.22^{bcd}
Opt.-Cu	20^{a}	0.063^{a}	40.1^{a}	4.29^{b}	2.46^{abc}
LSD (0.05)	**10.9**	**0.03**	**6.1**	**0.41**	**0.33**
CV (%)	**33.1**	**31.7**	**9.6**	**6.36**	**8.37**

Values followed by the same letter(s) within a column are not significantly different at $P \leq 0.05$, NNPP = number of nodules per plant, NDW = nodule dry weight, and DMY = dry matter yield

Dry matter yield of chickpea was highly ($P \leq 0.001$) influenced by the treatments in the soil. The highest dry matter yields were obtained from the optimum treatments. The reductions in dry matter yields were 53.3, 8.7, 52.2, 20.1, 11.6 and 9.3% in the control, omission of P, S, Zn and Cu in the soil, respectively as compared to optimum treatment. Thus, P, S, Zn and Cu in the soil were limiting nutrients to support good crop growth. This is attributed to the low levels of OC, available P, S, Zn, and Cu in the soils (Table 3) although the low total N was compensated by N fixing capacity of the test crop.

Most soils in the south central Rift Valley and Wolaita area are highly responsive to commercial fertilizers, especially P fertilizers (Sheleme *et al*., 2001). Ali *et al*. (2002) also reported that nutrient deficiencies cause yield losses of varying magnitude in chickpea, *e.g*., around 10% due to sub-optimal nodulation and hence nitrogen deficiency, 29–45% due to phosphorus, and 16-30% due to sulphur. The results are in agreement with the findings of Srinivasarao *et al*. (2006), who found that omission of optimum nutrients (N, P, S, and Zn) led to reduction of shoot biomass of urdbean and mungbean by 32% compared to untreated control under pot experiment. Similar results have also been reported by Ali *et al*. (2002), who found the yield losses due to deficiencies of N, P and S up to 10, 29-45 and 16-30%, respectively, in chickpea. Lifang *et al*. (2000) also reported that omission of N, P and K reduced dry matter yield by 56.4, 63.9 and 23.3%, respectively. Nagendra *et al.* (2007) found that omission of P, K and Zn in greenhouse experiments resulted in relative yields reduction by 66, 75 and 75%, respectively compared to the optimum treatment. As suggested by Ali *et al*. (2004), there is a high requirement of phosphorus by legumes as P affects growth of the host plant, and growth and function of the nodules. Additionally, Zinc fertilization to the soil, had a significant effect on chickpea plant height, biological yield, Zn concentration, and protein content (Khorgamy *et al*., 2009).

Dry matter yield of chickpea was positively and significantly ($P \leq 0.001$) correlated with nodule dry weight, uptake and concentrations of N and P and also significantly ($P \leq 0.01$) with number of nodules, plant height, grain yield and concentrations of Mn, Zn and Cu in plants grown in the soil.

Fertilizer application showed highly significant ($p \leq 0.001$) influence in grain yield. The highest grain yield was obtained from optimum treatment, whereas the lowest grain yields were recorded from the control, P- S- and Zn- omitted treatments which were significantly inferior to all other treatments (Table 4). The optimum nutrient treatment increased grain yield by 32 % over the control treatments. The magnitude of grain yield losses due to omission of P, S and Zn were 22-25, 18-24 and 15-26% respectively (Table 4). The results are in line with Ali *et al*. (2002) who reported that P and S deficiencies caused yield losses up to 29-45 and 16-30% respectively, in chickpea, Hussain (2010) also reported 15% decrease in seed yield of soybean due to omission of S application, whereas 22% yield increment chickpea was obtained due to S application (Shrinivasarao *et al.,* 2004).

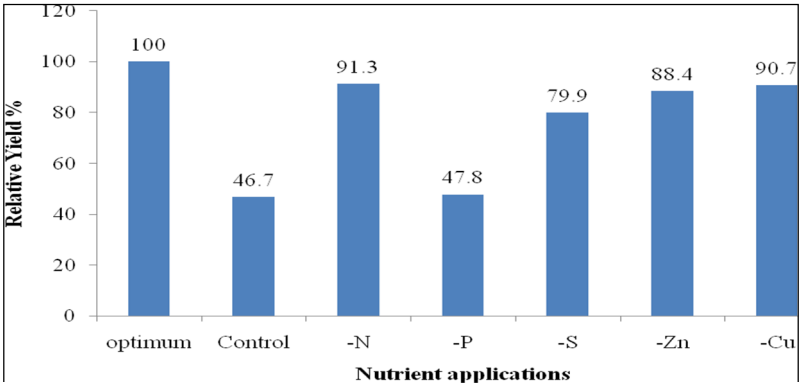

Figure 5 Relative biomass yields of chickpea as influenced by macro- and micro-nutrient application in Nitisols of Gununo under greenhouse condition

