



Green Grams (*Vigna radiata* L.) Performance in Post Mined Soils When Subjected to Organic and Inorganic Fertilizers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Though mining promotes global industrialization and economy, it disrupts natural ecosystem that calls for sustainable reclamation. A field study was conducted at Base titanium limited - Kwale County to investigate the potential of mined soils to support growth and yield of green grams when subjected to organic and inorganic fertilizers. A two factor experiment was laid down in RCBD with

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split plot arrangement and three replications. Green gram varieties (KS20 and N26) and amendments (organic and inorganic fertilizers) were tested. Obtained data that included: soil properties, plant height, leaf development, pods and grains per plant, root nodules, biomass, and grain yield was subjected to ANOVA using the Genstat statistical package and means ranked using Fisher's protected least significant difference at ($P \leq 0.05$). Results showed that combined manure and fertilizer significantly improved plant height by 36% and 36.4% for KS20 and N26 varieties respectively in season one and, 23% and 23% for KS20 and N26 varieties respectively in season two compared to unfertilized soils. Similarly, leaf development was increased by 54.9% and 52.2% for KS20 and N26 varieties respectively in season one and, 46.1% and 37% for KS20 and N26 varieties respectively in season two compared to unfertilized soils. Sole manure and sole fertilizer did not have significant effect on plant height and leaf development. Combined application of manure and fertilizer did not have significant influence on green gram nodulation, shoot and root dry matter accumulation, number of seeds per pod and pods per plant. It however increased N26 variety grain yield in the second season alone. Additionally, grains harvested from the post mined soils had adequate levels of phosphorus, Iron, Zinc, magnesium, potassium, and low levels of calcium and copper. Although the findings show that this post mined soils have the capacity to support green gram growth, further research to ascertain long term effects of green gram establishment on profile development, biological nitrogen fixation, enterprise return to capital and crops response after application of all limiting nutrients is recommended.

Keywords: Inorganic fertilizer; manure; mining; reclamation; green grams.

1. INTRODUCTION

Green gram (*Vigna radiate* L.) is an important pulses crop in Kenya. It is a short duration legume grown as the second most important legume after common bean and a key legume for climatically marginal areas [1]. According to Economic survey [2] report, about one million farmers in Kenya cultivate an average of 260,000 ha of green grams with five years average national production ranging from 61,000 MT to 121,000 MT compared to national consumption of between 58,000 MT to 127,000 MT. Most of the yields are consumed locally with less than 20% being exported [2]. Apart from grain production, green gram is used as livestock feed, cover and rotation crop, green manure source, as well as source of soil Nitrogen [3,4,5]. According to Marandu [6], the crop has the ability to add about 30-40kg N/ha in a single season through biological nitrogen fixation.

According to GOK [7], KIHBS [8], area under green gram production and consumption rate has been rising steadily from 1978, while production per unit area has been fluctuating creating a production-consumption deficit. Current countries average green gram yield lies within 0.5-0.6 tons/ha which fall far below the countries crop potential and compares unfavorably with the global average yields of 0.73 tons/ha [9,10]. This production is not able to meet countries demand forcing the country to take up about 80% of the green grams exported by Uganda and Tanzania.

Mining is a vital activity that supports income generation, employment creation, industrial raw materials and economic growth worldwide. Notwithstanding the noteworthy role played by the industry, mining is categorized as a form of land degradation that interrupt ecosystem balance through loss of above and below ground biodiversity and overall soil health [11]. Closure of mining process marks the commencement of post mining reclamation process which encompasses returning soil into exhumed areas and initiating soil reconstruction by allowing colonization by either indigenous plant species or new species more so trees and grasses. Although mined soils can be reclaimed through crop farming to contribute towards food security, this approach has not been exploited and documented widely across the globe. The current research therefore aimed at establishing the performance of green gram crop in post mined soils when subjected to manure and inorganic fertilizers.