3.4 Macronutrients concentrations and uptake

Nitrogen concentration and uptake were significantly ($P \leq 0.001$) influenced by treatments (Appendix 1 and 2). Shoot and grain N concentrations in the plants with optimum treatment were 1.11 and 6.04 % respectively in the soil; The lowest values for these parameters were obtained from the control and P-omitted treatments (Table 5 & 6). Varies 66 to 85% of the N was found in grains indicating that grain is an important N sink in the plant and showing chickpea as an important source of protein. Higher N concentration in the grain than in stem might be due to the remobilization of large amounts of N from vegetative parts to pods during reproductive stage. The highest N content under optimum nutrient treatment could be attributed to adequate supply of starter N to initiate roots and infection sites and is in agreement with the finding of Srinivasarao *et al*. (2006), who reported 2.67% of N content in shoot of urdbean and mungbean, due to supply of optimum nutrient N, P, K, S, and Zn.

Table 5: Effect of nutrient application on macronutrients concentrations by chickpea shoot

Treatment	N	P	K	Ca	Mg	S
			%			
Control	0.89^{ab}	0.17^{b}	0.84	0.10	0.18	0.13^{b}
Optimum	1.11^{a}	0.35^{a}	0.98	0.09	0.23	0.39^{a}
Opt.-N	0.81^{b}	0.36^{a}	1.28	0.11	0.25	0.33^{a}
Opt.-P	0.54^{c}	0.20^{b}	1.15	0.10	0.18	0.35^{a}
Opt.-S	1.06^{a}	0.34^{a}	1.24	0.10	0.23	0.14^{b}
Opt.-Zn	0.99^{ab}	0.32^{a}	0.96	0.10	0.19	0.36^{a}
Opt.-Cu	1.02^{a}	0.35^{a}	1.22	0.10	0.22	0.37^{a}
LSD (0.05)	0.21	0.06	NS	NS	NS	0.08
CV (%)	13.1	12.5	24.4	25.3	18.3	16.5

Values followed by the same letter(s) within a column for each treatment are not significantly different at $P \leq 0.05$, NS (Not-significant)

Nitrogen concentrations in dry matter and grain of P-omitted treatments were very low (Table 5 & 6). Phosphorus plays a key role in the symbiotic N_2 fixation process by increasing the percent and amount of N in the harvested portion of the host legume (Marschner, 1995). Shu-Jie *et al*. (2007) found that P application on legumes can also increase N concentrations in grain and total N uptake. Results of the present study also confirm the dependency of biological nitrogen fixation on soil available P and are in line with the findings of Rehm and Schmitt (2002) who indicated P is an essential ingredient for rhizobium bacteria to convert atmospheric N_2 into ammonium (NH_4) form useable by plants. Similarly, Ahlawat and Sharma (1996) found that phosphorus application had significant effect on N and P uptake in grain and stem of chickpea as well as on total N and P uptake. Additionally, the N uptake in P-omitted treatments was also very low owing to low dry matter yield obtained from this treatment (Table 5 & 6). The low concentration of N in grain of Zn-omitted treatment might be due to the influence of Zn in increasing the ability of roots to fix atmospheric N. Similarly, Misra *et al*. (2002) reported an increase of 55% in root nodulation and 26% in N content of nodules with 20 mg Zn kg^{-1} soil.

Phosphorus concentration and uptake in chickpea were significantly ($P \leq 0.001$) influenced by the treatments in the soil (Appendix 1 and 2). Shoot and grain P concentrations in optimum treatment plants were 0.35 and 0.54 % in the soil and were higher than P-omitted and control treatments (Table 5 & 6). These values are above the critical limit in accordance with the ratings of Din *et al*. (1999), who indicated the critical P concentrations in whole shoots of chickpea ranging from 0.18 to 0.27%. Olivera *et al*. (2004) also indicated that phosphorus application to legumes increase plant biomass including nodule biomass and shoot phosphorus content and uptake due to the increased rate of nitrogen fixation. The values of P uptake and concentration in the control and P-omitted treatment were

very low compared to the other treatments, signifying that P was one of the limiting nutrients in these soils. These are in line with the lower dry matter yields obtained in P-omitted treatments (Tables 4) and with the low available P contents of the soils (Table 3). Schulze *et al.* (2006) indicated that legumes generally have higher P requirement because the process of symbiotic nitrogen fixation consumes a lot of energy.

Similarly, Calcium concentration and uptake in grain were significantly ($P \leq 0.001$) influenced by the treatments in the soil, whereas significant differences in its concentration in shoot was obtained only in plants grown in the soil (Appendix 1 and 2). Grain Ca concentrations in optimum nutrient treatment were 0.08 %, indicating that all were below the critical range (Table 5 & 6). Marschner (1995) indicated that the Ca content of plants varies between 0.1 and 5% of dry weight, and is usually lower than that of K. This low Ca uptake occurs because Ca can be absorbed only by young root tips and the uptake of Ca can also be competitively depressed by the presence of other cations such as K and ammonium which are rapidly taken up by roots (Mengel and Kirkby, 1987).

Table 6: Effect of nutrient application on macronutrients concentrations by chickpea grain

Treatment	N	P	K	Ca	Mg	S
				%		
Control	2.84^{d}	0.30^{c}	0.66^{c}	0.04^{bc}	0.11	0.10^{e}
Optimum	6.04^{a}	0.54^{a}	1.24^{a}	0.08^{a}	0.11	0.26^{a}
Opt.-N	3.80^{bc}	0.61^{a}	1.12^{a}	0.02^{d}	0.13	0.18^{bc}
Opt.-P	3.49^{cd}	0.40^{b}	1.18^{a}	0.04^{bc}	0.13	0.16^{cd}
Opt.-S	4.13^{bc}	0.55^{a}	1.24^{a}	0.03^{bcd}	0.13	0.12^{de}
Opt.-Zn	3.78^{bc}	0.56^{a}	1.08^{a}	0.03^{bcd}	0.12	0.20^{abc}
Opt.-Cu	4.40^{b}	0.62^{a}	0.86^{b}	0.05^{b}	0.13	0.22^{ab}
LSD (0.05)	0.67	0.09	0.18	0.02	NS	0.05
CV (%)	9.41	10.3	9.6	26.0	21.1	14.4

Values followed by the same letter(s) within a column for each treatment are not significantly different at $P \leq 0.05$, NS =Not significant.

Sulfur concentration and uptake in shoot and grain were significantly ($P \leq 0.001$) different under various treatments in the soils. The plants with optimum nutrient treatments had respective shoot- and grain-S of 0.39 and 0.26 % (Table 5 & 6). Shoot S concentration under all the treatments was within the critical range, whereas the concentrations of S in grain were below the critical range except for optimum treatments in the soil. The result showed that low uptake and concentration of S in shoot in the control and S-omitted treatments in the soil may be due to inadequate available S in soils indicating the possibility of response to S application, particularly by sensitive crops in the study area. This finding corroborates the available S contents of the soils (Table 3).

Table 7: Micronutrients concentrations in the shoot and grain of chickpea as influenced by treatments in the soil

Values followed by the same letter(s) within a column in the treatment are not significantly different, NS =Not-significant.

Treatment	Fe		Mn		Zn		Cu	
	μg g^{-1}							
	Shoot	Grain	Shoot	Grain	Shoot	Grain	Shoot	Grain
Control	599	254^{c}	118	42^{bc}	36.0^{b}	29.4^{d}	8.5^{c}	10.7^{bc}
Optimum	902	325^{b}	117	56^{a}	63.3^{a}	53.1^{a}	13^{a}	14^{ab}
Opt.-N	611	223^{c}	121	31^{cd}	60.2^{a}	45.3^{ab}	10.8^{ab}	9.3^{c}
Opt.-P	809	224^{c}	104	56^{a}	35.0^{b}	42.3^{bc}	13^{a}	12^{ab}
Opt.-S	962	326^{b}	128	48^{ab}	63.1^{a}	54.6^{a}	10.3^{b}	15^{a}
Opt.-Zn	675	488^{a}	120	27^{d}	41.3^{b}	31.6^{cd}	10.0^{b}	10.7^{bc}
Opt.-Cu	512	462^{a}	95	41^{bc}	57.0^{a}	48.5^{ab}	4.8^{c}	6.3^{d}
LSD (0.05)	NS	40	NS	12.8	9.4	10.8	2.6	3.5
CV (%)	28.7	7.0	16.2	16.9	10.6	14.2	14.5	17.7