2. METHODOLOGY

2.1 Site Description

The study was carried out at Base Titanium Limited (BTL) Company located in Kwale County (4.1816°S,39.4606°E), Kenya. The area is generally warm throughout the year with temperatures ranging between 24.2°C during the coldest months (June and July) and 27°C during the hottest months (January and February). The

site experiences low, erratic and unevenly distributed annual rainfall ranging from 900-1350 mm per annum. The original soils within the area are well drained, red to dusky red, very friable, sandy clay loam to clay, with a topsoil of loamy sand to sandy: rhodic Ferralsols [12]. The mined soils were reconstructed and rehabilitated for crop production. This was done by overlaying sand on the parent rock followed by compacting (for soil stability) a layer of sand and clay mixture then covering with approximately 10-20cm thick top soil.

2.2 Test Materials

Planting materials used were two green gram varieties, KS20 and N26 obtained from KARLO research institute in Mtwapa, Kenya. These green gram varieties were used because they are commonly grown in Kwale county, high yielding, early maturing and drought tolerant [5]. Fertilizer materials used were DAP (18:46:0) as basal fertilizer to supply starter Nitrogen and Phosphorus while Muriate of Potash (MOP) was used to supply potassium which was found limiting in the mined soils after soil analysis. These inorganic fertilizers were obtained from Agrochemical shops in Mombasa, Kenya. Farm yard manure sourced from farmer's farms was also used in the trials.

2.3 Experimental Design, Layout and Crop Husbandry

Fields experiments were carried out from April to October, 2021. A two factor experiment was laid down in Randomized Complete Block Design (RCBD) with split plot arrangement with three replications. Green grams varieties (KS20 and N26) was tested at main plots level while soil amendments, namely starter NP (F), farmyard manure (M), starter NP + manure (M+F) and control (C) were tested at subplot level. During crop establishment, the green grams were planted at a spacing of 45cm by 30 cm. The main plots measured 11m x 3m and were separated by a path of 1.5 m while subplots measured 3m x 2m. The subplots were separated by 1m path. The total experimental field measured 22.5m x 11m with a plant population of 1080 plants.

Land preparation was done manually using jembes and pangas and planting holes dug to a depth of 10cm. Farm yard manure was applied One week before planting at the rate of 10 tons/ha (or a handful of 1kg per hole) and thoroughly mixed with soil. Diammonium

phosphate (DAP), fertilizer was used during planting as basal fertilizer at a rate of 210 kg/Ha (or two tea spoonful per hill) as a source of starter N, and P. During planting two seeds per hole were dibbled directly in the field at a spacing of 45x30 cm then in one week's time after germination, thinned to one seed per hole. Muriate of Potash was side applied 21 days after germination at a rate of 20 kg/ha (or five grams per hill) as a source of potassium while foliar fertilizer was applied to supplement nutrients such as magnesium, molybdenum, boron, copper and zinc. Foliar fertilizers were applied at vegetative and grain filling stage at the rate of 1.5 L/ha. Weeding were done two weeks after planting and five weeks thereafter, by manually uprooting visible weeds to ensure weed free conditions. Sprinkler irrigation was applied twice a week to supplement available soil moisture. Pests and diseases noted during the experimental period included aphids and powdery mildew which were controlled using ACTARA 50 EC and Metalaxyl W (Ridomil Gold) respectively.

2.4 Data Collection

Data collected include: Initial soil chemical properties, plant growth and yield parameters. Initial soil chemical properties were determined at the beginning of first cropping season. Soil samples were taken randomly from six points using soil auger at 0-20cm and 20-40cm depth. The samples were thoroughly mixed and a 1.0 kg composite sample for each depth packed and taken to the laboratory for analysis. The soil was analyzed for pH, total nitrogen (N), available phosphorus (P), available potassium(K), available calcium (Ca), available magnesium(Mg), available sulphur (S), available zinc(Zn), available copper(Cu) boron(B), available iron(Fe), available sodium(Na) and available manganese(Mn), CEC, sodium percentage and organic matter following procedures described by Okalebo et al. [13].

For crop growth and yield parameters, five plants were tagged randomly from the inner three rows per plot 14 days after sowing. The five plants were used for data collection during experimentation. Data collected included: plant height, number of leaves, nodulation rate, pods per plant, biomass accumulation, grain yield and nutrient analysis.

Green gram plant height was determined by measuring the height of each tagged plant, per plot from the base to the tip of leaf of the main

shoot of a plant using tape measure and recorded in centimeters (cm). The measurement was done on weekly bases from two weeks after emergence till physiological maturity.

Number of leaves was established by counting the total number of leaves in each tagged plant per plot on weekly bases two weeks after crop emergence till physiological maturity.

Simple random sampling technique was used in the collection of root nodule samples to increase the likelihood of obtaining representative nodule samples within the plant population. In each plot, two plants with intact roots were carefully dug up from the soil and the roots were washed carefully to remove sand and then placed in sterile plastic bag. Total number of nodules in each plant was counted and recorded. To determine the quality of the nodule; every single nodule was dissected to observe the color, pink /red indicated live (effective) nodules while other colors like green and white indicated dead (ineffective) nodules. The sampling was carried out three times from flowering to physiological maturity stage.

Number of grains per pods and pods per plant was determined by counting the number of grains and pods in the five tagged plants per sub-plot on weekly basis from the day of first pod formation till harvesting. For final yield determination, the total weight of grains obtained from pods harvested in each sub-plot during harvesting time was used to calculate total yield per plot and per area. During harvesting, both above ground and below ground biomass was weighed when fresh and then taken to the laboratory for dry matter testing. The root and plant samples of greengrams were oven dried at 65°C for about 48 hours until constant weight then weighed to determine plant biomass. Grain samples obtained from the yields were air dried to a moisture content of 15% and then taken to the laboratory for nutrient analysis. The following nutrients were analyzed; dry matter, Phosphorus, Potassium, Magnesium, Calcium, Sulphur, Manganese, Molybdenum Sodium, Boron, Iron, Copper, and Zinc. The total N, available P, exchangeable K and available S of plant samples were determined following semi micro Kjeldahl method, modified Olsen method, NH₄OAc extraction method and CaCl₂ extraction method, respectively [13].

2.5 Data Analysis

All obtained data was subjected to statistical analysis as per the procedure prescribed for

split-plot design (SPD) to obtain the analysis of variance (ANOVA) using the GenStat statistical package [14] and treatment effect was tested for significance using F-test at a 5% level of significance. Means were ranked using Fisher's protected least significant difference test at a 5% level of significance.

3. RESULTS

3.1 Initial Soil Fertility Status of Research Site

3.1.1 Initial soil chemical properties

Initial soil analysis showed that the soils had slightly acidic (pH6.1-6.4) reaction with optimal levels of available calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and Zinc (Zn) (Table 1). Available phosphorus (P), potassium (K), Sulphur (S), boron (B), copper (Cu), cation exchange capacity (CEC), total nitrogen (TN) and organic matter (OM) levels were however, observed to be low.

3.2 Green Gram Growth as Affected by Manure and Inorganic Fertilizer in Post Mined Soils

Green gram growth differed significantly ($P \leq 0.05$) on application of manure and / fertilizer in the post mined soils (Table 2). Combination of manure and inorganic fertilizer was observed to support the highest plant height and leaf development in all green gram varieties over the two cropping seasons while unfertilized soils supported the least. Manure + fertilizer increased plant height by 36% (KS20) and 36.4% (N26) varieties in season one and 23% (KS20) and 23% (N26) in season two compared to unfertilized soils. Similarly, leaf development was increased by 54.9% (KS20) and 52.2% (N26) in season one and, 46.1% (KS20) and 37% (N26) in season two compared to unfertilized soils. There was no observed difference between sole manure and sole organic fertilizer effects on plant height in season one and two for both KS20 and N26 varieties. Similarly, leaf development did not differ between sole manure and sole fertilizer for KS20 and N26 variety in both seasons.

3.3 Response of Green Grams' Nodule Development to Manure and Fertilizer in Post Mined Soils

Post mined soils were observed to have insignificant ($P \leq 0.05$) effect on nodule formation

even after manure and fertilizer application (Fig. 1). Although soils fertilized with manure + fertilizer seemed to support higher nodules compared to all other treatments, the difference was not significant.

3.4 Green Grams' Grain Dry Matter Accumulation and Yield (Tons/ha) as Influenced by Farmyard Manure and Inorganic Fertilizer in Post Mined Soils

Post mined soils showed varied influence on green gram-dry matter accumulation and grain yield after application of manure and fertilizer (Table 3). Application of manure and fertilizer on post mined soils did not have significant ($P \leq 0.05$) effect on shoot and root dry matter yield throughout the two cropping seasons. Pods per plant was only significantly different for KS20 in season one while grains per pod was significant only for N26 in season two.

3.5 Nutrients Levels of Green Gram Grains Grown in Post Mined Soils Treated with Manure and Inorganic Fertilizer

The concentration of phosphorus ($P > 0.38\%$), Iron ($Fe > 59$ mg/kg), Zinc ($Zn > 27$ mg/kg), Mg > 0.16 mg/kg, and potassium ($K > 0.96\%$) was observed to be optimal in grains from all treatments (Table 4). Conversely, the levels of calcium ($< 0.113\%$) and Copper ($Cu < 10$ mg/kg) were low in all treatments.

4. DISCUSSION

4.1 Initial Soil Properties

Initial soil analysis indicated that the soils had sandy loam texture with slightly acidic (6.0-6.4) Ph; attributable to original soils mineralogy [12]. The Observed levels of exchangeable phosphorus ($P < 20$ ppm), potassium ($K < 120$ ppm), Sulphur ($S < 20$ ppm), Boron ($Bo < 0.8$ ppm), Copper ($Cu < 1.5$ ppm), Total nitrogen ($< 0.2\%$) and CEC < 15 Cmolkg⁻¹ are classified as low by Landon [15] and unable to support optimal crop growth and productivity. Existence of such low nutrient levels

is a common occurrence in post mined soils [16]. This is a result of disruption of soil components such as above and below ground biodiversity, horizonation, aggregate stability, as well as crucial nutrient cycles [17,18,19].

4.2 Green Gram Growth and Yield as Affected by Manure and Inorganic Fertilizer in Post Mined Soils

Application of both sole manure and sole fertilizer was observed to have insignificant effect on green gram height and leaf count in the post mined soils. Combined application of manure and fertilizer however significantly increased green gram height and leaf development. The significant increase of height and number of leaves on application of manure and fertilizer can be attributed to farmyard manure - inorganic fertilizer synergistic effect on soil properties. While inorganic fertilizer has the ability to provide required nutrients in right amounts and forms, manure manipulates soil physical, chemical attributes by acting in the long run leading to improved temperature regulation, nutrient and water retention, biotic activities and nutrient uptake [20,21,22,23]. Owing to the fact that the post mined soils had sandy loam texture with compromised structure and horizonation, most of the nutrients applied through sole fertilizer might have not been fully utilized by plants due to losses through leaching, seepage and runoff. Since manure releases nutrients slowly to the soil solution for plant uptake, the sole manure treated plants might not have had access to adequate nutrients leading to the slow growth. Combination of manure and fertilizer on the other hand might have improved the soil attributes leading to relatively better temperature regulation, water and nutrient holding capacity, mineralization, biotic activities hence presence of readily available nutrients within the exchange complex for crop uptake. Correspondingly, the additive effect on nutrient supply and improvement of soil physical conditions, checks N losses and conserves soil N by forming organic-mineral complexes, thus ensuring continuous N availability [24]. This results collaborate findings by [19,25,26,27,28,29,30].

Table 1. Initial soil chemical properties of post mined soils

Variable	pH	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	CEC	TN	OM
Value	6.44	10.6	109	509	100	15.4	159	196	0.43	0.87	2.39	4.29	0.08	1.65
Remarks	O	L	L	O	O	L	O	O	L	L	O	L	L	L

P-phosphorus, *K*-Potasium, *Ca*-Calcium, *Mg*-Magnesium, *S*-Sulphur, *Fe*-Iron, *Mn*-Manganese, *B*-Boron, *Cu*-Copper, *Zn*-Zinc, *CEC*-Cation exchange capacity, *TN*-Total nitrogen, *OM*-Organic matter; *O*-Optimal; *L*-low

Table 2. Response of green grams’ height and leaf development to manure and fertilizer application in post mined soils

	Plant Height (cm)				Number of leaves			
	KS20 Variety		N26 Variety		KS20 Variety		N26 Variety	
	S1	S2	S1	S2	S1	S2	S1	S2
Control	49.60a	33.00a	63.20a	41.20a	9.20a	9.13a	12.73a	14.53a
Manure	64.40ab	34.00a	94.90b	42.70a	15.00ab	16.67b	18.73a	19.80b
Fertilizer	69.40b	39.30a	95.50b	48.60a	20.20b	17.70b	19.57a	20.67bc
Manure + Fertilizer	77.80bc	42.90b	99.30b	54.80b	20.40b	19.10b	23.60b	23.07bc
LSD	19.63	8.25	19.63	10.25	7.839	4.036	7.839	4.036
% CV	16.5	15.3	16.5	15.3	25.5	15.7	25.5	15.7

LSD- least significant difference; CV- covariate of variance; S1- season 1, S2- season 2

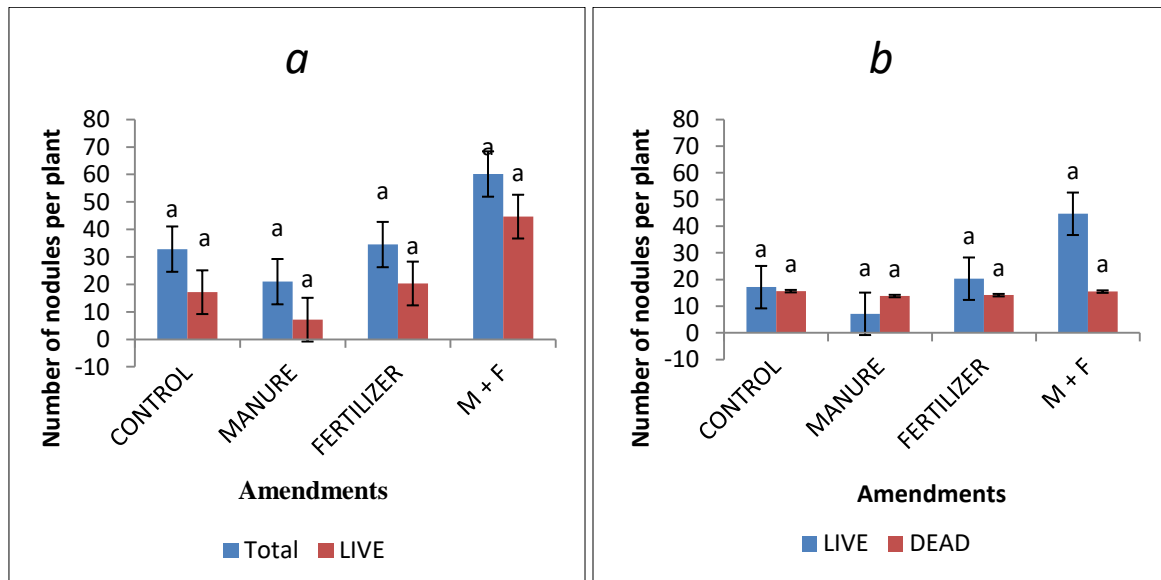


Fig. 1. Average number of root nodules in (a) KS20 and (b) N26 green gram varieties as influenced by manure and inorganic fertilizer in post mined soils after two cropping seasons

Nodule formation was not significantly affected by application of either manure or fertilizer in this post mined soils. It was also observed that the nodule numbers were also relatively low. The low nodulation rate in all treatments can be attributed to either prevailing low levels of rhizobium or critical nodulation nutrients in the disturbed soils. From the initial soil analysis, it was noted that the post mined soils were low in starter nitrogen, phosphorus and some essential nutrients such as calcium, cobalt, copper, iron, potassium, molybdenum, that according to [31,32,33,34] plays critical role in nodulation process. This study contradicts research findings by Muwal and Dhaked [35] who reported higher nodulation in soils fertilized with manure and fertilizer compared to sole manure or fertilizer.

Application of either manure or fertilizer or both did not have significant effect on overall dry

matter accumulation. Conversely, grain yields were only significant for N26 variety during the second season. The poor response of dry matter accumulation and grain yield to manure and/ or fertilizer application in this post mined soils can be attributed to inadequate uptake of nutrients and water from the soils during the critical growth stages. According to Esilaba [36], MALF&C [10], Singh [37] green gram requires adequate nutrition during vegetative growth, flower initiation and pod filling. Levels below optimum during this stages lead to either poor growth or yield. From the soil analysis reports, the post mined soils had initial low levels of nutrients and the sandy loam texture coupled with shallow depth might have negatively affected water and nutrient dynamics within the cropping season leading to reduced root development, vegetative growth, flowering, pod formation, seed set and ultimate grain

yield. This finding contradicts findings done on normal farm lands containing undisturbed soils by researchers such as [19,35,38,39,40,41,42] who observed that the combined use of manure and inorganic fertilizer resulted in higher yields than application of sole manure or sole fertilizer.

Adequate levels of phosphorus, Iron, Zinc, magnesium, potassium in grains harvested from all treatments can be ascribed to the crops ability to extract limited nutrients from the soils and translocate in the plant system. As a shallow

rooted dry land crop green grams are known to be hardy and have the ability to exploit available water and nutrients within soils. The low levels of calcium and copper in the grains can be attributed to the low initial levels of the nutrients within the post mined soils and the fact that planting fertilizers used were straight fertilizers lacking calcium and copper. This finding contradicts Muwal and Dhaked [35], who reported higher nutrient content in grains from soils treated with combination of manure and fertilizer compared to sole manure and sole fertilizer.

Table 3. Dry matter yield (Tons/Ha), pods per plant, grains per pod and grain yield (Tons/Ha) as influenced by manure and fertilizer in post mined soils

Season One	Dry Matter Yield (Tons/ha)		Pods per plant		Grains per pod		Grain Yield (Tons/ha)			
	Shoot	Root	Shoot	Root	KS20	N26	KS20	N26		
Variety/Treatment	KS20	N26	KS20	N26	KS20	N26	KS20	N26		
Control	1.41a	0.23a	1.74a	0.29a	19.50a	24.40a	12.60a	12.40a	0.60a	0.60a
Manure	1.99a	0.26a	3.04a	0.36a	31.00b	57.90b	12.90a	12.50a	0.80a	0.80a
Fertilizer	3.52a	0.39a	3.88a	0.39a	58.50c	49.30b	13.00a	12.70a	0.80a	0.80a
Manure + Fertilizer	4.16a	0.44a	3.65a	0.30a	63.70c	62.10b	13.70a	13.40a	0.80a	0.80a
LSD	2.70	0.19	2.70	0.19	9.90	9.90	1.70	1.70	0.50	0.10
%CV	45.60	27.10	45.60	27.10	51.30	51.30	8.40	8.40	48.9	21.1
Season two										
Control	0.65a	0.15a	0.92a	0.22a	13.30a	16.70a	11.80a	12.50a	0.40a	0.53a
Manure	0.76a	0.15a	1.04a	0.20a	14.70a	20.80a	12.00a	12.60a	0.60a	1.13b
Fertilizer	0.79a	0.17a	0.98a	0.25a	21.70a	26.00a	12.30a	12.70a	0.60a	1.13b
Manure + Fertilizer	1.09a	0.21a	1.55a	0.23a	25.30a	26.50a	12.50a	13.20a	0.60a	1.13b
LSD	0.32	0.11	0.32	0.11	9.60	9.60	2.35	2.35	0.50	0.40
%CV	20.90	23.30	20.90	23.30	28.50	28.50	8.20	8.20	48.90	21.1

LSD- least significant difference; CV- covariate of variance; S1- season 1, S2- season 2

Table 4. Nutrient content of green grams grown in post mined soils exposed to manure and inorganic fertilizers

	KS20 Variety				N26 Variety			
	Control	Manure	Fertilizer	M+F	Control	Manure	Fertilizer	M+F
Dry matter (%)	88.20	88.40	88.00	88.00	88.50	87.80	78.90	88.80
Phosphorus (%)	0.45	0.50	5.10	0.53	0.42	0.52	0.52	0.53
Magnesium (%)	0.19	0.20	0.18	0.19	0.19	0.20	0.20	0.20
Calcium (%)	0.09	0.11	0.09	0.08	0.09	0.09	0.09	0.09
Potassium (%)	1.51	1.54	1.43	1.53	1.43	1.53	1.47	1.49
Sulphur (mg/kg)	0.28	0.27	0.25	0.27	0.24	0.25	0.24	0.24
Manganese (mg/kg)	16.20	15.50	23.60	20.40	14.00	14.60	19.80	22.80
Molybdenum (mg/kg)	3.73	9.83	1.92	7.68	2.91	1.60	4.54	5.75
Sodium (mg/kg)	0.05	34.40	0.05	24.10	24.20	25.10	11.30	20.60
Boron (mg/kg)	18.50	17.70	17.40	17.50	14.30	14.90	15.80	13.80
Iron (mg/kg)	53.40	56.00	45.50	66.7	52.00	53.20	54.20	48.60
Copper (mg/kg)	8.59	6.85	3.49	4.55	8.12	4.93	3.32	3.17
Zinc (mg/kg)	33.20	31.20	29.80	29.20	31.90	28.90	29.60	29.10

M+F- manure + Fertilizer

5. CONCLUSIONS AND RECOMMENDATIONS

This study found that the post mined soils were sandy loam textured with adequate levels of available calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and Zinc (Zn) and low levels of exchangeable phosphorus (P), potassium (K), Sulphur (S), boron (B), copper (Cu), cation exchange capacity (CEC), total nitrogen (TN) and organic matter (OM). Combined application of manure and fertilizer was observed to increase plant height by 36% and 36.4% for KS20 and N26 varieties respectively in season one and, 23% and 23% for KS20 and N26 varieties respectively in season two compared to unfertilized soils. Similarly, leaf development was increased by 54.9% and 52.2% for KS20 and N26 varieties respectively in season one and, 46.1% and 37% for KS20 and N26 varieties respectively in season two compared to unfertilized soils. Sole manure and sole fertilizer did not have significant effect on plant height and number of leaves. Combined application of manure and fertilizer did not have significant influence on green gram nodulation, shoot and root dry matter accumulation, number of seeds per pod and pods per plant. It however increased N26 variety grain yield in the second season alone. Additionally, grains harvested from the post mined soils had adequate levels of phosphorus, Iron, Zinc, magnesium, potassium, and low levels of calcium and copper.

Although the findings show that this post mined soils have the capacity to support green gram production, there is need for further research to ascertain the following: (1). Long term effects of green gram establishment on profile development (2). Short and long term economics of green gram production in post mined soils (3). Crops response in post mined soils after application of all limiting nutrients within the soils (4). Effects of post mined soils on biological nitrogen fixation.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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