3.5 Micronutrient concentrations and uptake

Iron uptake in the soils and concentration in shoot of plants was not significant (Appendix 1 and 2). The critical Fe concentration in plant is about 200 μg g^{-1} dry weight (Marschner, 1995), and the results showed all treatments had above the critical range for chickpea (Table 7). Relatively high concentrations of Fe in grain were observed in Zn- and Cu-omitted treatments. In general, Zn and Cu application decreased Fe concentration, which might be due to ionic competition among these nutrients during nutrient uptake. Similarly, Asgelil *et al*. (2007) reported that crops grown in the soil didn't show deficiency of Fe in Ethiopia, and Zn fertilization affected accumulation of Fe and Mn in shoot dry matter of chickpea (Khan, 1998a). Earlier, in chickpea grown under greenhouse condition

application of 100 mg P kg^{-1} resulted in increased uptake of Fe in plants (Tufemkci *et al.,* 2005). Islam *et al.* (2009) also reported that P and S application increase Fe uptake in stem by 74 and 70% respectively, over the control.

Manganese uptake and concentration also differed significantly ($P \leq 0.001$) with varying treatments in the soil (Appendix 1 and 2). The concentration of Mn in shoot and grain for optimum nutrient treatment were 117 and 56 μg g^{-1} (Table 7). Srinivasarao *et al*. (2003) recorded Mn concentrations of 256-285 μg g^{-1} in shoot of chickpea. Islam *et al.* (2009) reported that P application increased Mn uptake in stem of chickpea by 34% over the control. The concentration of Mn in grain was affected by all treatments in the soils indicating that at higher nutrient application rates other cations may also compete with Mn for transport across membranes. The results corroborate with Asgelil *et al*. (2007) who reported absence of Mn deficiency on crops grown on Vertisols of Ethiopia.

Similar to Fe and Mn, Zn concentration and uptake in chickpea plants were also significantly ($P \leq 0.001$) affected by the treatments in the soil (Appendix 1 and 2). The concentration of Zn was higher in the soils with optimum nutrient supply and N, S and Cu omitted treatments in the soils than other treatments. Zinc concentration in shoot and grain in optimum nutrient treatment was 63.3 and 53.1 μg g^{-1}, which were above the critical levels of 20-21 μg g^{-1} in shoot as indicated by Khan *et al*. (1998). However, the concentrations of Zn in grain of control, P- and Zn-omitted treatment were lower than that of optimum treatment in the soils (Table 7), indicating the need for Zn application. Asgelil *et al*. (2007) also reported deficiency of Zn and Cu in 43 to 87% of the total plant samples from Vertisols and Nitisols of Ethiopia.

Similar to other micronutrients, the concentrations and uptake of Cu significantly ($P \leq 0.001$) differed due to different treatments in the soil (Appendix 1 and 2). Copper concentrations in shoot and grain in optimum nutrient treatment were 13 and 14 μg g^{-1} (Table 7), which were above the critical range for chickpea as per Cabrera *et al.* (2003) who indicated the critical levels of Cu ranging between 1.5 and 5.0 μg g^{-1} in legumes. Low uptake of Cu was observed in Zn-omitted treatment in the soil (Table 7).

4. Conclusion

Considering the laboratory analyses and sorption studies, P, Zn, and Cu had the highest potential in limiting yield, although there were also high probabilities of S in the soil to limit the yields. Nutrient deficiencies in Nitisols were in the order; P > S > Zn > N > Cu.

Based on the results, P, Zn and Cu in the soil were the most limiting nutrients to support good chickpea growth and development. These indicate that the soils of the study areas were inherently poor in P, Zn and Cu contents to support good chickpea growth. Also it is likely to have response to S application in the soils. Thus, S in the soil are probably the limiting nutrients to support good crop growth. Therefore, external supplies of fertilizers containing these nutrients could be recommended to improve chickpea production in the study areas.

In general, this research work has given a general picture of available nutrient status and the extent of their deficiencies in Gununo sub-watersheds. The information generated from the present study would assist in developing highly productive, sustainable and ecologically stable land use and management strategies for chickpea in the study areas. It is also expected that people living in the areas and any individual or organization who intend to invest and /or introduce new agricultural technologies in these areas will benefit from the generated data.

However, more detailed research and field experimentation are needed on P, S, Zn and Cu in the soils, as they are found to be deficient both in plants and soils. Additionally, determination of the soils' nutrient adsorbing capacities and residual release should be considered to develop a sound fertilizer recommendation.

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APPENDICES

Appendix Table 1. Inter-correlation coefficients (r) of number of nodules, nodule dry weight, plant height, dry matter yield, grain yield and nutrient concentration in shoot

	No. of nodule	Nodule dry weight	Plant height	Dry weight	Grain yield	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
NNPP	1.00														
NDW	0.85***	1.00													
PH	0.49*	0.57*	1.00												
DW	0.82***	0.75**	0.66**	1.00											
GY	0.39	0.49*	0.59**	0.65**	1.00										
N	0.44*	0.61***	0.35	0.54*	0.46*	1.00									
P	0.81***	0.78***	0.73***	0.85***	0.63**	0.43*	1.00								
K	0.30	0.31	0.28	0.36	0.39	-0.01	0.39	1.00							
Ca	0.13	0.11	-0.37	-0.04	0.11	0.21	-0.08	0.10	1.00						
Mg	0.45*	0.31	0.16	0.40	0.22	0.23	0.42	0.28	0.16	1.00					
S	0.28	0.15	0.26	0.47*	0.50*	-0.03	0.33	0.23	0.01	0.01	1.00				
Fe	-0.25	-0.15	0.25	-0.06	0.35	-0.11	0.09	0.43*	-0.06	-0.16	0.17	1.00			
Mn	0.21	0.23	0.07	-0.03	-0.07	0.25	0.09	0.15	0.37	0.42*	-0.26	0.21	1.00		
Zn	0.70***	0.63**	0.53*	0.73***	0.44*	0.54*	0.74***	0.31	-0.05	0.52*	0.21	-0.10	0.11	1.00	
Cu	-0.11	-0.14	-0.11	-0.08	0.07	-0.29	-0.08	0.67***	0.22	0.06	0.03	0.52*	0.34	-0.13	1.00

Number of observations (*n*)"24. *, ** and *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively.

Appendix Table 2. Inter-correlation coefficients (r) of number of nodules, nodule dry weight, plant height, dry matter yield, grain yield and nutrient uptake of shoot

	No. of nodule	Nodule dry weight	Plant height	Dry weight	Grain yield	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
NNPP	1.00														
NDW	0.85***	1.00													
PH	0.59*	0.57*	1.00												
DW	0.82***	0.74***	0.65**	1.00											
GY	0.38	0.49*	0.59**	0.65**	1.000										
N	0.71***	0.78***	0.60**	0.91***	0.69**	1.00									
P	0.83***	0.77***	0.70***	0.96***	0.66**	0.85***	1.00								
K	0.70***	0.66**	0.55**	0.87***	0.62**	0.79***	0.84***	1.00							
Ca	0.79***	0.68**	0.31	0.79***	0.49*	0.76***	0.74***	0.71***	1.00						
Mg	0.77***	0.65**	0.53*	0.87***	0.57**	0.80***	0.83***	0.83***	0.79***	1.00					
S	0.58**	0.45*	0.48*	0.80***	0.63**	0.70***	0.73***	0.63**	0.59**	0.61**	1.00				
Fe	0.36	0.39	0.64**	0.67***	0.69***	0.63**	0.60***	0.76***	0.39	0.56*	0.64**	1.00			
Mn	0.82***	0.76***	0.60**	0.85***	0.49*	0.78***	0.81***	0.83***	0.75***	0.87***	0.60**	0.66***	1.00		
Zn	0.78***	0.69***	0.63**	0.92***	0.59**	0.86***	0.91***	0.82***	0.75***	0.85***	0.71***	0.64**	0.81***	1.00	
Cu	0.50*	0.43*	0.36	0.65**	0.47*	0.53*	0.62**	0.85***	0.53***	0.62**	0.46*	0.74***	0.73***	0.60**	1.00

Number of observations (*n*)"21. *, ** and *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively