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Full Length Research Paper

Assessing the impact of different tillage systems and land uses on CO₂-C emissions in Eastern Amazonia

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The production and emission of CO₂ in native, pasture and cultivated areas is a result of microbiological activity and mineralization of organic matter, and depends on favorable environmental factors, such as temperature, availability of water and of land use. The results of this work show that the no tillage system (NT) has the potential to mitigate 37.7% of C-CO₂ efflux from cultivation of soy compared to conventional tillage (CT). The temperature of the soil accounted for 65% of the variability of the flux of CO₂-C in CT. The variation of soil moisture explained 73 and 51% of the flux of CO₂-C in CT and NT, respectively. These results indicate that soil moisture and soil temperature were controlling factors of CO₂-C emissions from soil to atmosphere because these parameters directly affect soil microbial activity. The results also show that the active pasture had the highest outflows of soil CO₂-C to the atmosphere in relation to forests and degraded pasture in Western Pará. Furthermore, it was shown that both the pastures and forests have seasonality in the flux, which mainly related to precipitation patterns and water potential between soil and air. We observed a strong correlation between the efflux and soil moisture of both capoeira and in the pastures, as the soil temperature was a controlling factor of the active efflux only in the pasture. The average flux of CO₂-C obtained in pasture active was 218.9 mg C m⁻² h⁻¹ value of 40.7% higher than the primary forests and 155.5 mg C m⁻² h⁻¹. Finally, the results presented here suggest that the conventional tillage and pasture management are activities strongly associated to human enhance biogeochemical changes in the balance of carbon in these ecosystems, since the efflux of CO₂-C is related to soil primary productivity of these ecosystems.

Key words: Greenhouse effect, land use change, carbon cycle, soil CO₂-C efflux.

INTRODUCTION

The concentration of greenhouse gases (GHG) in the atmosphere has increased substantially in recent years as a result of anthropogenic activities. The atmospheric concentrations of carbon dioxide (CO₂) have all increased since 1750 due to human activity. In 2011, the

concentrations of these three gases were 391 ppm, 1803 ppb and 324 ppb, which exceeded pré-industrial levels by 40, 150 and 20%, respectively. The concentrations of CO₂, CH₄ and N₂O now substantially the highest concentrations recorded in the ice cores during the last

800.000 years. The average rates of increase in concentrations Atmospheres in the last century are unprecedented in the last 22.000 years (IPCC, 2014). CO₂ emissions are primarily due to activities such as burning fossil fuels, the burning of forests and the loss of soil humus.

About half of cumulative anthropogenic CO₂ emissions between 1750 and 2010 have occurred in the last 40 years. In 1970, cumulative CO₂ emissions from fossil fuel combustion, cement production and flaring since 1750 were 420 ± 35 GtCO₂. In 2010, that cumulative total had tripled to 1300 ± 110 GtCO₂. Cumulative CO₂ emissions from forestry and other land use since 1750 increased from 490 ± 180 GtCO₂ in 1970 to 680 ± 300 GtCO₂ in 2010 (IPCC, 2014).

World agriculture is responsible for issuing significant amounts of CO₂, CH₄ and N₂O to atmosphere contributing 11, 47 and 58% of the total anthropogenic emissions of these gases, respectively (IPCC, 2007). In Brazil, the participation of agriculture to the total of anthropic emissions is accentuated, being 75, 78 and 91% for CO₂, CH₄ and N₂O, respectively (Cerri et al., 2007) when considering the conversion of natural areas to agriculture.

Deforestation in tropical regions is a major cause of global changes. The conversion of forests to pastures affects the biogeochemical cycles, carbon fluxes to the atmosphere, terrestrial biodiversity and also the social and economic viability of traditional forest peoples (Salimon et al., 2011; Giacomini et al., 2006; Foley et al., 2003). As a result, new scenarios of changes in land use are encroaching on natural areas within the Amazon region, causing unpredictable changes that increase the intensity of the impacts of this new production cycle (Gardner et al., 2013).

The advance of the agricultural frontier on these natural areas by removing the natural vegetation cover and even the impact of the use of new production techniques and new forms of management can change microbial processes. The use and even the preservation of these natural resources and ecological functions, together with the much desired sustainable development, is still far from being achieved (Fearnside, 2015; Foley et al., 2007).

In Eastern Amazonia, the agricultural frontier has expanded bringing changes to a scenario that has already changed greatly from previous waves of development which also aimed to increase food production. The expansion of the agricultural frontier changes the entire structure of the region it occupies. Furthermore, the impacts are not restricted to cultivated areas, but due to climatological patterns of the region, the effects caused by the unplanned use of fertilizers and

pesticides are felt in the natural areas that surround these areas of production (Schlesinger, 1997). Forests and water bodies are affected by compounds emitted by farming areas and these have modified their ecological functions and natural processes. Within the region, the diversity of environments and climatic conditions makes it necessary to take measurements of the long-term dynamics of the mechanisms controlling the interactions between the biosphere and atmosphere (Fearnside, 2002, 2015; Foley et al., 2007; Nepstad et al., 2014).

These scenarios are alternated within the impact area and therefore also alter the process and the rates at which they occur. Within Western Pará State, a frontier for agricultural expansion has been established and few studies have been done to assess the impact on disturbed sites and its effect on these new centers of production in what were recently natural areas.

On a regional scale, knowledge about this type of land cover change and land use is fundamental to evaluate the functioning of an ecosystem and also for landscape management. Deforestation in the Brazilian Amazon is mainly related to the conversion of forests into pastures (Fearnside, 2016; Nesptad et al., 2014). After using these pastures or after planting for a few years, the soils of this region become less productive and the most common practice is the abandonment of areas, entering a stage of secondary secession, that lead to secondary forests.

Thus there are three main soil covers in the Amazon region: primary forests, pastures and agricultural fields. Some researches indicate the consequences of deforestation and conversion to pasture on carbon stocks in vegetation and soils also (Salimon et al., 2004). However, the flow of soil carbon to the atmosphere has been little studied in the Amazon, except in a few places in Pará (Davidson et al., 2000; Davidson et al., 2004; Trumbore et al., 1995; Sotta Doff et al., 2004, 2007; Vasconcelos et al, 2004; Keller et al, 2005) and Rondônia (Feigl et al., 1995). In view of this scenario, the present study aims to evaluate the impact of different types of soil cover on the CO₂ emissions from the soil to the atmosphere.

Of these sources of carbon to the atmosphere, CO₂ is the most important in terms of mass, especially through burning and respiration of roots and soil microorganisms. In the face of this scenario, it becomes essential to have a better understanding of the effect of different tillage systems linked to agricultural practices on the biogeochemical cycling of carbon in the soil (Sugihara et al., 2012).

It is presumed that changes in this cycle resulting from change in land cover and climate changes can lead changes on local, regional and global levels. In order to understand these consequences and to mitigate

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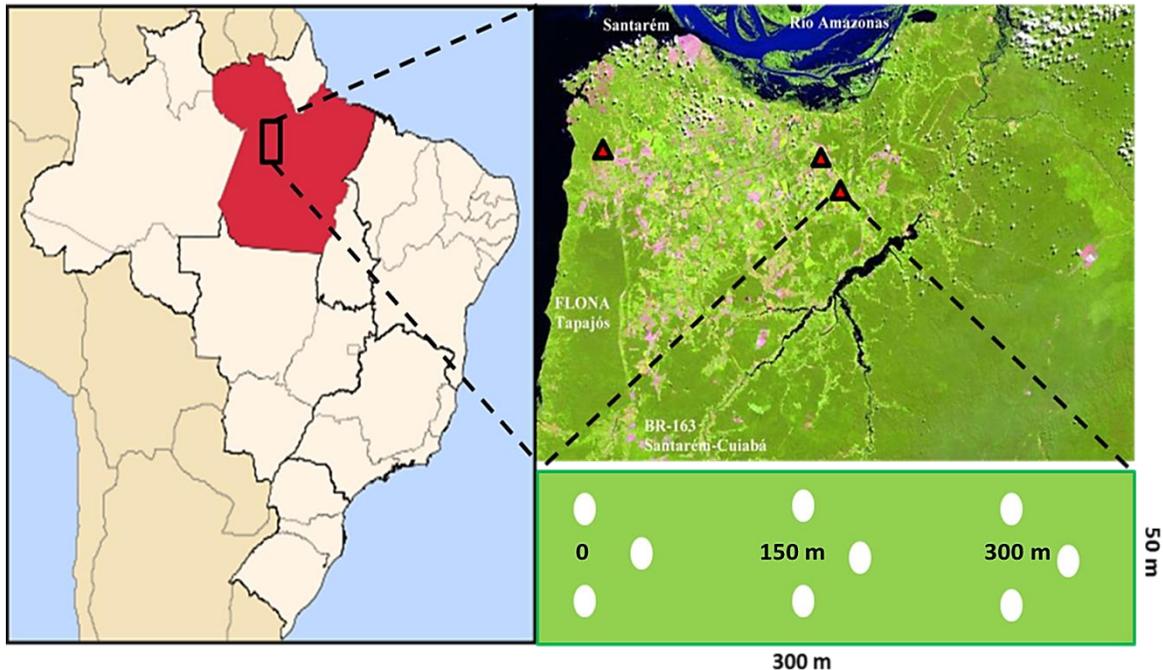


Figure 1. Area of study - triangles (measuring point) and rectangle in green (transect) blank circles (concentration chambers).

negative effects, the focus of this study was to examine C-CO₂ efflux from both forest and other forms of land use, such as secondary forest, grazing and agricultural areas.

MATERIALS AND METHODS

Study area

The study was conducted in Western Pará, on the Belterra plateau of the municipality of Santarém (Figure 1), in an agricultural field, secondary forests and degraded pasture situated 40 km from the PA-370 highway, whereas the active pasture is situated 25 km from the BR-163 highway. More details of the study sites are summarized in Table 1. These locations are characterized by a climate of type Am-megathermal humid tropical climate, according to the classification of Köppen; the average annual rainfall is 2096 mm annual average temperatures range from 24 to 28°C. All soils are dystrophic yellow Oxisols. The Physical and chemical characteristics of clayey Yellow Latossols in Belterra municipality are summarized in the in Table 2.

The selection of the watersheds was performed considering the principal axes of heterogeneity in both the environmental and socioeconomic aspects. The main criterion for selection of individual watersheds was the total remaining forest cover (or conversely, the accumulated historical deforestation) and that the selected areas form a gradient of deforestation.

Sample design

Using a stratified sampling design allows a wide variety of rural properties to be represented in the landscape, covering both small and large producers and also all major types of land use and

management practices in the region. In the areas of secondary forest, mature (forest that has not suffered significant disturbances or been exploited or influenced directly or indirectly by humans), active (that was managed to achieve a balance between yield and quality of the fodder produced) and degraded pastures, the latter being (defined as demonstrating a loss of the ability to maintain biological productivity, accumulate significant biomass, to cover the soil surface), the factor seasonality was considered and sampling was done on subsamples (50 m × 300 m) of watersheds for nine months from February until November 2012 covering the dry and wet periods with two monthly samplings with 9 repetitions in each transect, except for the month of August, where the measures were not carried out due to equipment failures.

In the agricultural field the sampling was of short duration (≈ 10 min) between the periods and comparative analysis between CT and NT was made with one sampling (9 replicates) done before cultivation and during sowing. Sampling was also done at the time of crop planting with two samplings during the first week after planting and one sampling in the second week after planting implements change in rates of CO₂-C efflux from the soil.

For the biodiversity and environmental data each sample point is a standardized transect 300 m long by 50 m wide. Nine chambers were distributed along a transect with a dimension (50 m × 300 m) and three chambers were installed for each point (0, 150 and 300 m), with three placed on the soil surface distant 10 meters (Figure 1) relative to one another for the measurements of CO₂-C efflux, totaling nine chambers for each transect.

Sampling system

Measurements of CO₂ emitted by the soil were made using the methodology of dynamic chambers with a coupled infrared gas analyzer (IRGA) model Licor-820 (Figure 2). The response signals of the detectors were captured at a frequency of 5 seconds and flux

Table 1. Specification of the study watersheds and their respective transects (land use).

Watershed	Land use	Extension Area (ha)	History	Previous use	Geographic coordinates (Lat./Long.)
112	Agricultural field (Conventional tillage and no tillage)	380	≈ 10 of years soybean farming under conventional tillage	Pasture	-54°29'57.6 W/ -2°43'18.54 S
	Secondary forests	10	Secondary forests with ≈ 15 years	Pasture	-54°29'33.2 W/ -2°44'19.11 S
	Degraded pasture	50	≈ 3 years management minus	Mature forest	-54°29'57.5 W/ -2°44'20.87 S
129	Active pasture	150	Pasture ≈ 7 years	Mature forest	-54°46'35.4 W/-2°43'33.03 S

Table 2. Physical and chemical characteristics of clayey Yellow Latossols in Belterra municipality, Pará State. Two experimental sites.

HOR	Depth (cm)		g/kg of soil				pH		cmol _c kg ⁻¹ soil				Silt/ clay	Ki	%		P (mg/kg)			
			Sand	ADA	Clay	C	Fe ₂ O ₃	H ₂ O	pH	Ca	Mg	K			S	Al		T	V	m
Typic Hapludox – coordinates: 02° 54'S and 54° 56'W																				
A1	0 -	11	30	640	890	19.8	61	3.7	-0.1	0.40	0.05	0.50	2.70	10.5	0.09	1.91	5	84	4	
AB	-	23	20	510	920	13.6	63	4.1	-0.3	0.30	0.03	0.45	2.10	7.8	0.07	1.86	5	84	2	
BA	-	45	20	0	930	9.6	66	4.3	-0.4	0.40	0.02	0.44	1.80	5.9	0.05	1.86	7	82	1	
Bw1	-	91	20	0	930	6.4	67	4.4	-0.4	0.30	0.01	0.32	1.70	4.4	0.05	1.88	7	85	1	
Bw2	-	160	10	0	930	4.2	67	4.7	-0.6	0.40	0.01	0.44	1.00	3.6	0.06	1.92	11	71	1	
Typic Hapludox – coordinates: 02° 45'S and 54° 54'W																				
O	3 -	0	160	50	680	55.9	41	4.8	-0.8	6.8	3.2	0.23	10.3	0.3	22.9	0.24	2.03	13	89	1
A1	0 -	15	100	50	820	18.1	49	4.5	-0.5	0.5	0.5	0.04	1.1	1.6	8.4	0.10	1.99	5	92	<1
AB	-	29	70	68	840	11.3	52	4.5	-0.5	0.4	0.02	0.44	1.7	6.4	0.11	1.98	1	90	<1	
BA	-	44	60	0	870	7.9	52	4.7	-0.6	0.50	0.02	0.53	1.5	4.6	0.08	1.94	1	88	<1	
Bw1	-	79	70	0	870	5.1	52	4.9	-0.9	0.40	0.01	0.42	1.3	4.0	0.07	1.94	1	91	<1	
Bw2	-	122	60	0	890	5.0	55	5.0	-1.0	0.40	0.01	0.42	1.3	3.5	0.06	1.92	1	-	-	
Bw3	-	200	50	0	880	4.0	54	5.0	-1.0	0.40	0.01	0.43	1.1	3.5	0.08	1.95	1	-	-	

was calculated by linear regression of concentration by time interval measurements.

Measurements were always conducted between 09:00 and 16:00 h. In order to evaluate whether the time of sampling was representative of soil respiration during the day, we also conducted samplings at 1 h intervals in pasture, mature and secondary forests, with nine flux measurements at each site and hour.

The flux was calculated from linear regression of the difference of CO₂ concentration over time. The measurements during the first minute were discarded from the regression to avoid any artifact of closing the chamber,

and only the data showing a linear increase in CO₂ concentration (usually during a 1 to 5 min interval) were used to calculate fluxes. The IRGA was calibrated every morning by using 'zero' air that had been run through a soda lime scrubber and by using certificated standard gas of 610 (±2%) ppmv of CO₂ (nitrogen as the balance gas).

CO₂ flow calculation

Soil respiration (*Rs*) was computed as the rate of change of the CO₂ concentration by time unit and area under the

region covered by the camera, as shown in the equation:

$$Rs = (C_n - C_{n-1}) / T_n \times (V/A) \quad (1)$$

where *Rs* = soil respiration (CO₂ μmol m⁻² s⁻¹); *C_{n-1}* = concentration (ppm) of CO₂ at the initial time (*n-1*); *C_n* = concentration (ppm) of CO₂ in the end time (*n*); *V* = chamber volume (m³); *A* = coverage area (m²); and *T_s* = time interval (s).

The calculation of the CO₂ flux described earlier was determined by means of a computer application (Licor - 2010) developed by Fagner (2010). These parameters

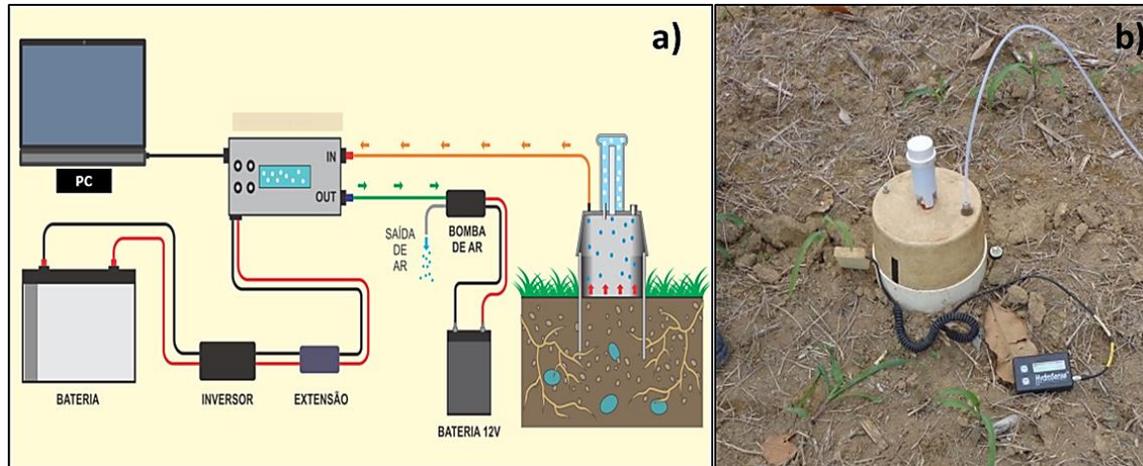


Figure 2. (a) Experimental arrangement and the (b) concentration chamber.

were inserted in Equation 1 in order to determine the flow of CO_2 from the soil. The average height of the chamber (cm) was obtained from three different points of the base ring. A conversion factor in the amount of 43.22 (this value is a conversion factor of the μmol unit to mg) was used to measure the efflux of $\text{CO}_2\text{-C}$ soil in $\text{mg CO}_2\text{-C m}^{-2} \text{h}^{-1}$.

Meteorological elements

To correlate the variation of soil CO_2 flux to the microclimate of the region parameters such as temperature of air and soil, soil moisture and precipitation were measured. For measurements of soil temperature a Taylor instruments digital thermometer was introduced into the soil to a depth of 5 cm, adjacent to the chamber. The same points were used for each sampling of soil CO_2 efflux.

Gravimetric soil moisture was measured at all points of measurements of CO_2 efflux at 10 cm depth using a soil auger. Samples were immediately placed in sealed plastic bags and weighed on the same day to obtain the wet weight of each soil sample and samples were dried at 105°C for approximately 48 h.

Waterfilled pore space (WFPS) was calculated as in the following equation:

$$\text{WFPS} = \% \text{Volumetric water} / \% \text{Soil porosity} \quad (2)$$

$$\% \text{Soil porosity} = 1 - (\text{soil bulk density} / 2.65)$$

where 2.65 is the assumed particle density.

Statistical analysis

Data were statistically analyzed with the software Statistica version 6.0 for Windows. Data were analyzed using ANOVA with site and season as fixed effects. Scheffe's (ou Tukey's) post hoc test was used to separate means, and a probability level of $\alpha = 0.05$ was used for all tests.

All data were analyzed for the distribution normality of data was checked by the Kolmogorov-Smirnov test and the tests showed that data were normally distributed.

The effect of and CO_2 emissions between conventional and no till systems was investigated by analysis of variance (ANOVA). When the ANOVA resulted in a significant effect ($p < 0.05$), the Tukey

multiple comparison test was used to separate means. The dependence of $\text{CO}_2\text{-C}$ efflux on temperature and soil moisture was evaluated from the significance of the correlation coefficients of linear regressions through the Pearson parametric test.

RESULTS AND DISCUSSION

Microclimate conditions in relation to no tillage and conventional tillage

The daily rainfall during the study period is presented in Figure 3. Dry soil is a favorable condition that ensures that soil preparation is carried out effectively, and for this reason dates wherein there was no precipitation (Figure 3) were recorded. There was no significant occurrence of drought, which maintained adequate levels of moisture in the soil, so it is assumed that this variable was not limiting to microbial activity responsible for the $\text{CO}_2\text{-C}$ efflux.

Comparison of CO_2 efflux between no tillage and conventional tillage

The no tillage system (NT) promoted less $\text{CO}_2\text{-C}$ efflux from the top soil ($p < 0.05$) than the conventional tillage system (CT). During all stages of tillage (plowing and harrowing, sowing and harvesting) there were higher levels of $\text{CO}_2\text{-C}$ efflux from the soil in the CT during the evaluation period, as shown in Reicosky et al. (1999) Figure 4. Throughout the measurement period greater efflux of $\text{CO}_2\text{-C}$ was observed in the system under CT (Figure 4) when compared with NT, averaging 196.1 ± 25.9 and $142.4 \pm 27 \text{ mg C m}^{-2} \cdot \text{h}^{-1}$, respectively (Table 3).

Similar values than those obtained in this work were reported in a study that evaluated the CO_2 emissions derived from plowing and harrowing, in which the

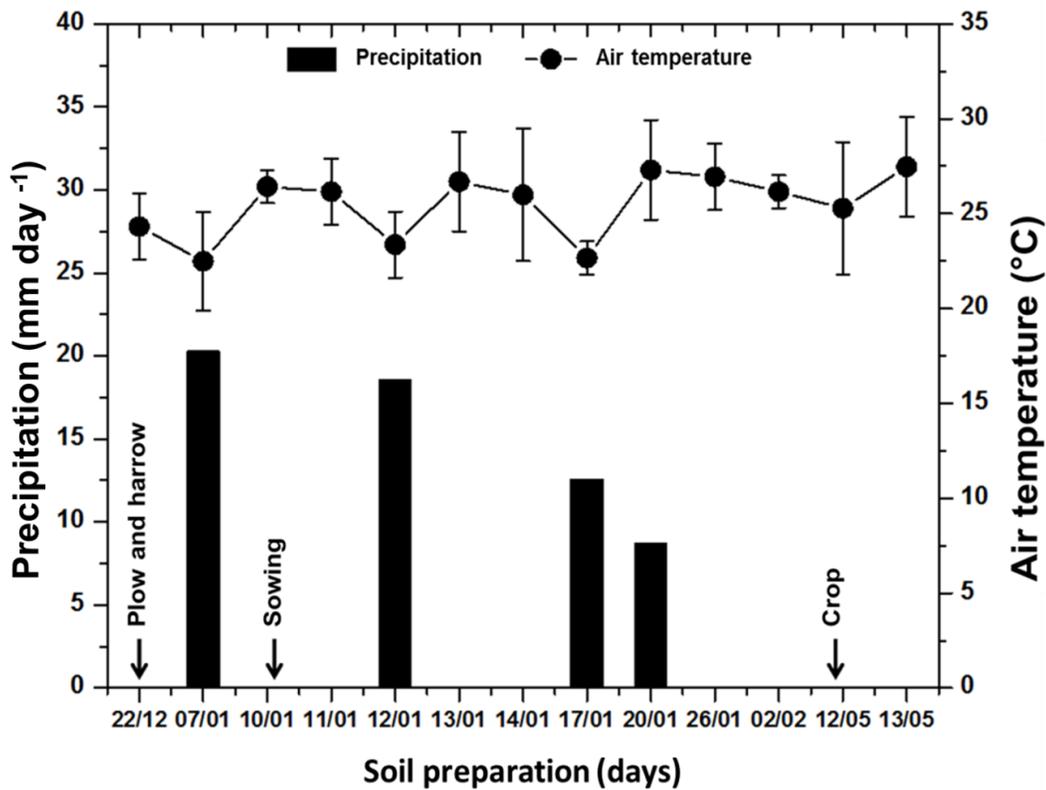


Figure 3. Distribution of day precipitation and variation of average air temperature during complete experimental period (22-Dec.-2010 to 13-May-2011).

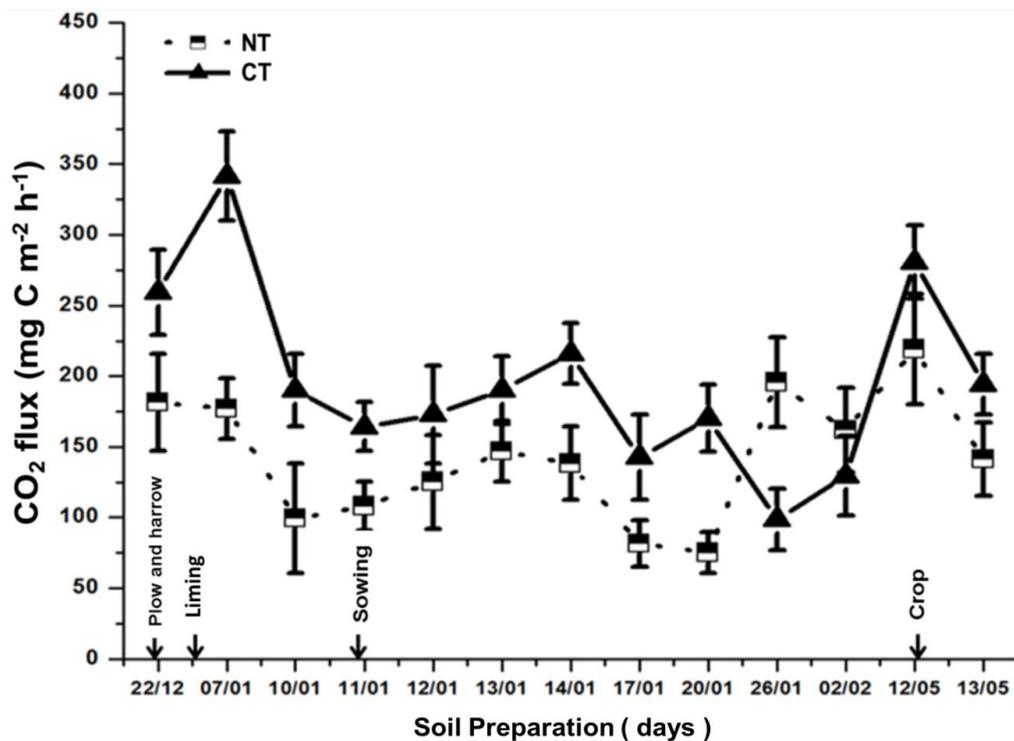


Figure 4. Soil CO₂-C efflux conventional tillage systems (CT) and no tillage (NT) in soybean/corn rotation. The bars indicate standard deviation.

Table 3. Mean values of CO₂-C (mg m⁻² h⁻¹) flux with standard deviation (SD), water filled pore space (WFPS%) and soil temperature (ST) at 5 cm depth under no-tillage and conventional tillage.

Date (Soil preparation)	No tillage				Conventional tillage			
	CO ₂ flux	SD	WFPS (%)	ST (°C)	CO ₂ flux	SD	WFPS (%)	ST (°C)
22 Dec. 2010	181.4	34.6	48.3	29.3	259.2	30.2	32.7	31.6
07 Jan. 2011	177.1	21.6	52.2	28.4	341.3	31.5	33.3	33.1
10 Jan. 2011	99.4	38.9	39.8	27.4	190.1	25.9	36.4	29.0
11 Jan. 2011	108.0	17.3	42.9	29.7	164.2	17.3	32.7	30.2
12 Jan. 2011	125.3	33.3	42.5	30.4	172.8	34.6	35.9	31.7
13 Jan. 2011	146.9	21.6	46.3	31.9	190.1	23.8	39.4	30.9
14 Jan. 2011	138.2	25.9	46.5	28.9	216.0	21.6	44.3	30.0
17 Jan. 2011	81.6	16.4	38.8	29.4	142.6	30.2	41.3	31.0
20 Jan. 2011	75.2	14.3	39.3	28.4	170.2	23.8	42.6	28.6
26 Jan. 2011	195.7	32.0	68.3	31.2	98.5	21.6	25.9	28.7
02 Feb. 2011	162.0	29.8	63.9	29.7	129.6	28.1	30.6	30.7
12 May 2011	219.0	38.9	64.3	31.8	280.8	25.9	30.1	29.7
13 May 2011	141.3	25.9	48.8	29.6	194.4	21.6	29.9	30.2
Media	142.4	27.0	49.4	29.7	196.1	25.9	35.0	30.4

average emissions of CO₂ efflux from the soil was greater in the CT treatment (La Scala et al., 2001).

It can also be seen in Figure 4 that the largest emission in CO₂-C efflux from the soil were recorded on 22-December-10 and 07-January-11 during soil preparation, with 250 and 345 mg C m⁻² h⁻¹ for the CT, respectively. These values occurred a few days after the execution of plowing and disking and application of limestone (CaCO₃ 4 t.ha⁻¹) on 07-January-11 generating a pulse (Figure 4) of CO₂-C of 341.3 ± 31.5 mg C m⁻² h⁻¹. This pulse was probably stimulated by liming and tillage from previous cultivation of soybean which possibly created favorable conditions for the decomposition and mineralization of soil C and therefore justifies the high efflux of CO₂-C (Anghinoni and Salet, 2000).

Subsequent soil tillage operations generated the highest value (341.3 mg C m⁻² h⁻¹) compared to the other days evaluated. In the seeding stage, the CO₂-C efflux values gradually increased in both NT and CT (Figure 4) which may be associated with intense autotrophic activity induced by application of fertilizer (NPK 308 kg.ha⁻¹) in this period.

Harrowing the soil breaks up the soil and frees the organic matter that can be found within the different soil aggregates, especially macroaggregates and microaggregates occluded within the macroaggregates (Six et al., 2000), making available the carbon within aggregates available for decomposition by microorganisms.

In temperate soils, the primary mechanism of protection of carbon in agroecosystems is the physical protection within aggregates. In tropical soils, on the other hand, such as Latosols, chemical protection may be the main mechanism of protection carbon (Zinnyl et al., 2005;

Denef et al., 2004). In this case, the impact of soil preparation of the efflux of CO₂-C may be less than the soils found in temperate biomes.

During soybean harvest (10-May-2011) CO₂-C efflux was measured in both management systems on subsequent days (12-May-2011), showing high values of efflux of CO₂-C. This pattern of emission of CO₂-C has been reported by some authors analyzing soybean several days after harvest (Fields, 2006; Oorts et al., 2007).

These high levels of emissions are apparently related to the higher concentrations of labile, C, which is consumed and used a substrate for growth of microbial populations (Dendooven et al., 2012). The roots of soybean remaining in the soil continue to emit CO₂-C during the process of decomposition, furthermore, and cutting of roots during the collection process can lead to increased efflux CO₂-C from the soil (Oorts et al, 2007; Varner et al., 2003).

Correlation between CO₂ efflux and soil temperature

The CO₂-C efflux from the soil is not influenced only by physical and biological processes in the soil, but also by environmental factors such as temperature and soil moisture, which are seen as important factors in controlling the rate of soil CO₂-C efflux. This study found a high coefficient of determination ($r^2=0.65$) and high significance ($P<0.01$) between the CO₂-C flux and soil temperature in CT, contrasting with the NT where there was no correlation between these variables (Figure 5a), indicating a more direct relationship of CO₂-C efflux in relation to soil temperature on, and that other factors

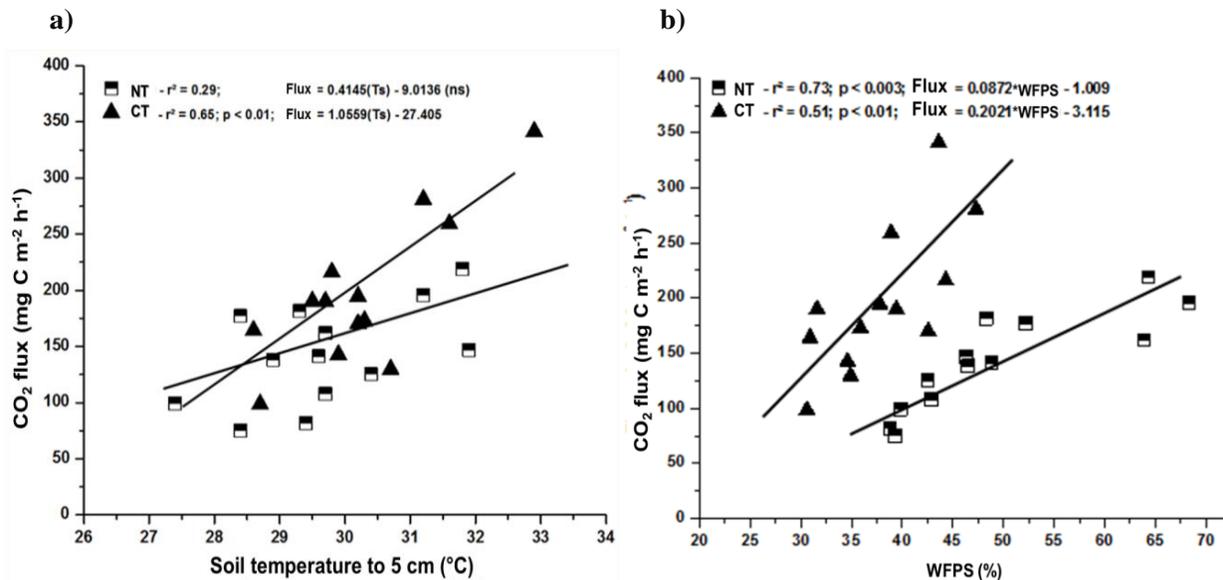


Figure 5. Correlation between the CO₂-C efflux and soil pores filled with water (WFPS) and soil temperature under conventional tillage (CT) and no tillage (NT) in soybean/corn rotational systems.

besides soil temperature may be influencing the efflux of CO₂-C soil in NT. Thus, the average daily temperature variation of the soil explained 65% of the variation in the soil CO₂-C efflux in CT.

Correlation between CO₂ efflux and soil temperature

In this case, the absence of ground cover in CT permitted direct incidence of sunlight and increased soil temperature, which, in turn, could have enhanced microbial activity, and thus the efflux of CO₂-C to the atmosphere (Moitinho et al., 2012).

Soil temperature as a factor controlling the CO₂-C efflux from the soil is an issue that has great importance, being used in analytical and statistical models in forecasting the emission of this gas from soils in various environments (Moncrieff and Fang, 2001). In the NT the input to the surface of organic debris decreased the incidence of direct sunlight on the ground and caused a consequent reduction in water loss (Salton and Mielniczuk, 1995) which acted to inhibit the increase in temperature CT ground and therefore to reduce the outflow of soil CO₂-C to the atmosphere.

The production of CO₂ in the soil is primarily a result of root and microbial activity which, when there is no limitation on other parameters (oxygen, moisture, pH, organic compounds, nutrients, etc.) is regulated by soil temperature.

Another important difference between CT and NT systems that influences production of CO₂ is microbial composition and its location in the soil profile, as highlighted by Vargas (2002).

These authors found largest populations of denitrifying organisms and fungi in soil layers in NT soil, and larger populations of aerobic microorganisms throughout the topsoil in CT.

These aspects are associated with the effects of soil management systems on the distribution of C and N throughout the soil profile (Steiner et al., 2012). However, physical changes triggered by tillage, and which reflect the ability of the soil to retain more or less water, have a strong effect on the composition and distribution of microorganisms in the soil profile (Bortolotto et al., 2015).

Correlation between CO₂ efflux and soil moisture

The NT system tends to have greater moisture and hence higher porosity filled with water (WFPS), accompanied by minor efflux of CO₂-C, relative to CT, and in the latter system, the soil moisture decreased drastically with tillage (Table 3). Soil moisture (WFPS) during the evaluation period explained 70 and 51%, respectively, in both systems (Figure 9) of the variability of the efflux of CO₂-C from the soil. This significant positive correlation (p < 0.05) for the NT system could be associated to the maintenance of residues in the soil, preventing direct incidence of solar radiation on the ground, and higher capacity for water storage in NT soil (Rethe et al, 2005; Costa, 2003) (Table 3), due to its larger stock of organic C, conditions which may explain part of the results obtained in the present study.

The CT also showed a significant linear correlation between CO₂-C efflux and soil moisture (r² = 0.51) (Figure 5b), which indicated greater dependence of CO₂-C

Table 4. Mean values of soil CO₂ efflux and standard deviation of the mean (spatial variation) in primary forest, secondary forests (n = 18), active pasture (n = 18) and degraded pasture (n = 18).

Year	Month	Precipitation	CO ₂ efflux (mg C m ⁻² h ⁻¹)		
			Secondary forest	Active pasture	Degraded pasture
2011	February	133.6	194.4 ± 17.3	315.4 ± 34.6	116.7 ± 21.6
	March	294.7	224.6 ± 38.9	302.4 ± 21.6	103.7 ± 34.6
	April	404.6	185.7 ± 30.2	345.6 ± 25.9	151.2 ± 30.2
	September	13.2	125.2 ± 21.6	116.6 ± 21.6	64.8 ± 25.9
	October	45.9	129.6 ± 30.2	103.7 ± 17.3	69.1 ± 21.6
	November	0	103.6 ± 38.9	129.6 ± 21.6	86.4 ± 34.6

efflux in soil CT on moisture, probably due to lack of crop residues on the soil surface, which increases its temperature. Some studies have shown that the CO₂-C efflux from the soil indicates that microbial activity in the soil increases exponentially or linearly with temperature and soil moisture and is used in mathematical models of ecosystems as a constant (Xu and Qi, 2001).

With respect to the effect of temperature and soil moisture on CO₂-C efflux (Ouyang and Zheng, 2000) emphasized that solar radiation is one of the important processes governing the diurnal cycles of soil temperature and water evaporation by controlling production rates from CO₂-C in the soil, and thus the CO₂-C emissions to the atmosphere from the soil (Rethe et al., 2005).

Temporal variation of CO₂-C efflux between land uses

Effluxes CO₂-C atmosphere for soil generally varied between 60 and 350 mg C m⁻² h⁻¹ being greater in the wet period (Table 4). These values are within the same range of magnitude observed by other authors for the Amazon region, between 80 and 400 mg C m⁻² h⁻¹ (Salimon et al., 2004; Fernandes et al., 2002) and also in temperate ecosystems where efflux values ranged between 50 and 300 mg m⁻² h⁻¹ (Jarvis and Rayment, 2000; Savage and Davidson, 2001).

Seasonal variation of the CO₂-C efflux in different types of land use

The highest fluxes were recorded during the wet season (February-April) with the largest peak observed in April. The lowest fluxes, in turn, were found in September, one of the months where in the dry season is most pronounced (Figure 6 and Table 4).

A seasonal pattern similar to this was also reported by Davidson et al. (2000) in forests and grasslands in eastern Pará and also by Fernandes et al. (2000) in Rondônia. This may have occurred in response to changes in temperature and soil moisture, reflecting chemical changes that make up the respiratory

processes occurring simultaneously at different depths within the soil profile. According to these authors the spatial variability can probably be explained by the unequal distribution of roots in soil depths.

The variability of soil CO₂-C efflux soil during the year following seasonal rainfall influences with the soil moisture and therefore this seasonal pattern in CO₂-C efflux is strongly associated with the physical process of water percolating into the soil. This water will tend to occupy the pore spaces that are filled by the gas causing the immediate expulsion of CO₂ stored in the soil to the atmosphere (Smith et al., 2003). In April (wet season), the fluxes recorded in the active pasture were four times higher compared to September (dry season). The dominant species of grass has nearly all its biomass in the form of leaves and when this biomass is reduced in the dry season the photosynthetic rate also decreases. The same process probably occurs with root and microbial respiration in the rhizosphere, resulting in a reduction in total CO₂ efflux from the soil (Salimon et al., 2004).

Salimon et al. (2004) measured fluxes in pasture in the wet season in Acre, that were 4.5 times higher than in the dry season. In forests, however, seasonal variations were smaller being 2.1 to 2.7 in the mature forest and 1.6 to 1.7 in the secondary forest.

In the current study, the seasonal variation of flux was also high, with a 2.8 times greater flow in the rain than in the dry season. In native ecosystems, however, there was a much more pronounced seasonal variation, with fluxes 1.6 and 1.7 times higher in the wet season compared to the dry season, in secondary forests and degraded pasture, respectively. In pastures in the Brazilian State of Acre studied by Salimon et al. (2004), annual fluxes were 70% higher when compared with mature forest.

In April all values of CO₂-C efflux were higher than the average of the outflows in the dry season (September, October and November). In these months of the dry season, the points of measurement had low CO₂-C efflux (Figure 6). This suggests that at the start of measurements, increased humidity favored the CO₂-C production at all measured points.

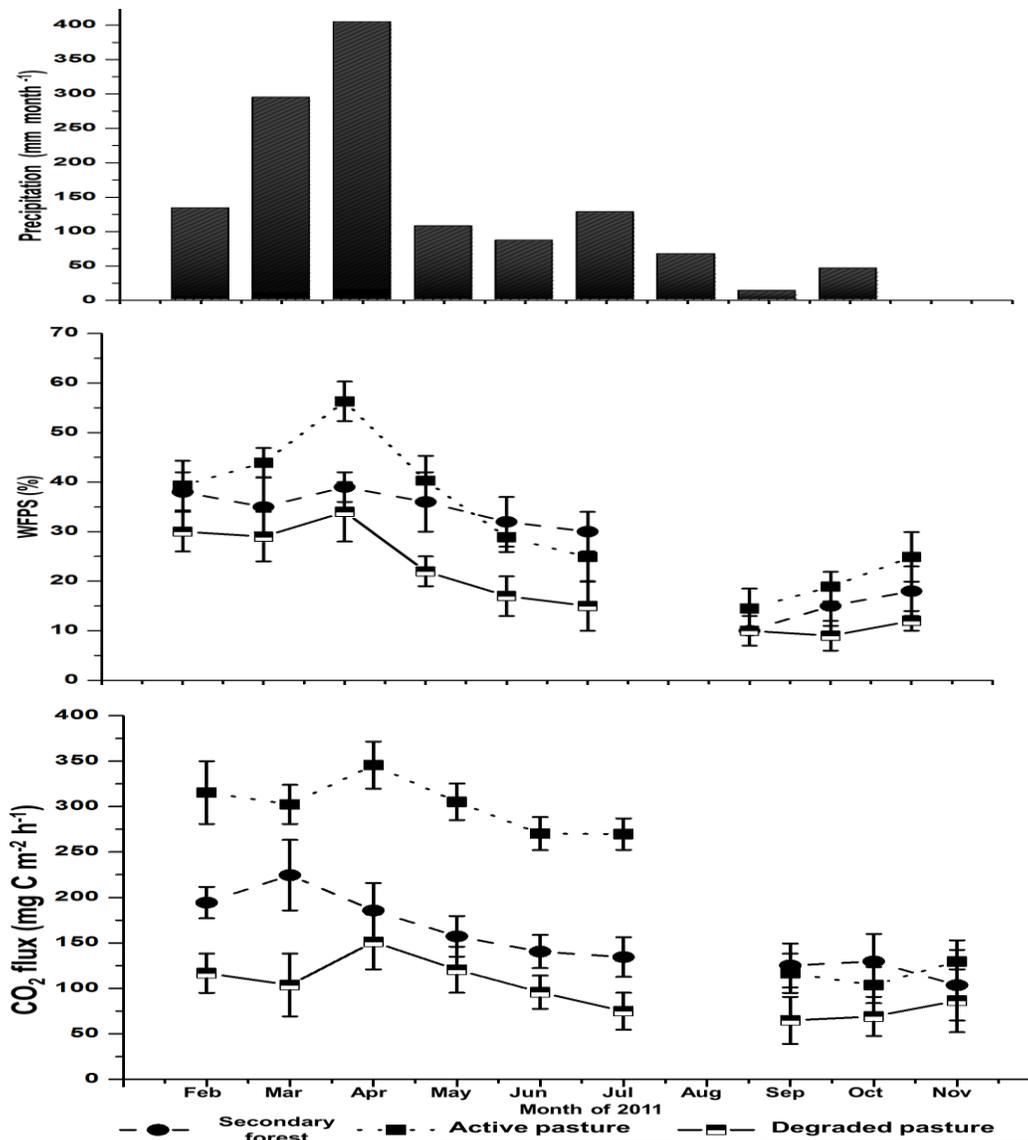


Figure 6. Seasonal variation of CO₂-C efflux from the soil, soil moisture and monthly rainfall. Error bars represent the standard spatial variation in secondary forest (n = 18), active pasture (n = 18) and degraded pasture (n = 18).

The possible increase in organic matter decomposition (with CO₂ production) may have altered the substrate thereby reducing CO₂-C emissions. Thus, the increase in humidity no longer showed homogeneous effect on CO₂ production. Incidentally, Davidson et al. (1998) and Savage and Davidson (2001) also find this pulse in CO₂-C emissions after the fast wetting of dry soil, similar to our study.

Although soil moisture (WFPS) was greater in pasture than in forest in the dry season (Figure 4), the largest reduction of the CO₂-C efflux from the soil in pastures indicates that grasses may be more sensitive to seasonal changes to water potential between the ground and air.

The surface soils under pasture are more clayey, but

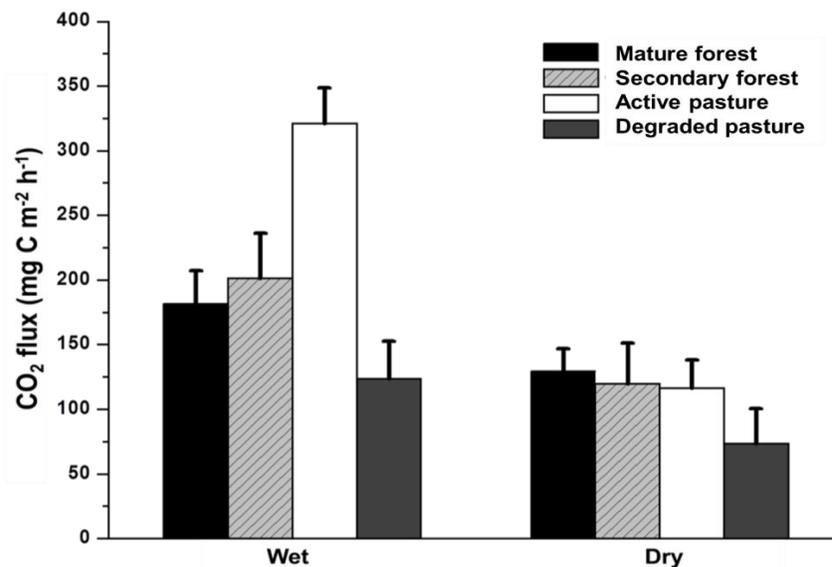
although there may be more water in the soil (Salimon et al., 2004), it may not be available to the plants. The forest vegetation has deeper roots, allowing access to deep soil water during the dry season, thereby reducing the seasonality of CO₂-C efflux in forests (Nesptad et al., 2008; 2014).

Therefore, the variation of water content in soil and the atmosphere should be primarily responsible for the seasonality observed and also the greater range of variation in the efflux soil of CO₂-C in active pasture. The influence of temperature on the soil CO₂-C efflux is minimal, since its range of variation is less than that observed in the flux due to rainfall.

Table 5. Average values of CO₂ efflux and standard deviations in mature forest, secondary forest, active pasture and degraded pasture active during the wet and dry season (mg m⁻² h⁻¹).

Seasonality	Land use			
	Mature forest	Secondary forest	Active pasture	Degraded pasture
Wet	181.4 ± 25.9 ^{aA}	201.6 ± 34.5 ^{aB}	321.2 ± 27.4 ^{aA}	123.8 ± 28.8 ^{aC}
Dry	129.6 ± 17.3 ^{bA}	120.0 ± 31.1 ^{bA}	116.6 ± 21.6 ^{bA}	73.4 ± 27.4 ^{bB}
Total average	155.5 ± 21.6	160.8 ± 32.8	218.9 ± 24.5	98.6 ± 28.1

*Different letters in the same column (lower case) and the same line (upper case) letters represent statistically significant differences (Tukey test, $\alpha = 0.05$).

**Figure 7.** Seasonal variation in CO₂-C efflux from the soil in mature forest, secondary forest, active and degraded pastures.

Comparison of CO₂ efflux between land uses

The values of soil CO₂-C efflux in secondary forest in the wet and dry periods varied between 168.5 and 216 mg C m⁻² h⁻¹ and 99.4 and 142.6 mg C m⁻² h⁻¹, and pasture values were between 254.9 and 311 mg C m⁻² h⁻¹ and 86.4 and 129.6 mg C m⁻² h⁻¹, and the degraded pasture values ranged between 121 and 172.8 mg C m⁻² h⁻¹ to 38.9 and 86.4 mg C m⁻² h⁻¹.

The lowest average value of CO₂-C efflux from the soil during the dry period was obtained in degraded pasture, and the highest was observed in the active pasture. For the wet season, meanwhile, the mean value of CO₂-C efflux from the soil was recorded in the active pasture and was lower in degraded pasture, as observed in Table 5.

The CO₂-C efflux from the soil, in general, is higher in the wet season, and soil moisture and temperature were the factors that primarily controlled the production of gas. In all types of soil cover statistically significant differences were observed relative to the seasonal factor (Table 5).

The active pasture had on average, higher CO₂-C efflux from the soil to the atmosphere in relation to forests and degraded pasture (Figure 7).

Pastures, on average, had higher CO₂-C efflux from the soil to the atmosphere than mature and secondary forests in the sampling period (Table 5). These results are similar but slightly higher than those from other studies conducted in the Southern and Western Amazon region (Salimon et al., 2004; Fernandes et al., 2002). Although, in Eastern Pará, Davidson et al. (2000), found that the forest had a greater flux than did a pasture.

Feigl (1994) working in a 13-year old pasture in the dry season, reported values between 91 and 182 mg C m⁻² h⁻¹, values that are close to those observed in the present study, for a similar period. Fernandes et al. (2002) also notes compatible values that are on average 100 mg C m⁻² h⁻¹ in the dry period and between 200 and 350 mg C m⁻² h⁻¹ in the wet season in pastures. Davidson et al. (2000) found values below 100 mg C m⁻² h⁻¹ during the dry period and between 200 and 400 mg C m⁻² h⁻¹ in the wet season.

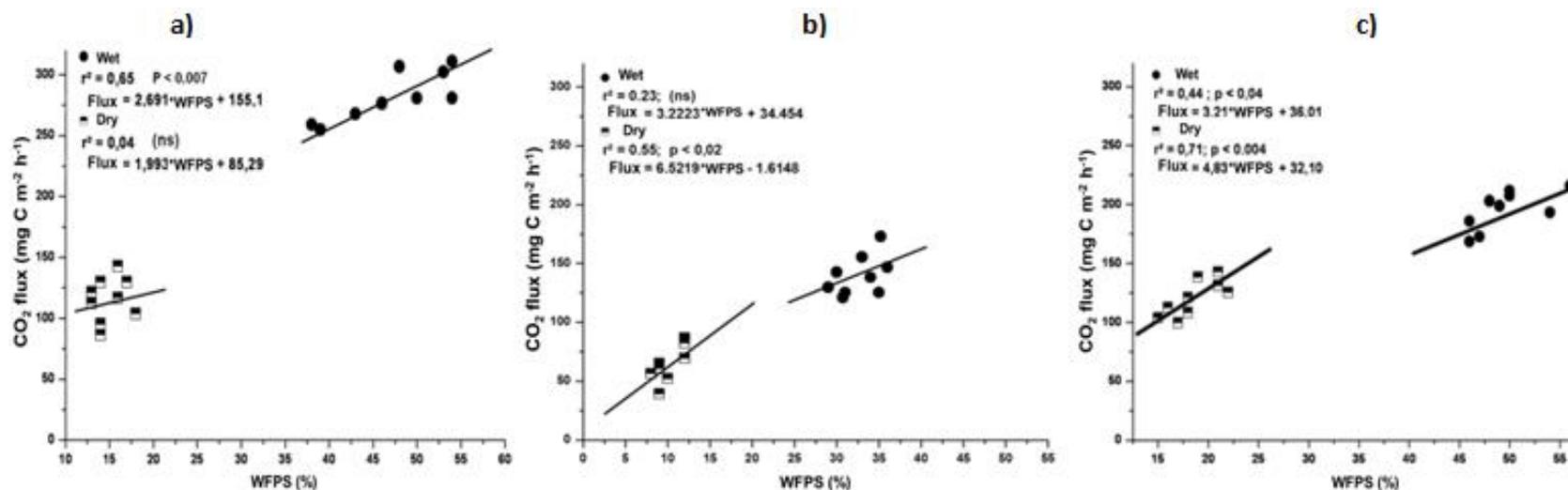


Figure 8. Correlation between the CO₂-C efflux from the soil and the percentage of pores filled by water (WFPS) during wet and dry seasons under the area of secondary forests (a), active pasture (b) and degraded pasture (c).

The discrepancy between the values observed in active pastures (Davidson et al., 2000) and the values of the present study, although both studies were conducted in the eastern Amazon, can be explained by fertilizer management in the active pasture of this study, which received 250 kg NPK ha⁻¹ that may have accelerated productivity and consequently induced the autotrophic component, thus increasing CO₂-C efflux from the soil to the atmosphere (Salimon et al., 2004; Fernandes et al., 2002).

Correlation between CO₂-C efflux and soil moisture in secondary forest, active and degraded pastures

In the dry period, the ratio of the efflux of CO₂-C and the percentage of pores filled with water was

significantly positive in the secondary forest ($r^2 = 0.71$, $p < 0.004$) and degraded pasture ($r^2 = 0.55$, $p < 0.02$), as can be shown in Figure 8b and c, respectively. In the wet season, in turn, only the secondary forest and active pasture showed statistically significant correlation ($r^2 = 0.44$, $p < 0.04$, $r^2 = 0.65$, $p < 0.007$) (Figure 8a), respectively.

Soil moisture interferes in the efflux of soil CO₂-C to the atmosphere through the supplement of nutrients that are available only to bacteria when dissolved in water films on the soil particles and also by the water content in the pores that controls the diffusion of gases through soil. When fully dry, the soil is composed only of solid particles and air. The total volume of space filled with air is called a pore. When there is drought conditions, soil particles lose their moisture films, hindering the diffusion of ions. This condition

facilitates the diffusion of gases, but alters the metabolism of bacteria and consequently the degradation of organic matter and the release of CO₂-C. When the amount of water contained in the soil increases, the total fraction of pores filled with water increases, restoring the diffusion of ions and hindering the diffusion of gases (Melillo et al., 2001).

Correlation between CO₂-C efflux and soil temperature in secondary forest, active and degraded pastures

In general the soil temperature varied little during the dry and wet seasons. The values of soil temperature at 5 cm depth in the different types of land cover were different, but there was a low temperature variation in both the wet and dry seasons.

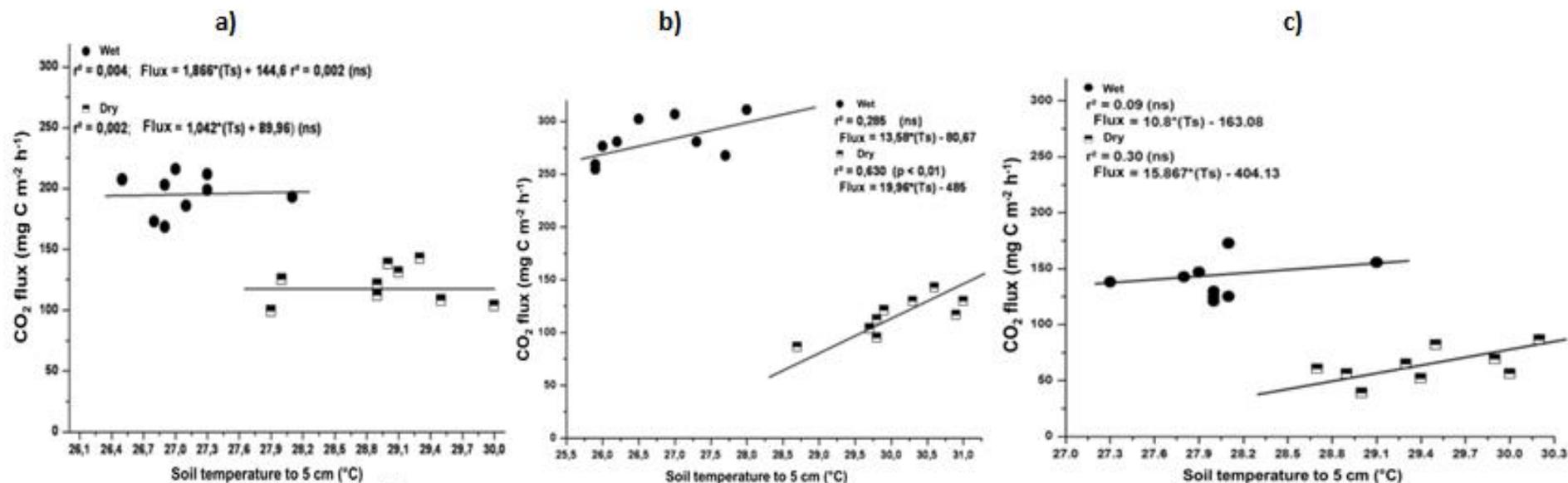


Figure 9. Correlation between the CO₂-C efflux from the soil and the soil temperature in the area of secondary forests (a), active pasture (b) and degraded pasture (c) during the wet and dry season.

The relationship between CO₂-C efflux and soil temperature, resulted in a significant linear relationship ($r^2 = 0.63$, $p < 0.05$) (Figure 9b) only in the area under active grazing as shown in Figure 8c.

In the area of secondary forest there was no pattern of dependence between the variation of the CO₂-C efflux and soil temperature, and this fact may indicate, hypothetically, that other parameters have exerted influence on variability of soil respiration in this area.

Some studies show that the microbial activity in the soil increases linearly with temperature (Bekku et al., 2003; Subke et al., 2003). On the other hand, high temperatures can influence the speed of enzymatic reactions of soil microbial communities, restricting their metabolic activity (Fang and Moncrief, 2001).

The data suggest a relationship between the efflux of CO₂-C and the temperature of the soil when there is high moisture. During the wet season, all plots studied showed a significant relationship between the efflux of CO₂-C and temperature. In the dry period, the portion under active pasture was the only one to show a significant relationship.

The dry season, showed higher soil moisture than other. It is possible that there is a minimum level of moisture in the soil so that the temperature increase causes an increase of microbial metabolism. In all plots studied soil temperature was not high, reaching a maximum of 33°C. This restriction precludes the possibility of enhanced microbial metabolism due to high temperatures.

Conclusion

With respect to the agricultural field with conventional tillage (CT) and no tillage (NT) systems

The results of this work show that the NT system has the potential to mitigate 37.7% of agricultural participation in CO₂-C efflux from soil-based cultivation of soy without the intense tillage as in CT. The soil temperature variation accounted for 65% of the variability of the flow of CO₂-C in CT. The variation of soil moisture explained 73 and 51% of variation in the flow of CO₂-C in NT and CT, respectively. These results indicate that moisture and soil temperature controlled emissions of CO₂-C soil to the atmosphere,

because these parameters directly affect the microbiological activity in the soil.

With respect to forest areas and pastures

The results also demonstrate that active grazing had the highest CO₂-C efflux from the soil to the atmosphere in relation to forests and degraded grasslands in the Western Region of Pará. Moreover, it was shown that both the pastures and forests exhibit a seasonality in this flow, which should be mainly related to precipitation patterns and water potential between soil and air.

There was a strong correlation between soil CO₂ efflux and soil moisture both in the pastures of secondary forests and soil temperature was a controlling factor in the efflux only in the active pasture. The mean value of the flux of CO₂-C obtained in the active pasture was 218.9 mg C m⁻² h⁻¹, a value 40.7% higher than in primary forest C (155.5 mg C m⁻² h⁻¹).

Finally, the results presented here suggest that conventional planting and the management of active grazing activities strongly associated with anthropogenic activities, leverage changes the in biogeochemical carbon balance of these ecosystems, since the CO₂-C efflux from the soil is related to primary productivity of these ecosystems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Repeatability analysis on morphological descriptors in the early stages of development

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The insufficiency of morphological descriptors reveals the importance of studies related to this topic for the genetic improvement of soybean, with attention to the possible descriptors measured in early stages of plant development which allows obtaining fast results. The aim of this work was to estimate the coefficient of repeatability of some morphological descriptors in the early stages of development of soybean and the minimum number of evaluations necessary to predict the real value of genotypes. Five (5) experiments were carried out with 124, 93, 90, 16 and 16 genotypes of soybean for the first, second, third, fourth, and fifth experiment, respectively, with characteristics combined at the stage V3. It was concluded that the length of the first internode requires fewer measurements compared to other measured characteristics. With six measurements, it was possible to obtain 95% and 90% reliability for plant height in V3 and first internode length, respectively, by the methods ANOVA, CP (correl), CP(cov) and AE(correl). With seven measurements, it was obtained 90% reliability for the epicotyl length for all methods used; 85% for the petiole length of unifoliate leaf by method of CP(cov) and 90% by methods ANOVA, CP(correl), AE(correl). With 15 measurements, it was possible to obtain 90% reliability for the hypocotyl length, petiole length of trifoliate leaf and the angle formed by the insertion of the petioles of the unifoliate leaf for all methods used; and, for the rachis length of the first trifoliate leaf, 37 measurements would be necessary for reliability of 90% by methods of ANOVA, CP(correl), CP(cov) and AE(correl).

Key words: *Glycine max* (L.), soybean breeding, number of evaluation, stability.

INTRODUCTION

One of the major components of the global financial market are the agricultural Commodities, within the soybean (*Glycine max* (L.) Merrill) stands out, in relation to the volume sold. Brazil is the world's second largest producer of soybeans with 102,110 million tons produced in 2015/2016, in a total planted area of 33,228 million

hectares, and presents the greater growth in planted area in the Brazilian agribusiness (Embrapa, 2016).

The genetic improvement programs of the oleaginous have been intensively acted in the development of new cultivars in Brazil, mainly after 1,997, when it was sanctioned the Law of Protection of Plant Varieties (LPC)

n°. 9,456, April 25, 1,997, regulated by Decree n°. 2,366, November 5, 1,997 (Neto et al., 2005). For a cultivar to be protected, it is necessary to prove that it is distinct, uniform and stable. The distinctiveness of a cultivar refers to a clear difference from any other variety whose existence on the date of the period of protection is recognized (Grilli, 2005).

The differentiation of cultivars is performed by a minimum margin of descriptors that are specific to each species (Neto et al., 2005). Currently, about 38 descriptors are used between the mandatory and the additional to differentiate genotypes of soybean, nevertheless, they are still insufficient to distinguish cultivars (Nogueira et al., 2008). Therefore, there is the need of expanding that list.

The identification of morphological descriptors evaluated in the early stages of plant development should be preferred, once it enables to obtain fast results and it is not necessary to wait for adult plants, thereby accelerating the work of the breeder.

Nevertheless, in literature, there is few detailed information about the amount of plants which should be measured to determine the number of evaluations needed in order to estimate the difference between the evaluated materials, in order for the selected genotype maintain its characteristic in future generations. According to Cruz et al. (2004) and Paula Ferreira et al. (2010), this expectation may be proved by the repeatability coefficient of the studied characteristic, and being possible to estimate when the measurement of the character is performed repeatedly in a particular individual.

The concept of repeatability can be stated as the correlation between measurements of a given character in the same individual, whose assessments were repeated in time or in space. It expresses the proportion of the total variance that is explained by the variations provided by genotype and by permanent changes attributed to the common environment (Cruz et al., 2004). Many authors such as da Silva et al. (2014), Lessa et al. (2014), Lira et al. (2009), Ribeiro et al. (2015) have been studying repeatability and morphological descriptors for breeding and preservation of cultivars for different crops, showing that the study has impact and importance worldwide.

In the tests involving regularly evaluated genotypes, it is possible to estimate the repeatability coefficients of the variables studied, that is, the probability that this result will be repeated in future evaluations. Also, it is possible to estimate the number of phenotypic observations required, for a certain character, which must be performed on each individual so that discrimination (or

selection) between the genotypes is carried out with a certain degree of reliability and time and labor economy (Cruz and Regazzi, 1997).

There are several methods used to estimate the repeatability, as the variance analysis, principal components and structural analysis (Abeywardena, 1972; Cruz and Regazzi, 2001; Mansour et al., 1981).

The objective of this study was to estimate the repeatability coefficient of some morphological descriptors in the early stages of development of soybean and the minimum number of evaluations necessary to predict the real value of genotypes.

MATERIALS AND METHODS

The experiments were conducted and evaluated in a greenhouse at the city of Viçosa, Minas Gerais – Brazil (20°45'14" S; 42°52'54" W; altitude of 408 m).

Five experiments were conducted in a completely randomized design, in which each experimental unit consisted of a plant, and the experiments one, two and three were conducted with five replicates for each treatment and the experiments four and five were conducted with sixteen repetitions. All experiments were grown in pots containing 3 dm³ of soil with 1/3 of organic matter and seeding depth standardized at 3 cm. After germination, the plants were conducted according to culture recommendations.

In Experiment 1, from September to October 2011, 124 genotypes (UV10-01, UV10-02, until UV10-122, Bossier and BRS Valiosa RR) were evaluated. In Experiment 2, from November to December 2011, 93 soybean genotypes (UV1-001, UV1-002, until UV1-090, BRS Valiosa RR, Bossier and MG/BR-46) were evaluated. In Experiment 3, from February to March 2012, 90 genotypes (UV100B01, UV100B02 up to UV88, Bossier and MG/BR-46) we evaluated. In Experiment 4, from December 2011 to April 2012, 16 genotypes (BRS Maxixe, BRS Candle, BRS 278 RR, BRS 271 RR, BRS Tracajá, UFV TN 105 AP, MGT 401 RR, BRS MG 68, MGT 801, FMT Tucunará, MGT 123 RR, MGT 127 RR, TMG 1176 RR, TMG 7188 RR, Msoy 7211 RR, Msoy 7908 RR) we evaluated. And, in Experiment 5, between June and November 2012, 16 genotypes (BRS Maxixe, BRS Candle, BRS 278 RR, BRS 271 RR, BRS Tracajá, UFVTN 105 AP, MGT 401 RR, BRS MG 68, MGT 801, FMT Tucunará, MGT 123 RR, MGT 127 RR, TMG 1176 RR, TMG 7188 RR, Msoy 7211 RR, Msoy 7908 RR) were also evaluated.

In all experiments, two trefoil completely developed, the variables hypocotyl length (CH), plant height (ALV3), epicotyl length (CE), the first internode (CPIN), the petiole of the first trifoliolate leaf (CPFT), the petiole of the unifoliolate leaf (CPFU) and the rachis of the first trifoliolate leaf (CRFT), were measured at V3. The evaluations were performed using a digital pachymeter. In Experiments 2, 3, 4 and 5 the angle formed by the insertion of the petioles of the unifoliolate leaf (AIFU), was also measured, with a protractor.

Initially, the variance analysis was performed, in order to identify the existence of genetic variability between genotypes, based on the characters analyzed in each experiment. Only for the characters with significant differences between the genotypes ($p < 0.05$), the repeatability study was conducted. The repeatability coefficients (r) were estimated by the variance analysis (ANOVA); principal

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Table 1. Variance analysis of morphological descriptors for soybeans: CH, ALV3, EC, CPIN, CPFT, CPFU, CRFT and AIFU.

V. S.	G.L	CH	ALV3	CE	CPIN	CPFU	CPFT	CRFC	AIFU
-----Experiment 1 (124 genotypes and 5 repetitions)-----									
Genotypes	123	2.01*	147.77*	8.49*	9.74*	0.95*	10.34*	0.71*	-
Residue	496	0.34	8.35	0.76	0.69	0.13	1.12	0.09	-
Overall average		2.89	29.54	6.49	6.08	2.16	10.12	0.81	-
CV%		20.08	9.78	13.47	13.66	16.65	10.45	37.21	-
-----Experiment 2 (93 genotypes and 5 repetitions)-----									
Genotypes	92	1.43*	47.63*	8.35*	8.63*	1.57*	17.70*	0.51*	541.60*
Residue	372	0.36	4.46	0.84	0.54	0.16	1.15	0.15	98.14
Overall average		3.59	18.54	8.06	5.04	2.28	10.44	0.85	62.35
CV%		16.80	11.38	11.39	14.60	17.68	10.27	45.94	15.89
-----Experiment 3 (90 genotypes and 5 repetitions)-----									
Genotypes	89	2.60*	101.51*	2.86*	4.97*	0.64*	10.02*	0.14*	454.80*
Residue	360	0.39	5.10	0.39	0.34	0.10	0.68	0.06	97.49
Overall average		3.59	18.54	8.06	5.04	2.28	10.44	0.85	62.35
CV%		14.95	8.51	9.61	12.29	19.56	11.12	43.78	15.32
-----Experiment 4 (16 genotypes and 16 repetitions)-----									
Genotypes	15	6.65*	505.98*	23.39*	24.38*	2.07*	8.87*	0.45*	2025.88*
Residue	240	0.22	13.96	0.48	0.54	0.08	0.84	0.06	110.73
Overall average		3.39	24.69	6.19	4.46	1.40	7.91	0.78	63.59
CV%		13.61	15.12	11.13	16.48	19.65	11.57	31.87	16.51
-----Experiment 5 (16 genotypes and 16 repetitions)-----									
Genotypes	15	6.26*	248.75*	23.79*	9.83*	3.30*	11.89*	0.55*	1476.42*
Residue	240	0.18	3.91	0.40	0.20	0.04	0.36	0.03	83.75
Overall average		3.45	17.52	4.91	2.94	1.52	6.21	0.72	54.89
CV%		12.20	11.28	12.92	15.10	13.55	9.67	24.32	16.67

* Significant at 5% probability by F-test.

components based on matrices of correlation [CP(correl)] and of variances and covariance phenotypic characteristics [CP(cov)]; and structural analysis, based on the correlation coefficient [AE(correl)] and of variances and covariance [AE(cov)]. The minimum number of measurements necessary to predict the real value of individuals, on the basis of the determination coefficients (R^2) pre-established (0.80, 0.85, 0.90, 0.95 and 0.99), was obtained according to the methodology described by Cruz et al. (2004). Statistical analyzes were performed in the Program Genes: Biometria (Cruz, 2013).

RESULTS AND DISCUSSION

All the characters studied had significant genotype effect ($p < 0.05$) in the experiments in which they were evaluated, indicating that the soybean genotypes differ among themselves, which reinforces the importance of repeatability studies to determine the minimum number of plants to be measured to predict their real value (Table 1). Nogueira et al. (2008) and Matsuo et al. (2012) also identified variability in CH, EC, and CPFT CRFT among

genotypes, in four different periods of seeding. Furthermore, Nogueira et al. (2008) reported large genetic influence for most of the characteristics in different periods, showing little environmental effect.

The repeatability coefficients for the CH, ranged from 0.369 to 0.705. The lowest value was obtained by ANOVA in experiment 2 and the largest one by CP(correl) in Experiment 5. For the ALV3, the lowest value (0.659) was obtained in experiment 2 by the methods of the AE(correl) and AE(cov) and the highest (0.823) in Experiment 5 by the method CP(cov). While for the EC the same coefficient was lower in experiment one (0.724) by the method of ANOVA and higher in experiment 5 (0.823) by the method of CP(cov). The magnitude of the determination coefficients for the CH and CE was greater than or equal to 75.20% by all methods in the five experiments evaluated, and lower than the lowest value obtained by Matsuo et al. (2012), equal to 82.5%. For the ALV3, the values for the

Table 2. Estimate of repeatability coefficients (r) and determination coefficients (Det), using the different methods for the morphological descriptors of soybean: CH, ALV3, CE, CPIN and CPFU.

Methods*	-----CH-----		-----ALV3-----		-----CE-----		-----PIN-----		-----CPFU-----	
	R^2	Det	R^2	Det	R^2	Det	R^2	Det	R^2	Det
-----Experiment 1 (124 genotypes and 5 repetitions)-----										
ANOVA	0.499	83.273	0.770	94.353	0.669	91.009	0.724	92.919	0.526	85.417
CP(cov)	0.533	85.096	0.774	94.487	0.671	91.085	0.728	93.053	0.610	85.904
CP(correl)	0.529	84.886	0.772	94.410	0.670	91.034	0.726	92.989	0.616	89.324
AE(correl)	0.529	84.862	0.771	94.406	0.670	91.021	0.726	92.980	0.679	89.048
AE(cov)	0.527	84.761	0.771	94.381	0.670	91.030	0.726	92.964	0.532	85.442
-----Experiment 2 (93 genotypes and 5 repetitions)-----										
ANOVA	0.369	74.516	0.660	90.644	0.641	89.907	0.750	93.735	0.636	89.714
CP(cov)	0.377	75.152	0.661	90.693	0.648	90.202	0.753	93.841	0.643	89.989
CP(correl)	0.377	75.179	0.660	90.662	0.647	90.147	0.753	93.846	0.639	89.857
AE(correl)	0.376	75.082	0.659	90.630	0.645	90.075	0.753	93.844	0.639	89.834
AE(cov)	0.372	74.794	0.659	90.624	0.644	90.061	0.752	93.801	0.636	89.741
-----Experiment 3 (90 genotypes and 5 repetitions)-----										
ANOVA	0.534	85.130	0.791	94.977	0.562	86.529	0.729	93.076	0.506	83.678
CP(cov)	0.539	85.397	0.795	95.091	0.583	87.477	0.742	93.510	0.532	85.038
CP(correl)	0.537	85.309	0.794	95.080	0.564	86.603	0.737	93.330	0.511	83.958
AE(correl)	0.537	85.283	0.794	95.072	0.561	86.459	0.737	93.323	0.508	83.754
AE(cov)	0.535	85.198	0.793	95.040	0.563	86.550	0.733	93.206	0.507	83.730
-----Experiment 4 (16 genotypes and 16 repetitions)-----										
ANOVA	0.652	96.767	0.688	97.240	0.750	97.961	0.732	97.767	0.623	96.360
CP(cov)	0.692	97.288	0.767	98.141	0.773	98.194	0.770	98.169	0.655	96.819
CP(correl)	0.692	97.295	0.746	97.916	0.771	98.172	0.760	98.062	0.631	96.471
AE(correl)	0.688	97.240	0.742	97.876	0.769	98.154	0.757	98.032	0.654	96.820
AE(cov)	0.680	97.142	0.728	97.713	0.761	98.078	0.742	97.871	0.658	96.857
-----Experiment 5 (16 genotypes and 16 repetitions)-----										
ANOVA	0.682	97.163	0.797	98.429	0.784	98.307	0.753	97.992	0.827	98.705
CP(cov)	0.702	97.415	0.823	98.669	0.804	98.495	0.791	98.378	0.849	98.898
CP(correl)	0.705	97.449	0.808	98.535	0.800	98.464	0.773	98.199	0.842	98.842
AE(correl)	0.701	97.402	0.804	98.502	0.799	98.451	0.769	98.157	0.842	98.837
AE(cov)	0.686	97.216	0.803	98.485	0.790	98.369	0.761	98.073	0.829	98.727

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

determination coefficients were greater than 90% (Table 2). The CPIN presented, in experiment 1, the lowest repeatability coefficient (0.724) by ANOVA and the highest (0.791) in Experiment 5 by CP(cov), associated with the determination coefficients greater than 93% (Table 2). The repeatability coefficient estimated for CPFU presented magnitude of 0.849 by CP(cov) to 0.506 by ANOVA, in the fifth and third experiment, respectively. The determination coefficients ranged from 98.898 to 83.678% (Table 2). Whereas, for the CPFT (Table 3), the

repeatability coefficients ranged from 0.375 (ANOVA), in Experiment 4, to 0.749 [CP(cov)], in Experiment 2; and the determination coefficients presented magnitude of 89.190% in Experiment 1 and 97.411% in Experiment 5.

For the CRFT, the repeatability coefficients ranged from 0.196 to 0.581, with the lowest one obtained by the method of the AE (cov), in Experiment 3, and the largest by methods CP(correl), AE(correl) and AE(cov), in Experiment 1. The same character had determination coefficients that ranged between 54.931 and 94.977%, in

Table 3. Estimate of repeatability coefficients (r) and determination coefficients (Det), using the different methods for the morphological descriptors of soybean: CPFT, CRFT, AIFU, COVG and DVG.

Methods*	-----CPFT-----		-----CRFT-----		-----AIFU-----	
	R^2	Det	R^2	Det	R^2	Det
-----Experiment 1 (124 genotypes and 5 repetitions)-----						
ANOVA	0.623	89.190	0.578	87.268	-	-
CP(cov)	0.631	89.531	0.583	87.467	-	-
CP(correl)	0.627	89.369	0.581	87.400	-	-
AE(correl)	0.627	89.345	0.581	87.384	-	-
AE(cov)	0.627	89.362	0.581	87.385	-	-
-----Experiment 2 (93 genotypes and 5 repetitions)-----						
ANOVA	0.742	93.504	0.319	70.084	0.475	81.880
CP(cov)	0.749	93.709	0.388	75.983	0.540	85.445
CP(correl)	0.745	93.581	0.408	77.507	0.565	86.655
AE(correl)	0.744	93.571	0.390	76.169	0.551	85.976
AE(cov)	0.744	93.555	0.380	75.364	0.523	84.587
-----Experiment 3 (90 genotypes and 5 repetitions)-----						
ANOVA	0.734	93.249	0.198	55.231	0.423	78.565
CP(cov)	0.736	93.298	0.199	55.395	0.491	82.818
CP(correl)	0.734	93.235	0.201	55.756	0.524	84.616
AE(correl)	0.733	93.224	0.197	55.156	0.506	83.671
AE(cov)	0.733	93.200	0.196	54.931	0.474	81.819
-----Experiment 4 (16 genotypes and 16 repetitions)-----						
ANOVA	0.375	90.564	0.283	86.347	0.405	77.309
CP(cov)	0.464	93.276	0.371	90.404	0.471	81.673
CP(correl)	0.439	92.607	0.331	88.799	0.502	83.460
AE(correl)	0.414	91.879	0.291	86.797	0.483	82.352
AE(cov)	0.401	91.454	0.295	86.998	0.451	80.439
-----Experiment 5 (16 genotypes and 16 repetitions)-----						
ANOVA	0.666	96.962	0.516	94.457	0.520	94.534
CP(cov)	0.702	97.411	0.540	94.946	0.600	95.993
CP(correl)	0.695	97.335	0.542	94.977	0.554	95.209
AE(correl)	0.691	97.275	0.530	94.752	0.533	94.815
AE(cov)	0.685	97.201	0.519	94.522	0.535	94.846

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

experiments 3 and 5, respectively. The AIFU presented magnitude of 0.405 by the method of ANOVA and 0.600 in the CP(cov) in Experiment 4 and 5, respectively, associated with the determination coefficient greater than 77.00% (Table 3).

The repeatability coefficients and the prediction of real value for the CH, CE, CPIN, CPFT and CRFT in soybean genotypes were studied by Matsuo et al. (2012). The authors reported that estimates of repeatability coefficients for the CH were low, ranging from 0.345 to

0.793, for the CE ranged from 0.478 to 0.914, for the CPIN, from 0.428 to 0.865, for CPFT, from 0.163 to 0.645 and for CRFC the coefficients ranged from 0.216 to 0.553, with prediction of real value average of 84.72%. The results obtained in Experiments 1 and 2 of this study were similar to that of Matsuo et al. (2012) for the variable CH, however, the magnitude of the repeatability coefficient found in the other experiments and for the other characteristics were superior. This difference in the magnitude of the values of repeatability varies with the

Table 4. Number of evaluations needed associated with different determination coefficients (R^2), estimated for the CH and ALV3 in five experiments based on different methodologies*.

R^2	-----CH-----					-----ALV3-----				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
-----ANOVA-----										
0.80	4.0	6.8	3.5	2.1	1.9	1.2	2.1	1.1	1.8	1.0
0.85	5.7	9.7	4.9	3.0	2.6	1.7	2.9	1.5	2.6	1.4
0.90	9.0	15.4	7.9	4.8	4.2	2.7	4.6	2.4	4.1	2.3
0.95	19.1	32.5	16.6	10.2	8.9	5.7	9.8	5.0	8.6	4.9
0.99	99.4	169.3	86.5	52.9	46.2	29.6	51.1	26.2	45.0	25.3
-----CP(cov)-----										
0.80	3.5	6.6	3.4	1.8	1.7	1.2	2.1	1.0	1.2	0.9
0.85	5.0	9.4	4.8	2.5	2.4	1.7	2.9	1.5	1.7	1.2
0.90	7.9	14.9	7.7	4.0	3.8	2.6	4.6	2.3	2.7	1.9
0.95	16.6	31.4	16.2	8.5	8.1	5.5	9.7	4.9	5.8	4.1
0.99	86.7	163.7	84.6	44.2	42.0	28.9	50.8	25.6	30.0	21.4
-----CP(correl)-----										
0.80	3.6	6.6	3.4	1.8	1.7	1.2	2.1	1.0	1.4	1.0
0.85	5.0	9.4	4.9	2.5	2.4	1.7	2.9	1.5	1.9	1.3
0.90	8.0	14.9	7.7	4.0	3.8	2.7	4.6	2.3	3.1	2.1
0.95	16.9	31.4	16.4	8.5	8.0	5.6	9.8	4.9	6.5	4.5
0.99	88.1	163.4	85.2	44.0	41.5	29.3	51.0	25.6	33.7	23.6
-----AE(cov)-----										
0.80	3.6	6.6	3.5	1.8	1.7	1.2	2.1	1.0	1.4	1.0
0.85	5.1	9.4	4.9	2.6	2.4	1.7	2.9	1.5	2.0	1.4
0.90	8.0	14.9	7.8	4.1	3.8	2.7	4.7	2.3	3.1	2.2
0.95	16.9	31.5	16.4	8.6	8.1	5.6	9.8	4.9	6.6	4.6
0.99	88.3	164.3	85.4	45.0	42.3	29.3	51.2	25.7	34.4	24.1

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix or correlation average; and AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix.

nature of the character, with the genetic properties of the population, with the conditions in which individuals are developed and if the genotype of the individual, where repeated measurements are made, is stabilized (Cruz et al., 2004).

The low estimates of the repeatability coefficients, in general, less than 0.4, result in difficulties for the breeder to identify the best genotypic values from the phenotypic average analysis obtained (Ferreira et al., 1999). According to Neto et al. (2002), these estimates indicate dissimilarity in the repetition of character between one assessment and another.

Neto et al. (2002) observed irregularities of behavior between successive evaluations for diameter, lap height and weight of liquid in heart of palm, due to low magnitude of repeatability coefficients, less than 0.4. Matsuo et al. (2009) stated that the selection of strains with resistance to powdery mildew, based on the results of the group of genotypes adapted in Goiás, Brazil, would not be a good alternative, since it showed repeatability coefficients of less than 0.4.

It was observed, in general, in the present study, that the greater the repetitions number, greater the results for the repeatability coefficient (r) and lower the numbers of evaluations needed. Nevertheless, this occurred in function of the genetic properties of analyzed genotypes. According to Danner et al. (2010), the repeatability and determination coefficients obtained among the individuals selected from a strawberry guava tree were high in relation to those found in surinam cherry tree, because the genotypes of strawberry guava tree presented greater phenotypic stability, due to the increased selection pressure and management used to plants.

Considering a confidence level of 90%, and taking into account the greatest value within each method and between experiments, to predict the real value of CH in soybean genotypes, would be necessary 16 evaluations by the method of ANOVA, and 15 by methods of CP(correl), CP(cov) and AE(cov). For the ALV3, would be necessary 5 evaluations based on all four methods used (Table 4). For CE and CPIN (Table 5) the number of measurements would be 7 and 4 respectively, by ANOVA,

Table 5. Number of evaluations needed associated with different R^2 , estimated for the CE and CPIN in five experiments based on different methodologies*.

R^2	-----CE-----					-----CPIN-----				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	-----ANOVA-----									
0.80	2.0	2.2	3.1	1.3	1.1	1.5	1.3	1.5	1.5	1.3
0.85	2.8	3.2	4.4	1.9	1.6	2.2	1.9	2.1	2.1	1.9
0.90	4.4	5.1	7.0	3.0	2.5	3.4	3.0	3.3	3.3	3.0
0.95	9.4	10.7	14.8	6.3	5.2	7.2	6.4	7.1	6.9	6.2
0.99	48.9	55.6	77.1	33.0	27.3	37.7	33.1	36.8	36.2	32.5
	-----CP(cov)-----									
0.80	2.0	2.2	2.9	1.2	1.0	1.5	1.3	1.4	1.2	1.1
0.85	2.8	3.1	4.1	1.7	1.4	2.1	1.9	2.0	1.7	1.5
0.90	4.4	4.9	6.4	2.6	2.2	3.4	3.0	3.1	2.7	2.4
0.95	9.3	10.3	13.6	5.6	4.6	7.1	6.2	6.6	5.7	5.0
0.99	48.4	53.8	70.9	29.1	24.2	37.0	32.5	34.4	29.6	26.1
	-----CP(correl)-----									
0.80	2.0	2.2	3.1	1.2	1.0	1.5	1.3	1.4	1.3	1.2
0.85	2.8	3.1	4.4	1.7	1.4	2.1	1.9	2.0	1.8	1.7
0.90	4.4	4.9	7.0	2.7	2.2	3.4	3.0	3.2	2.8	2.6
0.95	9.4	10.4	14.7	5.7	4.7	7.2	6.2	6.8	6.0	5.6
0.99	48.8	54.1	76.6	29.5	24.7	37.3	32.5	35.4	31.3	29.0
	-----AE(cov)-----									
0.80	2.0	2.2	3.1	1.2	1.0	1.5	1.3	1.4	1.3	1.2
0.85	2.8	3.1	4.4	1.7	1.4	2.1	1.9	2.0	1.8	1.7
0.90	4.4	5.0	7.0	2.7	2.3	3.4	3.0	3.2	2.9	2.7
0.95	9.4	10.5	14.9	5.7	4.8	7.2	6.2	6.8	6.1	5.7
0.99	48.8	54.5	77.5	29.8	24.9	37.4	32.5	35.4	31.8	29.7

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

CP(correl), CP(cov) and AE(cov). While for the character CPFU would be required at least 9 evaluations by ANOVA, 8 by CP(cov) and 9 by methods of the CP(correl) and AE(cov), and for the CPFT the real value would be obtained with evaluation of 15 individuals by the method of ANOVA, 11 by the CP(cov), 12 by the CP(correl) and 13 by AE(cov) (Table 6).

Taking into account the same confidence level (90%), the prediction of real value to the CRFC would be necessary to evaluate 36 individuals by the methods CP(cov) and CP(correl) and 37 individuals by ANOVA and AE(cov). While for the characteristic AIFU would be needed 13 evaluations by ANOVA, 10 by the CP(cov) and AE(cov) and 9 by CP(correl) (Table 7).

The results for CH, CE and CRFT corroborate with the obtained values by Matsuo et al. (2012), however they present lower predicted values to CPIN and CPFT, which

probably is due to the genetic properties of different genotypes analyzed in the two studies.

The increase in accuracy for 95% implies the need for a greater number of evaluations and this would increase the costs and time for obtaining the results, but if labor-intensive enough, these factors do not impede the realization of the test in any of the characteristics studied.

In general, based on estimates of repeatability and reliability of 95%, considering the methods and experiments average, would be necessary 17 evaluations for hypocotyl length, 6 for plant height in V3, 9 for epicotyl length, 7 for the first internode length, 12 for petiole length of unifoliate leaf and petiole length of trifoliate leaf, 36 for rachis length, 18 for opening angle of the petioles of unifoliate leaf.

Analyzing the relationship between repeatability and number of measurements, it can be stated that: when the

Table 6. Number of evaluations needed associated with different R^2 , estimated for the CPFU and CPFT in five experiments based on different methodologies*.

R^2	-----CPFU-----					-----CPFT-----				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	-----ANOVA-----									
0.80	3.1	2.3	3.9	2.4	0.8	2.4	1.4	1.4	6.7	2.0
0.85	4.5	3.2	5.5	3.4	1.2	3.4	2.0	2.1	9.4	2.8
0.90	7.1	5.2	8.8	5.4	1.9	5.5	3.1	3.3	15.0	4.5
0.95	14.9	10.9	18.5	11.5	4.0	11.5	6.6	6.9	31.7	9.5
0.99	77.8	56.8	96.6	59.8	20.8	60.0	34.4	35.8	165.0	49.6
	-----CP(cov)-----									
0.80	3.0	2.2	3.5	2.1	0.7	2.3	1.3	1.4	4.6	1.7
0.85	4.3	3.2	5.0	3.0	1.0	3.3	1.9	2.0	6.5	2.4
0.90	6.8	5.0	7.9	4.7	1.6	5.3	3.0	3.2	10.4	3.8
0.95	14.4	10.6	16.7	10.0	3.4	11.1	6.4	6.8	21.9	8.1
0.99	74.9	55.1	87.1	52.0	17.7	57.9	33.2	35.6	114.2	42.1
	-----CP(correl)-----									
0.80	3.1	2.3	3.8	2.1	0.8	2.4	1.4	1.5	5.1	1.8
0.85	4.4	3.2	5.4	2.9	1.1	3.4	1.9	2.1	7.2	2.5
0.90	7.0	5.1	8.6	4.7	1.7	5.4	3.1	3.3	11.5	3.9
0.95	14.8	10.7	18.2	9.9	3.6	11.3	6.5	6.9	24.3	8.3
0.99	77.0	55.9	94.6	51.4	18.6	58.9	34.0	35.9	126.4	43.4
	-----AE(cov)-----									
0.80	3.1	2.3	3.9	2.1	0.8	2.4	1.4	1.5	5.7	1.8
0.85	4.4	3.2	5.5	3.0	1.1	3.4	1.9	2.1	8.0	2.5
0.90	7.0	5.1	8.7	4.8	1.7	5.4	3.1	3.3	12.7	4.0
0.95	14.8	10.8	18.4	10.0	3.6	11.3	6.5	6.9	26.9	8.5
0.99	77.3	56.0	96.0	52.3	18.6	59.0	34.0	36.0	140.0	44.4

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

repeatability is high, the increase in the number of measurements will result in little increase in accuracy, in relation to which it would have if an individual was evaluated by means of a single observation. When repeatability is low, the increase of repeated measurements can result in a significant increase in precision; and, with intermediate levels of repeatability, is rarely beneficial to do more than three measures in each individual for each character (Cruz et al., 2004; Cargnelutti Filho and Gonçalves, 2011). Dos Santos et al. (2016) also found similar results to the present work.

The results indicate the need for further studies with the aim of better understanding about the effect of genotypes, the influence of environmental conditions and genotype x environment interaction for the additional descriptors, aiming to estimate repeatability and determination coefficients and the number of evaluations

needed in order to estimate the difference between the evaluated materials.

Conclusions

The length of the first internode requires a smaller amount of measurements in comparison to other characteristics measured in the early stages of soybean development for the same level of reliability.

With six measurements, reliability levels of 95 and 90% were obtained for plant height in V3 and length of the first internode, respectively, by the methods of the ANOVA, CP(correl), CP(cov) and AE(correl).

With seven measurements, 90% of accuracy was obtained for the epicotyl length, for all methods used; 85% for the petiole length of the unifoliate leaf, by method

Table 7. Number of evaluations needed associated with different R^2 , estimated for the CRFT and AIFU in five experiments based on different methodologies.*

R^2	-----CRFC-----					-----AIFU-----				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	-----ANOVA-----									
0.80	2.9	8.5	16.2	10.1	3.8	-	4.4	5.5	3.7	3.8
0.85	4.1	12.1	23.0	14.3	5.3	-	6.3	7.7	5.2	5.5
0.90	6.6	19.2	36.5	22.8	8.5	-	10.0	12.3	8.3	8.7
0.95	13.9	40.6	77.0	48.1	17.8	-	21.0	25.9	17.6	18.3
0.99	72.2	211.3	401.2	250.5	92.9	-	109.5	135.0	91.6	95.3
	-----CP(cov)-----									
0.80	2.9	6.3	16.1	6.8	3.4	4.5	3.4	4.1	2.7	3.1
0.85	4.1	9.0	22.8	9.6	4.8	6.4	4.8	5.9	3.8	4.4
0.90	6.4	14.2	36.2	15.3	7.7	10.1	7.7	9.3	6.0	6.9
0.95	13.6	30.0	76.5	32.3	16.2	21.3	16.2	19.7	12.7	14.7
0.99	70.9	156.5	398.6	168.1	84.3	111.1	84.3	102.7	66.1	76.4
	-----CP(correl)-----									
0.80	2.9	5.8	15.9	8.1	3.4	4.0	3.1	3.6	3.2	3.2
0.85	4.1	8.2	22.5	11.4	4.8	5.6	4.4	5.2	4.6	4.6
0.90	6.5	13.1	35.7	18.2	7.6	8.9	6.9	8.2	7.2	7.2
0.95	13.7	27.6	75.4	38.3	16.1	18.8	14.6	17.3	15.3	15.3
0.99	71.4	143.6	392.8	199.8	83.8	98.1	76.2	90.0	79.7	79.6
	-----AE(cov)-----									
0.80	2.9	6.3	16.3	9.7	3.5	4.3	3.3	3.9	3.5	3.5
0.85	4.1	8.9	23.0	13.8	5.0	6.1	4.6	5.5	5.0	5.0
0.90	6.5	14.1	36.6	21.9	8.0	9.6	7.3	8.8	7.9	7.9
0.95	13.7	29.7	77.2	46.2	16.8	20.4	15.5	18.5	16.6	16.7
0.99	71.5	154.9	402.5	241.0	87.7	106.1	80.7	96.6	86.6	87.2

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

of CP(cov) and 90% by methods of ANOVA, CP(correl), AE(correl).

With 15 measurements, it was possible to obtain 90% reliability for the hypocotyl length, petiole length of the trifoliolate leaf and the angle formed by the insertion of the petioles of the unifoliolate leaf for all methods used; and, for the rachis length of the first trifoliolate leaf, would be necessary 37 measurements for reliability of 90% by methods of ANOVA, CP(correl), CP(cov) and AE(correl).

CONFLICT OF INTERESTS

The authors have declared any conflict of interests.

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Full Length Research Paper

Evaluation of suitability of tube well water for irrigation in Maiduguri Metropolitan, Borno State, Nigeria

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Groundwater contamination is gaining more concerns due to its direct and/or indirect impact on public health. Groundwaters from various sources are commonly used to irrigate vegetables in Maiduguri, Borno State. In this study, the physicochemical qualities of groundwater collected from 20 randomly selected tube wells in Maiduguri were evaluated for their suitability or otherwise for irrigation purposes. Selected wells were extensively used as sources of irrigation water. Standard procedures were followed during the analysis. The results revealed that the mean values of pH (6.6 to 8.0), calcium (2.6 to 5.2 meq/l), magnesium (2.6 to 5.0 meq/l), sodium (2.2 to 7.5 meq/l), carbonate (0.5 to 1.3 meq/l), bicarbonate (1.8 to 5.2 meq/l), chloride (0.9 to 2.9 meq/l), sulphate (2.8 to 9.8 meq/l), potassium (0.3 to 0.9 mg/l), Boron (0.1 to 0.6 meq/l) and nitrogen (1.9 to 4.8 mg/l) were all found to be within the acceptable limit for suitability of water for irrigation use based on Food and Agriculture Organization (FAO) recommendations. The results further revealed that, the values of electrical conductivity (0.3 to 0.7 ds/m), total dissolved solids (192 to 448 mg/l), sodium adsorption ratio (0.40 to 3.62), residual sodium carbonate (-1.3 to -7.6 meq/l), magnesium adsorption ratio (35.80 to 49.06%) and Kelly ratio (0.10 to 0.90) of the samples were within the safe limit recommended for irrigation water use. The quality of tube well waters in the study area can thus be regarded as good, and suitable for irrigation purposes, but similar analysis should be conducted on a routine basis to monitor the qualities of the waters toward safeguarding public health.

Key words: Tube well waters, physicochemical qualities, irrigation suitability, public health, Maiduguri.

INTRODUCTION

The rise in the global population has resulted in a larger increase in water demand in recent decades. The increasing water demand from domestic and agricultural uses has led to overexploitation of groundwater,

particularly in arid and semi-arid regions that are commonly characterized by poor availability of water resources (Ebrahimi et al., 2016). Irrigated agriculture is the largest water consuming sector accounting for more

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than 70% of the total water consumption in most countries developed or otherwise, as opposed to the 30% used by industries and for domestic use (UN, 2009; FAO, 2011). In Nigeria, agriculture is equally the highest water consuming sector accounting for 69% of the total water withdrawal followed by the domestic sector with about 1.7 km³ (21%) and the industrial sector with 0.8 km³ (10%). The total irrigated area in Nigeria is about 975054 ha (Aquastat, 2010).

Ground water is generally assumed to be safe for usage because it is located below the soil surface and is usually not in contact with the atmosphere (Quist et al., 1988). However, ground water are often contaminated due to human activities such as improper waste disposal, poor sanitation, seepage of agrochemicals and mining close to boreholes and shallow wells (Salifu et al., 2013; Fianko et al., 2010; Jain et al., 2009; Carpenter et al., 1998).

Water quality is simply the characteristics of a water supply that will influence its suitability for a specific use such as irrigation or domestic use. Quality is a function of physical, chemical and biological properties of the water. In irrigation water evaluation, emphasis is given only on physical and chemical characteristics of water. Water quality concerns have often been neglected because good quality water supplies have been abundant and readily available. This situation is now changing in many parts of the world due to the increasing demand for fresh water to meet the demand for food and fibre for the growing population as well as the increasing competition from other non-agricultural sectors (FAO, 2011). High quality crops can be produced only by using high-quality irrigation water keeping other inputs optimal. Therefore, a better understanding of the irrigation water quality is critical to the management of water for long term productivity and for public health concerns. The quality of irrigation waters can vary considerably irrespective of their sources, depending on the type and concentrations of the pollutants in it (James et al., 2012; Al-Ahmad, 2013). The presence of appreciable quantities of chemical substances in solution above certain fairly well defined limit can reduce crop yield and deteriorate soil fertility.

The suitability of water for irrigation depends upon the quality of the water, the soil type, the salt tolerant characteristics of the plants, climate and drainage characteristics of the soil. Irrigation using water of questionable quality will lead to problems of salinity, permeability and specific toxicity. A salinity problem occurs when the total quantity of dissolved salts in irrigation water is high enough that it accumulates in the root zone making it difficult for the plants to extract water resulting in reduced growth and wilting. The permeability problem with respect to water quality is noticed when the rate of infiltration is reduced by some salts to an extent that the plant is not given the right amount of water required. A toxicity problem occurs as a result of uptake

and accumulation of certain constituents from irrigation even when salinity is low.

In Maiduguri municipality, the common sources of water for irrigation are usually shallow rivers/ponds, and groundwater from tube wells and wash bores. Most of the rivers/ponds often get dried up untimely; thus inflicting a moisture stress on crops resulting in low yield and economic loss. Farmers in this region therefore, primarily rely solely on tube wells for irrigating their crops. The common crops irrigated include maize, onion, tomatoes, pepper, carrot, lettuce, cabbage and amaranthus among others. But groundwater contamination in relation to irrigation is one area that has not received adequate attention at in Maiduguri. Because of their toxicological importance in ecosystems and impact on public health, it is imperative to examine the quality of any water used for irrigation. This study was therefore carried out to examine the quality of groundwater from tube wells water in Maiduguri Metropolitan area of Borno State, Nigeria with a view to determine their suitability or otherwise for irrigation purposes in semi-arid environment. The study will also find out areas with potential risk where water treatment could be needed before irrigation.

MATERIALS AND METHODS

The study was carried out in Maiduguri Metropolitan area of Borno State, Nigeria, which is situated in the Sudano-Sahelian region of Northern Nigeria and located between latitude N11° 46'18" to N 11° 53' 21" and longitude E13° 03' 23" to E 13° 14' 19" (Figure 1). The area which is about 355 m above sea level lies within the Lake Chad Basin formation, which is an area formed as a result of down-warping during the Pleistocene period. This area is known for its dryness, with semi-arid climate or tropical grasslands vegetation. The climate is characterized by short wet season that last for about four months (July to October) and long dry season of 6 to 7 months (November to May). The average annual rainfall is around 640 mm and the temperature is high ranging between 20 and 40°C. The soil is predominantly sandy loam texture (Arku et al., 2011).

Water sampling and analysis

Water samples were collected from twenty (20) different randomly selected tube wells that were extensively used sources of water irrigation in the study area. The water samples were taken using test bottles which were filled with water, labeled, corked and transferred to the laboratory for physico-chemical analysis. The samples were analyzed for pH, Calcium, Magnesium (Mg²⁺), Sodium (Na⁺), Carbonate (Co³⁻), Bicarbonate (Hco³⁻), Chloride (Cl⁻), Sulphate (So⁴⁻), Potassium (K⁺), Boron (B), Nitrogen (N), and Electrical conductivity (EC) using the procedures recommended in the standard methods for the examination of water and waste water (APHA, 1999). The pH and electrical conductivity were measured in the field using a pH and conductivity meters respectively. Calcium and magnesium were determined using Varian AA240 Fast Sequential Atomic Absorption Spectrometer. Chloride and Nitrogen were analysed using ICS-90 chromatography. Sodium and potassium were determined using a flame emission photometer. Carbonate and bicarbonate were determined by titration with HCL Sulphate was analysed by ion chromatography (DX-120, dionex) and Boron was analysed using ICP-MS (Ultra mass 700, Varian).



Figure 1. Study area.

While values of Total dissolved solids (TDS), Sodium adsorption ratio (SAR), Magnesium adsorption ratio (MAR) Residual sodium carbonate (RSC) and Kelly ratio (KR) were calculated using the relationships shown below.

The values of total dissolved solids (TDS) were obtained using Equation 1 as contained in Food and Agriculture Organization (FAO, 1985):

$$TDS(mgl^{-1}) = EC(dSm^{-1}) * 640 \quad (1)$$

While the concentrations of SAR in the water samples were computed using Equation 2 (Richards, 1954).

$$SAR = \frac{Na}{\sqrt{\frac{(Ca^{2+} + Mg^{2+})}{2}}} \quad (2)$$

Equation 3 as contained in Raghunath (1987) was employed to calculate MAR:

$$MAR = \frac{Mg^{2+}}{Ca^{2+} + Mg^{2+}} \quad (3)$$

The values of RSC were evaluated using Equation 4 (Eaton, 1950):

$$RSC = (HCO_3^- + CO_3^-) - (Ca^{2+} + Mg^{2+}) \quad (4)$$

While KR was evaluated using the expression recommended by Kelly (1963) as in Equation 5:

$$KR = \frac{Na^{2+}}{Ca^{2+} + Mg^{2+}} \quad (5)$$

The results were subjected to statistical analysis using descriptive analysis.

RESULTS AND DISCUSSION

The physicochemical properties of the tube wells water analyzed were presented in Table 1. The mean pH values of the analyzed water samples ranged from 6.6 to 8.0. The pH is commonly known as a measure of acidity or alkalinity of water. A pH scale is in the range of 1 to 14, with pH 1 to 7 being acidic and pH greater than 7 being alkaline. The normal range 6.5 to 8.4 (Table 2) this implies that the samples were alkaline. Lower pH values are undesirable as they trigger corrosion of irrigation

Table 1. The mean values of physicochemical properties of tube well waters in the study area.

Tubewell number	Depth (m)	pH	Ca ²⁺ (meq/l)	Mg ²⁺ (meq/l)	Na ⁺ (meq/l)	Co ₃ ⁻ (meq/l)	HCo ₃ ⁻ (meq/l)	Cl ⁻	So ₄ ⁻	K ⁺ (mg/l)	B (mg/l)	N (mg/l)
1	45	7.2	4.6	5.0	7.5	0.5	5.2	2.8	8.2	0.8	0.6	4.1
2	45	6.7	4.3	4.8	5.2	0.8	4.8	2.6	9.6	0.5	0.4	4.8
3	60	7.3	3.8	4.1	4.8	0.5	3.2	2.2	9.8	0.3	0.1	4.4
4	66	7.1	4.3	3.8	3.8	0.9	4.6	1.8	5.0	0.7	0.5	3.8
5	63	6.7	5.2	4.4	2.6	1.0	2.8	1.6	4.2	0.7	0.3	3.6
6	48	7.1	3.8	4.6	3.8	1.0	5.0	1.9	2.8	0.9	0.4	3.9
7	45	7.2	3.9	3.4	3.2	1.1	2.2	2.0	5.6	0.6	0.2	4.7
8	42	6.8	3.4	3.2	2.9	1.1	3.4	2.9	6.4	0.6	0.6	2.8
9	69	7.5	5.2	2.9	2.2	0.7	3.4	1.4	8.0	0.5	0.3	2.5
10	66	7.7	4.0	4.3	3.0	0.6	4.0	1.3	6.8	0.4	0.5	3.2
11	60	7.5	3.6	5.0	5.0	0.8	4.0	1.2	7.3	0.6	0.2	3.0
12	48	7.3	3.4	5.0	4.3	0.9	4.6	1.5	7.6	0.6	0.2	4.0
13	51	6.7	2.8	3.8	2.3	1.0	4.6	2.5	3.9	0.9	0.5	4.1
14	54	7.2	3.0	4.1	2.4	1.1	2.0	2.4	4.7	0.9	0.1	2.6
15	57	7.8	4.2	2.6	6.0	1.3	1.8	1.0	5.8	0.3	0.2	2.7
16	60	7.5	2.6	3.4	5.5	1.1	2.2	0.9	9.0	0.3	0.3	1.9
17	60	6.6	3.7	4.8	2.8	0.9	2.4	1.3	9.5	0.8	0.6	2.0
18	63	8.0	5.0	4.6	3.5	0.8	4.6	2.8	6.8	0.8	0.4	3.2
19	66	7.4	4.3	3.6	4.8	0.6	3.6	2.7	7.7	0.6	0.6	4.3
20	72	7.1	4.5	4.9	4.3	0.8	4.4	2.3	7.0	0.5	0.5	4.7
SEE±	2.042	0.888	0.167	0.315	0.049	0.205	0.1446	0.446	0.044	0.0388	0.201	0.332
S.D	9.131	0.381	0.746	1.408	0.219	1.099	0.663	1.994	0.199	0.172	0.901	1.022

SEE ± standard error of estimates and SD=Standard deviation.

pipes where they occur. High pH values above 8.5 are in most cases caused by high concentrations of carbonate and bicarbonate which is referred to as alkalinity. The values are similar to pH values of 6.3 to 8.1 for ground water reported by Salifu et al. (2017) in western region of Ghana.

Calcium

The calcium concentrations of the analyzed samples were in the range of 2.6 to 2.5 meq/l. (Table 1). The values obtained were far below the recommended maximum usual range of 0 to 20 meq/l in irrigation water (Ayers and Westcott, 1985). Calcium contents relate to the hardness of water and it is an important water quality evaluation parameter that guides in the classification of water as either soft or hard. This implies that the groundwater samples analyzed are therefore suitable for irrigation. The values are in agreement with the calcium concentrations of ground water reported by Hakim et al. (2009)

Magnesium

The magnesium concentrations in the water samples were in the ranged between 2.6 to 5.0 meq/l. The values

are within the acceptable range (0 to 5 meq/l) of values for suitability of water for irrigation as shown in Table 2. Narany et al. (2012) reported similar findings of magnesium concentrations (1.58 to 5 meq/l) in irrigation water sample. The water collected from the tube wells in Maiduguri can therefore be free of magnesium problem.

Sodium

The sodium levels for the analyzed samples ranged from 2.2 to 7.5 meq/l (Table 1). These values are within the normal accepted ranges for sodium in irrigation water (Tables 2 and 3). Apparently, the ground waters in the tube wells are devoid of sodium problem. High sodium ions concentration in irrigation water will result into salinity problem that affects the permeability of the soil and results in infiltration problems. As continuous quantities of soluble salts accumulate in the root zone, it becomes difficult for the plants to extract water from the salty salt solution and this will result in poor growth performance and lower yields.

Carbonate and bicarbonate

The levels of carbonate and bicarbonate in the water samples were found to ranged between 0.5 and 1.3 meq/l

Table 2. FAO guidelines for irrigation water quality interpretation (Ayers and Westcott, 1985).

Water constituents	Units	Degree of restriction on use		
		None	Slight to moderate	Severe
Salinity				
EC _w	dS/m	<0.7	0.7-3.0	>3.0
TDS	mg/l	<450	450-2000	>2000
Salinity, infiltration influence				
SAR=0-3 and EC _w =		>0.7	0.7-0.2	<0.2
SAR=3-6 and EC _w =		>1.2	1.2-0.3	<0.3
SAR=6-12 and EC _w =		>1.9	1.9-0.5	<0.5
SAR=12-20 and EC _w =		>2.9	2.9-1.3	<1.3
SAR=20-40 and EC _w =		>5.0	5.0-2.9	<2.9
Specific iron toxicity				
Sodium (Na ⁺)	-	-	-	-
Surface irrigation	SAR	<3	3-9	>9
Sprinkler irrigation	meq/l	<3	>3	-
Chloride (Cl⁻)				
Surface irrigation		<4	4-10	>10
Sprinkler irrigation		<3	>3	-
Boron (B)		<0.7	<0.7-3.0	>3.0
Miscellaneous effects				
Nitrogen		<5	5-30	>30
Bicarbonate (HCO ₃)		<1.5	1.5-7.5	>7.5
pH		Normal range 6.5 to 8.4		-

and 1.8 and 5.2 meq/l, respectively. These values and both within the acceptable limits are provided in Table 2. A high concentration of carbonate and bicarbonate in water increases the sodium adsorption ratio which translates to salinity problem

Chloride

The chloride contents of the water samples analyzed ranged from 0.9 to 2.9 meq/l. The values obtained were less than 4 meq/l which indicated no restriction in the use of the source of water for irrigation based on the recommendation of Ayers and Westcott (1985) presented in Tables 2 and 3. Chloride is an essential element to plants, but needed at very low concentrations and it can be toxic to crops at high concentrations. High concentrations of chlorides affect the growth of plants by increasing the osmotic pressure, reduce water availability to plants and hence reduced crop growth and productivity.

Sulphate

The sulphate concentrations of the tube wells water

ranged from 2.8 to 9.8 meq/l. Sulphate ion is a major contributor to salinity. However, sulphate is generally considered to be non-toxic except at very high concentrations where high sulphate may interfere with uptake of other nutrients. The source of water can safely be used for irrigation since the values obtained are within the 0 to 20 meq/l acceptable limit in irrigation water by FAO (Table 2).

Potassium

The potassium levels in the analyzed water samples ranged between 0.3 and 0.9 mg/l and these values are within the acceptable range of 0 to 2 mg/l (Table 2) provided by FAO for irrigation purposes. Potassium is an important element when evaluating the suitability of ground water for irrigation. Reddy (2013) reported similar values for ground water in India.

Boron

Boron concentrations for the water samples in this study

Table 3. Laboratory determinations needed to evaluate common irrigation water quality problems (Ayers and Westcott, 1985).

Water parameter	Symbol	Unit	Usual range in irrigation water
Salinity			
Salt content	-	-	-
Electrical conductivity	EC _w	dS/m	0 - 3
Total dissolved solids	TDS	mg/l	0 - 2000
Cations and anions			
Calcium	Ca ²⁺	meq/l	0 - 20
Magnesium	Mg ²⁺	meq/l	0 - 5
Sodium	Na ⁺	meq/l	0 - 40
Carbonate	CO ₃ ⁻	meq/l	0 - 1
Bicarbonate	HCO ₃ ⁻	meq/l	0 - 10
Chloride	Cl ⁻	meq/l	0 - 30
Sulphate	SO ₄ ⁻	meq/l	0 - 20
Nutrients			
Nitrate-nitrogen	NO ₃ ⁻ -N	mg/l	0 - 10
Ammonium-nitrogen	NH ₄ ⁻ -N	mg/l	0 - 5
Phosphate-phosphorus	PO ₄ ⁻ -P	mg/l	0 - 2
Potassium	K ⁺	mg/l	0 - 2
Miscellaneous			
Boron	B	mg/l	0 - 2
Acid/Basicity	pH	1-14	6.0 - 8.5
Sodium adsorption ratio	SAR	-	0 - 15

ranged from 0.1 to 0.6 meq/l. Boron is one of the essential elements for plants growth that is required in relatively small quantities. But at higher concentration boron becomes toxic. Boron toxicity can be observed on sensitive crops at concentrations less than 1 mg/l and it was suggested that irrigation water particularly from ground water ought to be analyzed for the concentration of boron before crops are irrigated. Based on the recommendation of Ayers and Westcott (1985), irrigation water with boron concentrations of less than 0.7 meq/l can be safely used for irrigation without restriction (Tables 2 and 3). The values obtained therefore showed that the source of water is suitable for irrigation with regard to Boron problems.

Nitrogen

The nitrogen levels in the analyzed samples were in the range of 1.9 to 4.8 mg/l (Table 1). Nitrogen in irrigation water is generally a fertility issue. Higher concentrations of nitrogen in irrigation water can result into quality problems in crops such as barley and sugar beets and excessive vegetative growth in some vegetables. However, these problems can generally be taken care

using appropriate fertilizer application and irrigation best management practices. The values obtained were found to be within the allowable limit for the use of water for irrigation presented in Table 2.

Electrical conductivity (EC)

The EC values of the analyzed samples in this study ranged from 0.3 to 0.7 dS/m (Table 4). According to Ayers and Westcott (1985), groundwater with EC value greater than 3 dS/m is termed "Fair" and would greatly affect crop productivity or yield. Water is termed "Good" if the EC is in the range of 0.7 to 3 dS/m. While water with EC value of less than 0.7 dS/m is classified as "Excellent" for irrigation.

Electrical conductivity is one of the most important parameter for determining the suitability of water for irrigation. It is a measure of EC simply refers to the ability of a substance to conduct electric current. Water that is free from salt strongly resists the passage of an electric current. Most of the salts in water are present in their ionic forms and are capable of conducting current. The more the quantities of dissolved salts in water the higher

Table 4. Values of EC (dS/m), TDS, SAR, MAR, RSC and KR.

S/N	EC	TDS	SAR	MAR	RSC	KR
1	0.3	192	0.54	45.28	-4.9	0.11
2	0.4	256	0.54	44.07	-6.2	0.11
3	0.3	192	0.45	43.16	-5.8	0.10
4	0.4	256	0.50	46.19	-2.6	0.12
5	0.3	192	0.40	45.83	-5.8	0.10
6	0.6	384	0.92	48.04	-4.2	0.21
7	0.5	320	0.65	38.10	-7.2	0.14
8	0.6	384	1.16	48.48	-2.1	0.32
9	0.6	384	1.09	35.80	-4.0	0.27
10	0.7	448	1.52	48.72	-3.7	0.44
11	0.6	384	2.50	44.33	-4.9	0.27
12	0.6	384	1.17	49.06	-5.1	0.41
13	0.6	384	0.69	37.50	-2.4	0.63
14	0.6	384	1.17	39.29	-5.3	0.29
15	0.5	320	0.69	48.60	-7.6	0.15
16	0.4	256	2.40	39.08	-5.4	0.57
17	0.6	384	1.25	48.00	-6.7	0.28
18	0.7	448	1.64	47.92	-4.2	0.38
19	0.3	192	3.62	45.45	-1.3	0.90
20	0.6	384	0.68	48.00	-7.3	0.14

its conductivity value. EC is frequently expressed in decisiemens per meter (dS/m) and microsiemens per centimeters ($\mu\text{S/cm}$). Since the values of EC obtained were less than 0.7 ds/m (Table 2), the groundwater in the sampled tube wells can be classified as excellent for irrigation.

Total dissolved solids (TDS)

TDS of the tube wells water in the study area were in the range of 192 to 448 mg/l (Table 4). Total dissolved solids (TDS) simply refer to any dissolved minerals, salts, metals, cations, or anions in water (Lamgenegger, 1990). It is an important parameter that indicates the general nature of salinity of the water. The use of a water source with very high salinity level increases the osmotic potential of the soil water. An increase in the osmotic pressure of the soil solution increases the amount of energy, which plants must use to take up water from the soil. As a result, respiration is increased and the growth and yield of most plants decline progressively as osmotic pressure increases. Based on Ayers and Westcott (1985) degree of restriction on use of water classification, water with TDS values less than 450 mg/l are described as "None" and considered suitable or good for irrigation (Tables 2 and 3). Evidently, the sampled waters can be described as excellent for irrigating purposes.

Sodium absorption ratio (SAR)

SAR values of the water samples in this study ranged

from 0.40 to 3.62 (Table 4). Todd (1980) and Sadashivaiah et al. (2008) classified irrigation water with SAR value less than 10 meq/l as "Excellent" for irrigation usage and those with SAR values in the range of 10 and 18 meq/l are termed "Good". While water with SAR values ranging from 18 and 26 meq/l and greater than 26 meq/l are classified as "Doubtful" and "Unsuitable", respectively. SAR is an important water quality parameter that shows the effect of relative cation concentration on sodium accumulation in the soil. Therefore, SAR is a more reliable method for the assessment of water quality for irrigation with respect to sodium hazard based on the fact that, it is more closely related to exchangeable sodium percentage compared to simple sodium percentage (Tiwari and Mansour, 1988). High concentrations of sodium affect the physical and soil structure by dispersing the clay particles which results in the formation of crusts, waterlogging and reduced permeability (Kelly, 1951; Suarez et al., 2006; Vasanthavigar et al., 2012). The values obtained are less than the SAR value of 10 which is designated as excellent for irrigation use by Todd (1980) and Sadashivaiah et al. (2008). Al-Ahmadi (2013) reported lower SAR values (0.3 to 3.5) similar to the results of this study.

Residual sodium carbonate (RSC)

Table 4 also shows that the residual sodium carbonate values of the samples ranged from -1.3 to -7.6 meq/l.

RSC is a term used by Eaton (1950) to express bicarbonate hazard in water in order to ascertain the suitability or otherwise of the source of water for irrigation. Water with high bicarbonate concentration may result in the precipitation of calcium and magnesium as well as increase in the relative proportion of sodium in the water in the form of sodium carbonate. Eaton (1950) and Richard (1954) termed water with RSC values of less than 1.25 meq/l as safe for irrigation. While water with an RSC values in the range of 1.25 and 2.5 meq/l is termed marginal and could be used with good irrigation management techniques and soil salinity monitored by laboratory analysis. But water with RSC values greater than 2.5 meq/l are classified as unsuitable for irrigation. The values obtained are less than 0 and this revealed that the source of water is very good for irrigating crops based on Bishnoi et al. (1984), Eaton (1950) and Richard (1954) classification of water quality for irrigation. The RSC values of this study are similar to the findings of Zaidi et al. (2016) which reported lower RSC values of less than 1.25.

Magnesium adsorption ratio (MAR)

MAR values for the analyzed samples were found to have ranged between 35.80 and 49.06% (Table 4). Szabolics and Darab (1964) and Raghunath (1987) considered magnesium content as an important parameter in evaluating the suitability of water for irrigation. Calcium and magnesium do not behave the same in the soil, and magnesium was found to deteriorate the soil structure especially when the water is highly saline. FAO (2008) reported that high concentration of magnesium promotes a higher development of exchangeable sodium in irrigated soils. The values obtained were less than 50% that was recommended by Gupta and Gupta (1987) and Raghunath (1987) as suitable for irrigation.

Kelly's ratio (KR)

The result finally revealed that the KR values of the samples were in the range of 0.10 and 0.90. KR is an important parameter recommended by Kelly (1963) for the evaluation of water quality for irrigation. The parameter is based on the concentration of Na^{2+} , Ca^{2+} and Mg^{2+} in the water. According to this evaluation method, irrigation water with a KR value greater than one indicates an excessive level of sodium in the water and is therefore considered unfit for irrigation. On the other hand, irrigation water with a KR value of less than 1 indicates that the water is free from sodicity hazard, and is suitable to be used for irrigation purpose. This indicates that tube wells from where water samples were collected are good sources of irrigation water.

Conclusion

The study evaluated the suitability of tube well waters for irrigation purposes and found that, there were no conflict between the values of the parameters studied and the standard values. The pH level of water samples from tube wells analyzed were within the acceptable limit for suitability of water for irrigation based on FAO recommendations. The concentrations of Calcium, Magnesium, Sodium, Carbonate and Bicarbonate, Chloride, Sulphate, Potassium, Boron and Nitrogen contents of the analysed water samples were all found to be within the acceptable limit recommended by Ayers and Westcott (1985) for suitability of water for irrigation. EC and TDS values of the water samples analyzed were all found to be within the recommended limits for suitability of water for irrigation based on Ayers and Westcott (1985) classification. SAR values of the analyzed water samples were less than the SAR value of 10 recommended as excellent for irrigation usage. The values of RSC, MAR and KR all fall within the allowable limits. This suggests that the source of water can safely be used for irrigation and there is indication of the need to conduct any pre-irrigation treatment. Public health is thus safe.

CONFLICT OF INTERESTS

The authors have declared any conflict of interests.

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Full Length Research Paper

Assessment of factors influencing smallholder farmers' adoption of mushroom for livelihood diversification in Western Kenya

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Poverty is a critical issue in developing countries. It has become almost impossible to carry out any profitable agricultural production as a means of livelihood diversification in areas with small land acreage. Therefore, there is need to identify enterprises that can be incorporated into small holder farmers' production processes. This study assessed the factors that influence small holder farmers' adoption of mushroom for livelihood diversification from a sample of 240 smallholder farmers in Vihiga County in Western Kenya. Both descriptive methods and a binomial logit model were applied in the analysis. Results indicate that about three quarters of the farmers in the area were aware of mushroom production in the area and four fifth of them were willing to engage in mushroom production as a livelihood diversification option. Empirical results indicated that marital status, formal education, group membership, consumption of mushroom, availability of market for mushroom in the area, previous involvement/experience in mushroom production and total land acreage had a positive effect on farmers' awareness of mushroom production. Age, gender, awareness level, consumption of mushroom and total land available had a positive effect on the farmers' willingness to engage in mushroom production.

Key words: Poverty, land constraint, livelihood diversification, mushroom production.

INTRODUCTION

Agriculture can be used to deliver an annual economic growth rate of 10% in Kenya, if the right policies and framework are put in place (UN, 2000). This can be achieved through diversification to high value crops and transformation of the smallholder agricultural sector from subsistence to an innovative and commercially oriented sector. The World Bank (2007) identifies three key areas

where improvements are critical if strong economic performance is to be sustained; infrastructure, agricultural productivity, and the investment climate. Diversification of rural livelihoods is the subject of scientific research because income from farming has come under pressure due to population explosion (Barrett et al., 2001). Rapid population growth and subdivision of land has also

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resulted in small land acreage leading to a concern that contribution to household incomes from agricultural activities may no longer be meaningful (Marenya et al., 2003). Governments throughout the developing world have for many years had a keen and sustained interest in diversifying their rural economies and the economic activities of rural residents (Delgado and Siamwalla, 1997). Households combine and explore diverse strategies to act, cope and adapt to fast-changing local and regional drivers (Valbuena et al., 2015).

Ellis (1998) defines livelihood diversification as the process by which rural families engage in different activities and social support capabilities in order to improve their standards of living. This is the phenomenon where rural households engage in multiple activities in order to survive and to improve their standard of living. These activities are either on-farm or off-farm and it includes both agricultural and non-agricultural activities. On-farm diversification includes the introduction of new crops into farming systems or farmers investing in livestock, hunting, and fisheries. This is distinguished from 'off-farm' activities which generally refer to activities undertaken away from the household's own farm such as wage employment on other farms (Ellis and Freeman, 2005). Livelihood diversification is a serious long term issue for policies concerned with reducing poverty in low income developing countries. However, farmers in rural areas in the developing countries are most vulnerable because of their lack of access to education, longer distance from markets to their homes, their low wealth status and small household size and may have the fewest opportunities to diversify in spite of the acknowledged importance of diversification as a strategy to accumulate income for consumption and/or investment and to spread risk (Ellis, 2000).

Generally, from the perspective of managing risk and associated vulnerability of rural households, and in some cases from a desire to increase incomes, farm diversification makes sense as a policy goal (Kimenju and Tschirley, 2009). Better off rural households may diversify their farming practices and their non-agricultural employment to balance risks of possible market failure where the economy lacks adequate insurance mechanisms (Von and Pandya-Lorch, 1991; Ellis, 1998). Diversification is one strategy that smallholder farmers may employ to reduce their vulnerability in the face of global environmental change (Paul et al., 2015).

Opportunities may arise, to significantly improve up an existing but considerably small activity, in response to a sudden change in circumstances. Developing more generic livelihood skills together with the provision of generic business services will improve individual abilities to identify and seize new livelihood opportunities in a range of sectors (Gordon et al., 2010). Household level diversification has implications for rural poverty reduction policies because the conventional approaches aimed at increasing employment, incomes and productivity in

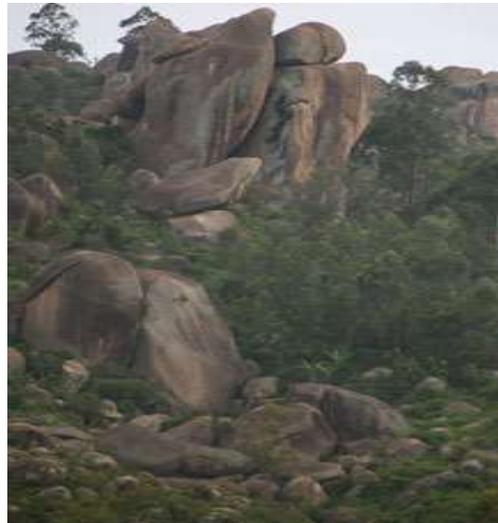


Figure 1. A picture showing the rocky terrain and steep landscape in Vihiga County, Kenya.

single occupations, like farming, may be missing their targets. Household members especially from peasant families often refrain from adopting beneficial technologies and engage in production of low value crops that require extensive labor. This often results to them having to sacrifice quick monetary gains in favor of achieving long term sustainability of their livelihood systems (Stakhanov, 2010).

In low income countries in Asia, Latin America and Africa, people diversify their productive activities, sources of income, and households' resources to secure their wellbeing and/or to respond to a crisis. For instance, better off rural households may diversify their farming practices and their non-agricultural employment to balance risks of possible market failure where the economy lacks adequate insurance mechanisms. They also may diversify sources of off-farm employment to increase household income when the economy is improving. Poor farmers who cannot rely solely on agriculture commonly use off-farm income diversification as a form of self-insurance (Barrett et al., 2001). Chambers (1997) argued that poor people have to diversify sources of livelihood in order to survive in a risk-prone and uncertain world.

Decreasing land availability has necessitated research in new technologies that require less land for profitable agricultural production especially in areas where the existing farming practices have led to increasingly low production (Figure 1).

Mushroom in Kenya is one of the high value crops that can be grown alongside other crops as a diversification option for both small holder and large scale farmers. It is an important cash crop, though still mostly produced at a small-scale level within the country. Button (*Agaricus*



Figure 2. A picture of the local mushroom variety.

bisporus) and Oyster (*Pleurotus* species) are the two main commercially produced mushroom varieties in Kenya. A three square meter plot of land can produce up to 1,000 mushroom sets in small polythene bags. Harvesting can be done fortnightly with a kilo of mushroom going for as much as 800 Kenyan shillings. Use of idle structures, production all year round the first harvest being 28 to 35 days after planting the crop, use of agricultural waste as substrate and its ability to biodegrade offers opportunity for its production and this provides a more economical and environmentally friendly disposal system (Figure 2) (Isikhuemhen et al., 2000).

Kenya is yet to achieve rapid growth in incomes in rural economy and in the economy as a whole, and this can be done by first embracing agricultural transformation, where individual farms are to shift from highly diversified subsistence-oriented production towards more specialized production oriented towards the market or other systems of exchange (Kimenju and Tschirley, 2009). The current local mushroom supply of 484.5 tones is way below its demand of 1,200 tones necessitating importation. However, mushroom being an emerging crop limited research has been done on its use as a livelihood diversification option for farmers in areas where land is a constraint to production. Mushrooms have the potential to steer a country to achieve the MDGs of poverty and hunger eradication, improved health, improved environment and potential to boost the overall national economy (Gateri, 2012). However, limited research has been undertaken on mushroom to provide clear information about mushroom production and marketing (Odeno et al., 2012). This calls for the joint participation of players and all stakeholders in production, extension, research, policy and marketing in order to optimize the mushroom value chain (Figure 3).

METHODOLOGY

This paper is based on small holder farmers' survey data from a random sample of 240 farmers in Western, Kenya. The study used both qualitative and quantitative data collected in the survey. Part of the qualitative data was obtained from the focus group discussion which constituted farmers and an extension officer from the Ministry of Agriculture, through oral discussions. Data was also collected using semi-structured questionnaires which were administered to the households by trained enumerators who interviewed the farmers in their respective homes. The data collected included household characteristics, farm characteristics, farm enterprise investments and non-farm enterprise investments. However, there are some potential limitations in the data based on the fact that farmers in the study area kept very little records on their farm and non-farm enterprise activities and this meant that most of the data was based on farmers' memory recall. These limitations were overcome by engaging the farmers in lengthy discussions on their production over time. Different socio-economic characteristics were described using percentages and means that were obtained and graphs were used to describe their distributions. Binomial logit was applied in regression analysis for farmers' awareness of mushroom production and willingness to engage in mushroom production as a livelihood diversification option.

RESULTS AND DISCUSSION

Socio-economic characteristics

The average sampled household in the study area is generally led by a male with the average age of 30 years. Development programs being introduced in the area should mostly target the youth because most of the people currently living in the area are aged between 19 and 35 years. Sampled households in the area had an average of 5 members, this was attributed mostly to the fact the study defined a household as people living and eating in the same house. The largest sampled household

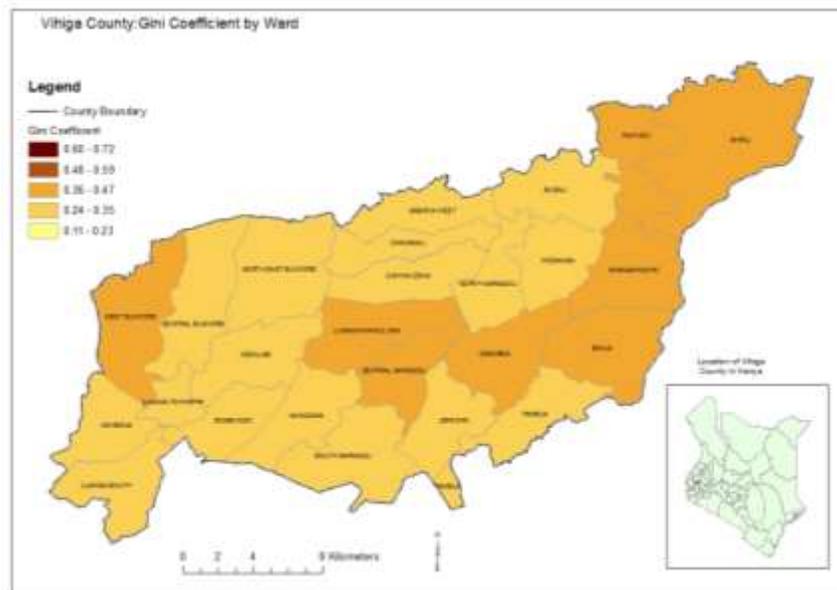


Figure 3. A map of Vihiga County Kenya.

in the survey had 11 members. The households with only one household member were either unmarried men or senior citizens who were living alone with the rest of their families living either in urban centers or further away from their current location in search of jobs or education. In all the households, the proportion of women in the household composition was lower than that of men. For the interviews conducted, most of the respondents were the heads of the households; this can be used to explain the high poverty levels because the household head that is mostly looked upon to provide for the family stays at home for lack of engagement in any productive economic activity. Only 58% of the household heads in the survey were married, with rest widowed, separated or divorced. Less than a quarter of the people interviewed belonged to farmer groups, attending an average of 4 meetings per year. A large proportion of the members in the households interviewed were farmers with an average of 1 acre per household.

A large percentage of the respondents were aware of mushroom production in the area (69%) though only 3% of them were mushroom farmers. Mushroom was being consumed by nearly all of the interviewed households. Less than 10% of the people currently not producing mushrooms have been previously involved in production but stopped mainly because of poor access to input and lack of credit. People who have not been involved in production were willing to start production mainly because of home consumption and income diversification because they were of the opinion that there is an existing market in the area, therefore it would be an alternative source of income for them and this would in turn improve their livelihoods. The major setback of information

dissemination in the area was that only the people in development groups obtained information directly from the source, all the rest received it through third parties.

The land allocated for maize production dropped in 2013, but went up greatly in 2014 most likely so as to increase production to cater for the needs of the increasing population but the production still continued dropping in the 3 years making it impossible to cater for the high population. Given that 40% of the households depend on farming as their main source of income, a lot of households were adversely affected by the decrease in maize production because it is the enterprise that is depended on mostly by people in this region. The results show there are challenges that call for policies that support alternative and more remunerative livelihoods that assist farmers exit the poverty web.

Results of the binomial logit regression model

The negative elasticity on age, gender, crop total, total livestock unit, and average acreage under maize production imply that a unit addition on any of these variables has a negative effect on the awareness of mushroom production (Table 1).

Age had a negative effect on awareness of mushroom production. This implies that the older the person was, the less likely they were aware of mushroom production in the area. This can be attributed to the fact that as people age they tend to be more risk averse hence are comfortable with their current portfolio and would not want to engage in any new ventures for the risks involved.

Table 1. Factors affecting farmers' awareness of mushroom as a diversification option.

Parameter	Coeff.	Std. Err.	Z	P> z
Age	-0.01	0.01	-0.78	0.44
Gender	-0.38	0.39	-0.97	0.33
Marital status	1.08	0.45	2.42	0.02**
Highest grade	0.64	0.41	1.56	0.12
Group membership	0.09	0.39	0.23	0.82
Consumption	2.88	1.69	1.70	0.09*
Mushroom market	1.69	0.41	4.11	0.00***
Previously produced mushroom	1.90	1.09	1.75	0.08*
Cropping land	-1.70	0.83	-2.05	0.04**
Total land	1.29	0.73	1.78	0.08*
Total Livestock unit	-0.03	0.13	-0.27	0.79
Log cash on food per week	0.70	0.33	1.99	0.05**
Log farming amount	0.28	0.14	2.03	0.04**
Average maize acreage	-2.40	1.36	-1.77	0.08*
Average bean average	0.60	0.92	0.65	0.51

*Significant at 10%, **Significant at 5% and ***Significant at 1%.

Gender had a negative effect. More women were more aware of mushroom production compared to men. This can be attributed to the fact more women are in development groups than men therefore women access production information more than men during their group meetings.

Married people are also more likely to be aware of mushroom production than single parents. This is because as they each goes about their day to day activities they meet different people therefore each of them has access to different information which they share when they get back to their homes.

The more educated the respondent the more likely they were aware of mushroom production. It can be viewed as the more educated a person is the more they are ready to learn therefore they get information on new production in the region and also in other regions. Also, the people who are in development groups were more aware than those not in groups because most group members get more information as they go for meetings and also most donors target groups for awareness campaigns and projects.

People who consume mushroom were more aware of its production in the area because they were either producing it or they purchase it for consumption, they were even aware of the people producing it in the area. Also people who had previously produced mushroom were more aware of its production in the area because they stopped production mostly because of lack of spawns.

People with larger pieces of land under crop production are more risk averse and are mostly producing what has previously been grown; maize and beans. People with smaller pieces of land are willing to try out new crops that

will enable them to get the most from their small pieces of land. They were more willing to grow crops that they eventually sell and use the proceeds from those sales to purchase maize because it is uneconomical for them to produce it under the small land acreage.

The number of animals a household keeps has a negative effect on its awareness of mushroom production because farmers who kept more animals preferred them to crop production because they found crop production to be uneconomical therefore they never sought information on crop production making it impossible for them to be aware of some of the crops that are grown in the area (Table 2).

The negative coefficient on marital status, work status, total land under cropping, total livestock unit and total amount spent on food per week imply that a unit addition on any of these variables had a negative effect on the willingness of the farmers to diversify into mushroom production.

Age has a positive effect on willingness to start production. The older people were more willing to start mushroom production majorly because it was not considered as an enterprise that requires a lot of energy and can even be practiced out with people with disabilities. This supports the findings by Olale et al. (2010) and Wanyama et al. (2010) that households experience on livelihood options and the desire to diversify increase with age. A group of people living with disabilities that was interviewed in Hamisi was engaging in mushroom production as a group venture. Gender was also a positive factor in that more men were willing to start production majorly because they are considered as the bread winners in most families therefore they are

Table 2. Determinants of farmers' willingness to diversify into mushroom production

Parameter	Coeff.	Std. Err.	Z	P> z
Age	0.09	0.04	2.01	0.05**
Gender	2.10	1.05	2.01	0.05**
Marital status	-2.47	1.40	-1.77	0.08*
Work	-2.09	1.17	-1.79	0.07*
Log meetings	0.15	0.38	0.39	0.69
Highest grade	1.19	0.91	1.31	0.19
Awareness of production	0.06	0.92	0.07	0.94
Mushroom consumption	0.37	1.80	0.20	0.84
Mushroom market	2.20	0.87	2.55	0.01***
Cropping land	-2.92	1.60	-1.82	0.07*
Total land	1.53	1.33	1.15	0.25
Total livestock unit	-0.19	0.28	-0.70	0.49
Total on food per week	-0.00	0.00	-1.02	0.31
Farming amount	0.00	0.00	1.70	0.09*
Average bean acreage	3.77	2.80	1.35	0.18

*Significant at 10%, **Significant at 5% and ***Significant at 1%.

more willing to engage in enterprises that would be considered as income diversification so as to supplement their income. Marital status had an effect; the household heads that were not married were more willing to engage in mushroom production because they are considered as the sole bread winners in their families hence they are more willing to engage in enterprises that they consider to receive a higher pay off which can be used as a source of income from their families.

Households with farming as the main source of income were more willing to engage in mushroom production than households that had other alternative sources of income for example households engaging in business and the employed. The more educated people were more willing to engage in mushroom production because it is often assumed that the more educated a person, the more risk loving they are. And most educated people, for example the teachers and the people working in the county office, were willing to engage in risky ventures whose pay off is high. They were the ones who had several diversification options in their portfolio. This supports the findings of Olale et al. (2010) of positive influence of education on livelihood strategy diversification.

People that are aware of mushroom production are willing to engage in its production. This is also the case for people consuming mushroom because they are aware of the mushroom market in the area and the current supply does not meet the demand.

The larger the cropping area and the higher the amount of money spent on food per week, the less likely the farmer was willing to engage in mushroom production. This is mostly because most farmers with large pieces of land already have their mind set to production of a given

type of crop mainly maize, beans, sweet potatoes and bananas and they assume they have specialized in only these type of crops therefore they are not willing to engage in other enterprises. Families that spend less on food per week were more willing to engage in the production of mushroom because, such families mostly depend on consuming what they produce on their own farms and purchase only what they cannot produce or what is in insufficient supply because they are mostly very poor. Since mushroom is grown for subsistence and commercial purposes, such families would engage in it so as to get food for consumption and also money to purchase what they lack.

CONCLUSIONS AND POLICY IMPLICATIONS

From the study it was concluded that diversification is vital for the well being of the smallholder farmers in Western Kenya. Mushroom as a livelihood diversification option should be adopted by farmers in Western Kenya because of the land and topographic challenges. Factors such as consumption of mushroom, market for mushroom and land acreage should be used to create awareness of mushroom production in the area while age, sex, work status and mushroom market in the area should be used in designing policies for adoption of mushroom as a livelihood diversification option for in Western Kenya County.

Currently, most farmers in Western Kenya County are aware of mushroom production and market in the area but are not actively engaged in its production. The County Government's office for agriculture should continue with the mushroom awareness campaign that is

currently ongoing in the area, as this will continue to encourage more farmers to engage in its production because there exists a market both in the area and in other areas and this can serve as a way of the people in the county improving their livelihoods and reduce the poverty levels in the county.

Some farmers in the area were not actively engaged in group membership and some were not members of any development group. Farmers should also be advised on the importance of being members of farmers groups and development groups in the area as it is a channel for information access on new agricultural production practices. Being members of such groups also puts the farmers in better positions to access credit that can be used in agricultural production thereby improving their welfare. The county government should also continue with encouraging more farmers to join development and farmer groups as this puts them in a better position to obtain knowledge and at times inputs to use in agricultural production.

Many farmers were willing to engage in mushroom production but lacked skills to engage in this enterprise, therefore, the County Agricultural office should come up with strategies that include farmer field days and extensive extension programs with all the stakeholders in the region that will ensure the farmers are well trained on different agricultural production techniques. Currently in Western Kenya, farmer field days are planned by the ministry of Agriculture office but some farmers still fail to attend. Awareness campaigns on the importance of attending such forums should be put in place by the county government of Western Kenya. Increased turn out in such events will encourage the donors to allocate more funds for awareness campaigns and trainings to ensure the farmers have knowledge on production of mushroom and also subsidize the cost of spawn that is supplied to farmers.

LIMITATIONS OF THE STUDY AND SUGGESTION FOR FURTHER RESEARCH

Studies have been done on mushroom markets and its value chain but little has been done on its use as a diversification option for farmers in different areas facing different production challenges. Given the decreasing land sizes necessitating diversification in the country, more research should be done on livelihood diversification for farmers in different areas, in order to come up with the best production mix for farmers in different areas. This will ensure that farmers come up with the best production mix that will ensure maximum profitability given the resources at their disposal.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth of *Jatropha curcas* L. plants under salt and nutrition stress

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The objective of this study was to evaluate the effects of nutrition and salt stress on the growth and accumulation of mineral elements in *Jatropha curcas* plants. The work was conducted in greenhouse with 100% solar radiation interception, at Goiás State University, Ipameri Campus, Goiás, Brazil. The experiment was set up in five-liter containers following the completely randomized design in 4 x 2 factorial arrangement (I: Clark's complete nutrient solution, Clark's nutrient solution modified to prevent contents of ii: nitrogen, iii: potassium or iv: magnesium, and two salinity treatments, distilled water and saline water [2 dS m⁻¹] obtained by NaCl addition). On even days, the plants received nutrient solution and on odd days, they received saline or distilled water. The plants were irrigated with complete nutrient solution up to the 20th day after germination and the treatments were applied from the 21st to the 60th day. The results show that there was no toxic effect of salt on *J. curcas* plants; however, salinity caused nutritional unbalance and significantly hindered Ca²⁺ and K⁺ absorption and slightly reduced vegetative growth due to the small change in water status. *J. curcas* plants are moderately tolerant to irrigation water salinity.

Key words: Mineral nutrition, biofuel, oleaginous, osmotic stress, water restriction.

INTRODUCTION

Concern for natural resources has intensified the search for renewable energy alternatives, like biofuels. Brazil stands out in the world scenario for its growing, vigorous renewable energy matrix, since 45% of the energy and 18% of the fuels consumed in Brazil are renewable, while in the rest of the world, only 14% of consumed energy comes from renewable source (ANP, 2015).

The Brazilian program for production and use of biofuel has stimulated the cultivation of raw materials and marketing of vegetable oil. However, the program is founded on only one raw material, soybeans, accounting for 73.92% of all vegetable oil for biodiesel production (ANP, 2014). Therefore, diversifying the sources of raw material is required in order to make the system less

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vulnerable to weather and economic conditions, by introducing promising species such as *Jatropha curcas* L.

J. curcas is a perennial lactiferous Euphorbiaceae, native to Mexico in Central America, where high genetic diversity was reported (Henning, 2008). The plants are deciduous with juicy stem and fast growth. The species has great economic potential, either in landscaping as hedge, pharmacology, and especially in the production of biodiesel oil. The seeds are oleaginous with about 40% of the oil easily processed into biodiesel. *J. curcas* seed biodiesel has 84% of diesel oil calorific value (Matos, 2010; Ferreira, 2015). The oil has about 80% of unsaturated fatty acids and low freezing point (-10°C) and can be marketed in various regions of the world (Matos, 2010), which represents great advantage, especially in colder regions. These features have contributed to increased commercial interest in this culture.

The *J. curcas* plant develops in several kinds of soils, including sandy, stony, saline, alkaline and rocky ones, which, from the physical nutritional point of view, are restrictive to full root development (Carvalho et al., 2013). It is a fast-developing shrub and can start production already in the first year of planting and remain productive for about 40 years. Its productive climax occurs at the fourth year in the field (Dias et al., 2007). Commercial plantations of *Jatropha* in Brazil are still in early stages due to the low scientific knowledge of the plant. With the development of new research, the culture is expected to turn from potential to actual raw material for the biodiesel market (Andréo-Souza et al., 2010). *J. curcas* species lacks genetic improvement, in that it shows significantly uneven growth, architecture, development and fruit ripening. Although, rustic and tolerant to drought, the crop needs agronomic knowledge, as basic information such as fertilizing recommendation, salinity tolerance and productivity is not available on the species (Matos et al., 2014a).

Although, it is drought tolerant and can survive with 200 mm per year for three years using water stored in stems and roots, it is presumed that its maximum genetic potential is expressed in adequate conditions of water availability. Adequate water supply enables significant productivity gains. However, water scarcity in many inland reservoirs intensifies the need for research to assess the possibility of the species cultivation with saline water irrigation. The use of saline water for irrigation has become an important alternative given the scarcity of good quality water worldwide. The quality of many water sources is low, especially water wells and surface reservoirs. Because it contains soluble salts, water used for irrigation entails periodic salt incorporation to the soil profile. In the absence of leaching, the salt is deposited in the root area and soil surface due to water evaporation (Veras et al., 2011).

Recent studies point to reasonable sensitivity of *J. curcas* to salinity with significant reduction in biomass, leaf number and stem diameter (Oliveira et al., 2015; Sousa et al., 2011). The adverse effects of salinity are

the result of three primary factors: i) inhibition of water absorption caused by the solution low water potential; ii) toxic effects of Na⁺ and Cl⁻ ions at the cellular level; and iii) changes in nutritional balance altering the absorption of K⁺, Ca²⁺, Mg²⁺, NO₃⁻, Cl⁻, Na⁺ and other chemical elements (Diaz-Lopez et al., 2012; Mansour, 2013). The combination of these factors causes changes in cell metabolism with significant reduction in grain yield. Plants develop innumerable salt stress tolerating mechanisms that include osmotic adjustment, toxic ion compartmentalization, and absorption inhibition and/or exclusion. Their salinity tolerance is difficult to measure due to the dependence on many factors such as weather, growth stage and soil fertility (Taiz and Zeiger, 2013). Besides, many genetical and environmental effects were reported in growth of different plant species from seed collection to harvest (Yazici, 2010; Dilaver et al., 2015; Tebes et al., 2015; Cercioğlu and Bilir, 2016).

Under natural conditions, abiotic stresses may occur simultaneously, sometimes causing irreversible damage to the plants. With a view to better physiological understanding of salinity and nutrient deficiency occurring simultaneously, this study aimed at evaluating the effects of nutrition and salt stress on the growth and accumulation of mineral elements of *J. curcas* plants.

MATERIALS AND METHODS

Experimental design

The work was conducted in greenhouse with 100% solar radiation interception at Goiás State University, Ipameri Campus (17°43'19" S, 48°09'35" W, 773 m), Ipameri, Goiás. According to Köppen classification, the region has tropical savanna climate (Aw), with rainy summers and dry winters. The experiment was carried out in five-liter containers following the completely randomized design in a 4 × 2 factorial arrangement (i: Clark complete nutrient solution, Clark's nutrient solution modified to prevent contents of ii: nitrogen, iii: potassium or iv: magnesium, and two salinity treatments, distilled water and saline water [2 dS m⁻¹] obtained by NaCl addition). On even days, the plants were treated with nutrient solution and on odd days, with saline or distilled water. The plants were irrigated with the complete nutrient solution until the 20th day after germination and the treatments from the 21st to the 60th days using the salts as shown in Table 1.

The solution volume to be applied was determined based on the container weight differences. Initially, the solution was placed in the containers until drainage started and 60 min later, the same containers were weighed for the first time. Exactly 24 h after the first weighing, the containers were weighed again and the difference in mass between the first and second weighing corresponded to the solution volume to be applied daily per container (150 ml). 60 days after germination, the following analyses were performed: leaf number, plant height, stem diameter, transpiration, leaf area, relative water content, total biomass and nitrogen concentration (N), calcium (Ca) potassium (K), magnesium (Mg) and sodium (Na) in leaves and roots.

Growth variables

The number of leaves was obtained by counting. The plant height

Table 1. Composition and indication of presence (yes) or absence (no) of macro and micronutrients of the Clark's nutrient solution modified to provide different levels of nutrients.

Supply solution salts	mM	Complete	- N	- Mg	-K
Ca(NO ₃) ₂ .4H ₂ O	1.04	Yes	No	Yes	Yes
NH ₄ NO ₃	1.00	Yes	No	Yes	Yes
KNO ₃	0.80	Yes	No	Yes	No
KH ₂ PO ₄	0.069	Yes	Yes	Yes	No
KCl	0.931	Yes	Yes	Yes	No
MgSO ₄ .7H ₂ O	0.60	Yes	Yes	No	Yes
CaCl ₂	1.04	Yes	Yes	Yes	Yes
NH ₄ H ₂ PO ₄	2.00	Yes	Yes	Yes	Yes
ZnSO ₄ .7H ₂ O	0.002	Yes	Yes	Yes	Yes
CuSO ₄ .5H ₂ O	0.0005	Yes	Yes	Yes	Yes
MnCl ₂ .4H ₂ O	0.007	Yes	Yes	Yes	Yes
Na ₂ MoO ₄ .4H ₂ O	0.0006	Yes	Yes	Yes	Yes
H ₃ BO ₃	0.019	Yes	Yes	Yes	Yes
FeEDTA	0.045	Yes	Yes	Yes	Yes

and stem diameter were measured using a graduated ruler and a digital pachymeter, respectively. For leaf area evaluation, a graduated tape (cm) was used, measuring the length and width of all leaves in each plant and then the leaf area was calculated following the equation proposed by Severino et al. (2007). The leaves, roots and stems were plucked and oven-dried at 72°C to reach constant dry mass and then weighed separately. The mass data enabled calculation of the leaf mass ratio (LMR), root mass ratio (RMR), stem mass ratio (SMR), shoot/root system ratio (S/R) and total biomass.

Leaf relative water content (RWC)

In order to obtain the relative water content, twenty 12-mm leaf discs were taken, weighed, and saturated for 24 h in Petri dishes with distilled water. Then, the discs were weighed again and dried at 70°C for 72 h. Subsequently, the dry matter was obtained.

Photosynthetic pigments

In order to determine the total chlorophyll and carotenoid concentration, leaf discs were taken from known areas and put into jars containing dimethyl sulfoxide (DMSO). Afterwards, extraction was made in 65°C water bath for one hour. Aliquots were taken for spectrophotometric reading at 480, 649, 1 and 665 nm. Chlorophyll a (Chl a) and chlorophyll b (Chl b) contents were determined according to the equation proposed by Wellburn (1994).

Nutritional analyses

Samples of roots and fully expanded leaves were collected, and then total N concentration was determined according to Kjeldahl sulfuric digestion method. In order to determine the contents of potassium, calcium, magnesium and sodium, the methodology proposed by Johnson and Ulrich (1959) was used.

Statistical procedures

The experiment was set up in five-liter containers following the

completely randomized design in a 4 × 2 factorial arrangement and six replications. The experimental plot corresponded to one plant per container. In order to evaluate the effects of nutritional deficiency and salinity on the growth and mineral accumulation in plants, variance analysis was used (ANOVA). Assumptions of normal distribution and variance homogeneity were always tested. Whenever the ANOVA results were significant, further comparisons of Newman-Keuls were performed to detect differences between the treatment averages.

In order to evaluate the effect of the analyzed variables on root biomass, multiple regression analyses were performed using the forward stepwise selection model (Sokal and Rolf, 1994). The forward stepwise model selects the independent variables in the regression model according to the amount of explained variation in the dependent variable; the first independent variable introduced in the model explains most of the variation; the second one, most of the remaining variation, and so forth. Adding variables to the model ends when the next variable to be included does not have a significant partial correlation ($p > 0.05$). The same analytical procedure was performed to evaluate the effect of variables on the plant total biomass. All statistical analyzes used in this work were performed under the procedures of R software (R CORE TEAM, 2015).

RESULTS

Biomass, stem diameter and relative water content were, respectively, 16, 9 and 11% lower in the plants irrigated with saline solution as compared to plants irrigated with non-saline solution. Only plant height showed statistical significance due to the nutritional treatment applied. This variable was approximately 9% lower in treatment lacking nitrogen, when compared with the average of the other treatments containing nitrogen. The summary of the variance analysis for growth and water status variables is shown in Table 2.

Potassium concentration (K⁺) was 18% higher in the roots of plants treated with the complete nutrient solution

Table 2. Variance analysis and average test for biomass, number of leaves, plant height, stem diameter (SD), relative water content (RWC) and leaf area (LA) in *J. curcas* plants subjected to nutritional and saline deficiency.

Variation source	GL	Mean squares					
		Biomass (g)	Number leaves	Height (cm)	SD (cm)	RWC (%)	LA (cm ²)
Nutrient	3	0.40 ^{ns}	0.79 ^{ns}	4.30*	0.03 ^{ns}	35.25 ^{ns}	139.0 ^{ns}
Salt	1	4.41**	0.06 ^{ns}	0.14 ^{ns}	0.16**	681.4**	0.90 ^{ns}
Nutrient*salt	3	1.25 ^{ns}	0.41 ^{ns}	2.53 ^{ns}	0.02 ^{ns}	94.14 ^{ns}	268.8 ^{ns}
CV (%)		12.83	35.6	7.97	7.99	6.30	32.96
Treatments		Averages					
N absence		4.73 ^a	2.25 ^a	9.87 ^b	1.50 ^a	80.6 ^a	64.7 ^a
K absence		5.10 ^a	2.63 ^a	10.65 ^a	1.53 ^a	78.6 ^a	66.9 ^a
Mg absence		4.74 ^a	2.87 ^a	10.42 ^a	1.41 ^a	79.8 ^a	65.7 ^a
Complete Solution		4.82 ^a	2.00 ^a	11.22 ^a	1.50 ^a	83.8 ^a	55.1 ^a
Salt		Averages					
With salt		4.49 ^b	2.36 ^a	10.45 ^a	1.43 ^b	76.4 ^b	63.0 ^a
Without salt		5.29 ^a	2.52 ^a	10.70 ^a	1.56 ^a	85.3 ^a	63.4 ^a

*Significant at 5% and at **1% probability; ns = not significant by F test. Averages followed by the same small letter in the column do not differ at 5% probability by Newman-Keuls test.

Table 3. Variance analysis and average test for phosphorus (P), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), sodium (Na⁺) and ratio between sodium and potassium (Na⁺/K⁺) in roots of *J. curcas* plant subjected to nutritional and salinity deficiency.

Variation source	GL	Mean squares					
		P	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	Na ⁺ /K ⁺
Nutrient	3	0.0004 ^{ns}	0.213**	0.01**	0.008 ^{ns}	0.29**	0.046 ^{ns}
Salt	1	0.0002 ^{ns}	2.00**	0.029**	0.008 ^{ns}	4.21**	3.60**
Nutrient*salt	3	0.0001 ^{ns}	0.007 ^{ns}	0.010 ^{ns}	0.009 ^{ns}	0.09 ^{ns}	0.22 ^{ns}
CV (%)		9.96	9.70	19.42	16.64	14.17	21.04
Treatments		Averages (dag/kg)					
N absence		0.062 ^a	1.55 ^b	0.26 ^b	0.23 ^a	0.83 ^b	0.58 ^a
K absence		0.063 ^a	1.50 ^b	0.30 ^b	0.22 ^a	0.81 ^b	0.57 ^a
Mg absence		0.064 ^a	1.43 ^b	0.34 ^a	0.24 ^a	0.86 ^b	0.65 ^a
Complete Solution		0.063 ^a	1.81 ^a	0.30 ^b	0.23 ^a	1.15 ^a	0.68 ^a
Salt		Averages (dag/kg)					
With salt		0.065 ^a	1.33 ^b	0.26 ^b	0.22 ^a	1.24 ^a	0.93 ^a
Without salt		0.061 ^a	1.78 ^a	0.33 ^a	0.24 ^a	0.62 ^b	0.35 ^b

*Significant at 5% and at **1% probability; ns = not significant by F test. Averages followed by the same small letter in the column do not differ at 5% probability by Newman-Keuls test.

when compared with the average of plants treated with lacking nutrients. Calcium concentration (Ca²⁺) was 17% lower in the roots of plants treated with nutrient solution without nitrogen when compared with the average of other treatments. Sodium concentration (Na⁺) was 28% higher in the roots of plants treated with the complete nutrient solution when compared with the average of plants treated without nutrients. K⁺ and Ca²⁺ concentrations were, respectively, 26 and 22% lower in

plants irrigated with saline solution as compared to plants irrigated with non-saline water. Na⁺ concentration and Na⁺/K⁺ ratio were, respectively, 50 and 63% lower in plants irrigated with non-saline water when compared with the plants irrigated with saline solution. The summary of the variance analysis for nutrient concentrations in the root system is shown in Table 3.

Phosphorus concentration was 35% higher in the leaves of plants treated with nutrient solution without K⁺

Table 4. Variance analysis and average test for phosphorus (P), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), sodium (Na⁺) and sodium and potassium ratio (Na⁺/K⁺), and nitrogen (N) in *J. curcas* plant leaves subjected to nutritional and salinity deficiency.

Variation source	GL	Mean squares						
		P	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	Na ⁺ /K ⁺	N
Nutrient	3	1.4x10 ^{-3*}	0.25 ^{ns}	0.01 [*]	9x10 ^{-4ns}	7x10 ^{-2ns}	0.27 ^{ns}	0.03 ^{ns}
Salt	1	0.017 [*]	2.78 [*]	0.02 [*]	10 ^{-5ns}	1,32 [*]	5.71 ^{**}	0.001 ^{ns}
Nutrient*salt	3	10 ^{-4ns}	0.06 ^{ns}	8x10 ^{-3ns}	8x10 ^{-4ns}	0.05 ^{ns}	0.28 ^{ns}	0.011 ^{ns}
CV (%)		17.91	31.59	24.32	24.33	50.51	26.84	14.65
Treatments		Averages (dag/kg)						
N absence		0.062 ^b	1.15 ^a	0.25 ^{ab}	0.12 ^a	0.66 ^a	0.90 ^a	1.02 ^a
K absence		0.084 ^a	1.13 ^a	0.34 ^a	0.14 ^a	0.86 ^a	0.86 ^a	1.22 ^a
Mg absence		0.059 ^b	0.92 ^a	0.26 ^{ab}	0.13 ^a	0.46 ^a	0.65 ^a	1.32 ^a
Complete solution		0.043 ^b	0.71 ^a	0.15 ^b	0.07 ^a	0.59 ^a	0.88 ^a	1.20 ^a
Salt		Averages (dag/kg)						
With salt		0.055 ^a	0.58 ^b	0.19 ^b	0.11 ^a	1.09 ^a	1.72 ^a	1.23 ^a
Without salt		0.068 ^a	1.35 ^a	0.31 ^a	0.13 ^a	0.43 ^b	0.34 ^b	1.17 ^a

*Significant at 5% and at **1% probability; ns = not significant by F test. Averages followed by the same small letter in the column do not differ at 5% probability by Newman-Keuls test.

Table 5. Multiple regression model to evaluate the effect of root biomass and root nutrients in the total biomass accumulation of *J. curcas* plants subjected to nutritional and salinity deficiency.

Total biomass	Model explanation	F	P			
	R ² = 0.679	F _(3, 44) =34.165	p<0.001			
	Beta	Beta standard error	B	B standard error	t ₍₄₄₎	p-level
Intercept			3.582	0.612	5.851	<0.001
Root biomass	0.713	0.092	1.869	0.242	7.721	<0.001
Na ⁺ /K ⁺	-0.215	0.094	-0.544	0.238	-2.282	0.027
Total P	-0.046	0.084	-4.580	8.506	-0.538	0.593

when compared with the average of the other treatments. Ca²⁺ concentration was 47% lower in the leaves of plants treated with the complete nutrient solution when compared with the average of the other nutrient-deficient treatments. Sodium concentration (Na⁺) was 28% higher in the roots of plants treated with the complete nutrient solution when compared with the average of plants treated with those lacking nutrients. K⁺ and Ca²⁺ concentrations were, respectively, 68 and 49% lower in plants irrigated with saline solution as compared to plants irrigated with non-saline water. Na⁺ concentration and Na⁺/K⁺ ratio were, respectively, 61 and 81% lower in plants irrigated with non-saline water when compared with the plants irrigated with saline water. The summary of the variance analysis for nutrient concentrations in leaves is shown in Table 4.

Multivariate regression showed that the nutrients present in roots and root biomass explained 67% of the total biomass variation (model R²: 0.738; p<0.001) (Table

5). The partial coefficients indicate that the values were significant only for root biomass and Na⁺/K⁺, the relation being positive for the former and negative for the latter.

DISCUSSION

Under natural conditions, abiotic stresses often occur simultaneously and involve complex damage and repair mechanisms, causing either reversible or irreversible harm to the plants. The nutritional unbalance caused by salinity in irrigation water shows that *J. curcas* is moderately tolerant to irrigation water salinity.

The absence of significant changes in root mass, stem and leaf ratios, total carotenoid and chlorophyll leaf concentrations (data not shown) caused by irrigation water salinity and/or nutritional deficiency points to the absence of toxic effects on chloroplasts. On the other hand, variations in the concentration of photosynthetic

pigments in plants exposed to salinity are indicative of toxicity in chloroplasts (Souza et al., 2015). Salinity promoted small changes to vegetative growth (biomass, height, diameter, number of leaves and leaf area) of *J. curcas* plants. These variations were mainly due to low osmotic potential of irrigation solution, which slightly changed the plant water status. The reduction in the relative water content in plants grown under salinity possibly limited (even mildly) cell expansion and vegetative growth. Approximately, 2/3 of plant growth is due to cell expansion, which depends on cell turgor provided by tissue hydration (Taiz and Zaiger, 2013). Reduction in growth variables in *J. curcas* plants grown under salinity and/or water limitation has been reported in literature in numerous studies (Carvalho et al., 2013; Carvalho Junior et al., 2014; Oliveira et al., 2010, 2015). Despite the long treatment time, it is not possible to assert that there was water deficit, but rather a small change in water status. The succulent stem of *J. Curcas* plants works as a water buffer, supplying the leaves under reduced water availability (Matos et al., 2014b). Moreover, *J. curcas* plants absorb NaCl from saline solutions and use Na⁺ and Cl⁻ to accomplish osmotic adjustment and continue absorbing water (Da Silva et al., 2009). Lower plant height under nitrogen deficiency is due to the importance of this nutrient to a vigorous vegetative stage. Nitrogen is essential for *J. curcas* plant vegetative growth (Do Carmo et al., 2014). Quite possibly, a longer treatment would result in significant variations in other growth variables.

Lower concentrations of K⁺, Ca²⁺ and Na⁺ in the roots of plants grown under nutrient deficiency when compared with plants treated with the complete solution are due to the importance of K⁺ and Ca²⁺ for the balance of organic and inorganic anions in the plant and to increased presence of salts in the complete solution. Higher foliar Ca²⁺ concentration in plants grown under nutritional deficiency can be associated with Ca²⁺ secondary messenger role in the transfer of information to the response elicitor target. Despite the reduction in foliar Ca²⁺ concentration under salt stress, this nutrient is essential for membrane stability and maintenance of Na⁺/K⁺ relationship at suitable levels for the plants (Lacerda et al., 2004). The chemical similarity between phosphorus and cadmium can result in greater absorption of the latter because of changes to membrane selectivity in the presence of Na⁺ (Taiz and Zaiger, 2013). Higher concentration of total P in leaves under K⁺ deficiency and no difference in the concentration of this nutrient in leaves and roots of plants treated with saline solution are indicative of maintenance of plasma membrane integrity as to total P absorption.

Salinity caused nutritional unbalance in *J. curcas* plants. High intracellular Na⁺ concentration has possibly enhanced the competition for absorption sites with low and/or high K⁺ affinity and reduced concentrations of this chemical element in plant tissues (root and leaves).

According to Taiz and Zaiger (2013), Na⁺ high denaturing potential hinders membrane proteins and contributes to the reduction of K⁺ internal concentration due to the competition with Na⁺ for the same absorption site. In addition, intracellular reductions of Ca²⁺ in plants grown under salinity may be associated with increased influx of Na⁺ through nonselective cationic channels. Epstein and Bloom (2005) stated that excessive Na⁺ reduces Ca²⁺ influx and prevents Na⁺ efflux cellular detoxification mechanism activated by Ca²⁺.

The Na⁺/K⁺ ratio (0.93 and 1.72, respectively) in leaves and roots of *J. curcas* plants irrigated with saline solution was always higher than that of plants irrigated with non-saline water due to Na⁺ high accumulation and K⁺ reduced absorption. According to Dias-López et al. (2012), it is common for the Na⁺/K⁺ ratio in *J. curcas* to be higher than 1.2 and lower than 2.5, but for optimal cellular metabolism, it should always be lower than 0.6. Multiple regressions indicate that the root biomass had a positive and fundamental importance for total biomass accumulation. However, Na⁺ root concentration and Na⁺/K⁺ ratio were the variables that most negatively contributed to the plant total biomass accumulation. Na⁺/K⁺ ratio is specifically an important indicator of salt stress tolerance or susceptibility of *J. curcas* plants.

Conclusions

1. In the present study, it was not possible to identify the toxic effects of salt in *J. curcas*. However, there was nutritional unbalance due to high accumulation of Na⁺ in the tissues and mild vegetative growth reduction caused by small variation in water status.
2. *J. curcas* plants are moderately tolerant to the salinity of irrigation water.
3. Salinity causes nutritional unbalance in *J. curcas* plants with significant damage to Ca²⁺ and K⁺ absorption.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Improving geotechnical properties of a sand-clay soil by cement stabilization for base course in forest roads

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The study evaluated the chemical stabilization of soils with cement toward application in improving forest roads, as constructive alternative of low cost. The study was carried out with fine soils from the region of Niquelândia-GO, where the sand-clay-silt particle proportion was predominant, and Portland cement CP-II-Z-32 used as a stabilizing agent at 2% under dry soil weight. Geotechnical tests were conducted in different compaction energies (normal, intermediate and modified) and curing periods (1, 7 and 28 days), and the data were analyzed statistically by Analysis of Variance (ANOVA) and Tukey test. The soil-cement mixture presented higher unconfined compressive strength (UCS) than the compacted local soil, reaching a highest value of 650.52 kPa on modified compaction energy. Also, California bearing ratio (CBR) with values of 44.1% in the intermediate compaction energy and 41.7% in the modified compaction energy reached higher values compared to lateritic soil sampled. Soil-cement mixture was prescribed as improved layer for forest road surface, as well as reached indication for subgrade reinforcement material and sub-base in conventional paved roads.

Key words: Forest management, chemical stabilizing, lateritic soil, unpaved roads.

INTRODUCTION

Road infrastructures for forest activities are complex engineering structures that involve design, building, and maintenance (Sessions, 2007). The technical constraints for planning and building a forest road network are the condition and type of traffic, terrain, climate and building standard. Also, importance should be given to soil type, especially for its availability and bearing capacity as

material of road construction (Machado and Malinovsky, 1986; Nogami and Villibor, 1995).

Forest roads serve a single purpose and have a distinct function from other roads. The low traffic volume of long and heavy trucks in one direction characterizes these routes. In addition, they have differentiated structure in their cross section, with two almost indistinct layers,

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which are the surface or natural soil, and an improved layer, which should support the demands of forest transport and resist hazardous weather (Sessions and Heinrich, 1993).

Dias Junior et al. (2005) pointed out the vehicle and machinery traffic in the wood harvest and transport operations, and the low constructive pattern of forest roads as constraints to achieve sustainable forest development. For the authors, the improvement in the conditions of these roads, seeking greater durability and bearing capacity, represents an important component for economic sustainability of forestry undertakings. Forest road infrastructure should include economic viability in addition to technical parameters, prioritizing the acquisition of materials suitable for road construction with longer service life, minimizing maintenance costs of roads and forest transport vehicles and ensuring the efficient flow of forest goods.

The expansion of forest activities and increase in wood demand have required a forest road network of good quality, which is drivable all year round and has longer service life (Sessions, 2007). However, in many situations, the problem is in the local soil, which does not have the conditions required for roadwork for being little resistant, very compressible or having characteristics that fall short economically (Trindade et al., 2008). For Ingles and Metcalf (1972) this fact leads to the need to develop stabilization processes which make it possible to improve certain geotechnical properties of these soils, especially at a regional level, in order to fit them within the technical specifications applicable to the paving of roads.

Soil stabilization represents any artificial modification introduced in its behavior in order to make it employable in engineering works, acquiring a quantitative character through parameters inherent to certain design criteria, such as shear strength and deformation under the action of loads (Lima, 1981). From the road point of view, soil stabilization refers to the construction methods in which soils are treated so that they have surface, subbases and bases, occasionally coatings able to withstand traffic loads during the road life, resisting to the weather without degradation (Baptista, 1978). Mechanical and chemical stabilization techniques may be employed for improving the local soil properties in forest road construction. Mechanical stabilization is achieved by soil compacting, granulometric correction, and chemical stabilization, by adding luting materials to the soil.

Cement is used as a chemical stabilizer, and its addition to soil produces a mixture or material known as soil-cement. The results of the chemical processes of soil, cement and water are decreased porosity, plasticity and increased mechanical strength and durability (Kézdi and Rétháti, 1988). Small cement content, of the order of 1 to 2% is sufficient to reduce volume changes and increase the soil bearing capacity (Ingles, 1968). Portelinha et al. (2012) mentioned that the addition of 2 and 3% of lime or cement to laterite soil was sufficient to

alter its workability characteristics and mechanical strength for road paving purposes.

Cement addition becomes an attractive technique when the design requires improving the local soil. Cement and local soil can be applied in the construction of pavement foundations, in the earth dam slope protection, and as a support for the layer of shallow foundations (Consoli et al., 2007). However, it is essential to carry out experimental programs in laboratory to get to know the mechanical behavior of soils when adding cement.

The objective of this study was to investigate in the laboratory the technical potential of cement use in improving geotechnical characteristics of a local soil from the surface of an unpaved forest road, as an economic alternative for the construction of flexible pavement layers.

Study area

The study was performed in a section of a forest road located in the municipality of Niquelandia, Goiás State, Brazil (14°26'41"S; 48°44'26"W). This section was set up along of main access of a road network used for timber transportation by trucks loading about 25 tons. The origin and destination of the logs was the eucalyptus plantation (1566 ha) and iron-nickel alloy beneficiation plant, respectively, managed by Anglo American Company.

MATERIALS AND METHODS

The materials used were lateritic soil, with sand-clay-silt particle proportion and classified by Brazilian Agricultural Research Corporation - Embrapa (2009) as an Oxisol (LVAd30) from the forest road and cement. Nineteen soil samples were collected, 45 m from each other, at a depth of 20 cm in three collecting points by sample, roadside and center, using a pick due to the high degree of soil compaction. Samples were packed in plastic bags, identified and sent to the Geotechnical Laboratory of University of Brasília - UnB. The cement used was Portland CP-II-Z-32 of chemical composition clinker + gypsum (76 to 94%), limestone (0 to 10%) and pozzolan (6 to 14%). Before starting the laboratory tests, gross twigs, herbs, and organic debris were removed manually. Additionally, the soils were homogenized and sieved (2 mm mesh), as described in preparing sample methods for soil characterization tests (DNER – National Department of Roadways, 1994a).

Laboratory experimental program

In the laboratory, soil samples were first air-dried for over 72 h (ABNT, Brazilian Association of Technical Standards, 1984a) toward determining the following physical parameters: (a) Unit weight of soil grains (ABNT, 1984b); (b) Full grain-size distribution (ABNT, 1984c); (c) Liquidity limit (ABNT, 1984d); and (d) Plasticity limit (ABNT, 1984e). By knowing these parameters, the soil and the mixture were classified as to the geotechnical behavior by the American road system TRB (Transportation Research Board), according to National Department of Terrestrial Infrastructure (DNIT, 2006), and by the MCT Brazilian method (Miniature Compacted Tropical) according to Nogami and Villibor (1995).

The limits established by ABNT (1995) were adopted for the granulometric distribution of the soil fractions, which were: (a) clay: $\phi \leq 0.002$ mm; (b) silt: $0.002 \text{ mm} < \phi \leq 0.06$ mm; (c) fine sand: $0.06 \text{ mm} < \phi \leq 0.2$ mm; (d) medium sand: $0.2 \text{ mm} < \phi \leq 0.6$ mm; (e) coarse sand: $0.6 \text{ mm} < \phi \leq 2$ mm; and (f) gravel: $\phi > 2$ mm.

The soil-cement mixture was made by adding cement and water to the soil in pre-defined quantities to achieve the desired moisture contents, followed by hand homogenization and screening (# 4.8 mm). The mixture produced was wrapped in a plastic bag and sealed for later use in trials. The cement content was 2% calculated on the dry soil mass, based on minimum amount of material to be added for soil improvement by chemical stabilization according to National Department of Terrestrial Infrastructure (DNIT, 2006).

After making the soil-cement mixture, the following mechanical tests were performed: (a) Compaction (ABNT, 1986); (b) Unconfined compressive strength - UCS (DNER, 1994a), and (c) California bearing ratio - CBR (DNER, 1994b). These trials were also carried out for the local soil without addition of cement.

The determination of compaction curves and optimal parameters, maximum dry unit weight (γ_{dmax}) and optimal humidity (w_{opt}) were performed according to the Proctor method. Proctor compaction was carried out through a dynamic process using a cylinder mold (10-cm internal diameter, 12.7-cm height and 1,000-cm³ volume). The compaction energies tested were normal, intermediate, and modified in quest of acceptable performance in terms of support capacity and low cost building for forest road application.

To determine the UCS, cylindrical soil proofs ($\phi = 5$ cm and height = 10 ± 0.05 cm) were molded in accord to compaction parameters (w_{opt} and γ_{dmax}), applying static compaction by using an electrical press, with compaction degree of 0.3%. After preparation, the proofs were packaged, named and submitted to damp curing periods of 1, 7 and 28 days in a chamber with 100% humidity and temperature of 21°C. Three proofs were molded for each compaction energy and curing time. The proofs have been compressed yet break or point of maximum compression strength of the soil, using a simple compaction press at the speed of 1.143 mm/min. The UCS values were obtained by averaging the three breakdown tension points for energy and curing.

The values of expansion and CSI were obtained from compacted mixture proofs based on the compaction parameters (w_{opt} and γ_{dmax}), on the modified and intermediate energies and for the cure period with best response in the UCS trial. Compaction was performed in five inner layers with a socket of 4.5 kg, in California cylinder ($\phi = 15.2$ cm and height = 12.7 cm), and the proof bodies prepared like this were subjected to a period of 96 h of immersion in water (DNER, 1994b) for the daily measurement of expansion values. Later, proof bodies were broken in triplicate at a specific press, through penetration of a standardized piston (area = 3 in²), at the speed 1.143 mm/min, obtaining the CSI value.

Statistical analysis

To determine the best results of the UCS trials, the analysis of variance (two-way ANOVA) was used, and the averages with significant effects were compared by the Tukey test at 1% significance, using the GENES software (Cruz, 2013). The factors considered were compaction energy and curing periods for local soil and soil-cement mixture, as follow: (a) Local soil + water + compaction + 0% cement + uncured; (b) Local soil + 2% cement + water + compaction + 1 curing day; (c) Local soil + 2% cement + water + compaction + 7 curing days; and (d) Local soil + 2% cement + water + compaction + 28 curing days. The results of the CSI trials were compared with the recommendations of DNIT (2006) for materials to be used in road paving layers, which are: (a) Sub-roadbed (CSI $\geq 2\%$ and expansion $\leq 2\%$); (B) Strengthening of the sub-roadbed (CSI $\geq 2\%$ and expansion $\leq 1\%$); (c) Sub-base (CSI \geq

20% and expansion $\leq 1\%$); and (d) Base (CSI $\geq 80\%$ and expansion $\leq 0.5\%$).

RESULTS AND DISCUSSION

Physical parameters and road soil classification

The granulometric characteristics of the local soil led to the following results in average percentage of its fractions, each with its standard deviation: (a) Clay ($21.8 \pm 7.15\%$); (b) Silt ($12.7 \pm 2.43\%$); (C) Fine sand ($46.6 \pm 7.56\%$); (d) Medium sand ($9.9 \pm 2.58\%$); (e) Coarse sand ($3.74 \pm 1.27\%$); and (f) Gravel ($5.19 \pm 2.58\%$). There was a predominance of the sand fraction in samples ($> 60\%$), and its texture was characterized as sandy-clayey-silty. This soil did not fit in any of the granulometric ranges recommended by the DNIT (2006) for use in granular base pavements, because it does not have 25% of particles larger than 2 mm, referring to the gravel fraction (5.19%).

The average value of soil grains unit mass was $2.69 \pm 0.03 \text{ g cm}^{-3}$. Pinto (2000) mentioned that this value is important in the calculation of other physical indices and varies little from soil to soil, with values close to 2.75 g cm^{-3} . There is an association between the typical vs values and the indication of the mineralogical nature of soils.

The average values of liquid limit, LL ($25.2 \pm 4.13\%$), plasticity limit, PL ($18.6 \pm 0.56\%$) and plasticity index, PI ($6.6 \pm 3.78\%$) of the soil corroborate with the typical values of Brazilian soil "variegated loamy sand of São Paulo" (Pinto, 2000). LL and PI values are related to fluctuations in the volume of compacted soils, by contraction or expansion, undesirable characteristics for the construction of roads. The maximum acceptance limits established by the DNIT (2006) for flexible pavements base materials are LL, 25% and IP, 6%.

The local soil was classified as A-4 (1) by the TRB system, indicating a satisfactory overall quality as material to be used in sub-roadbeds, and LA' (sandy laterite) by the MCT system, and the preferred order of use as the basis for local roads, sub-roadbed reinforcement, and compacted sub-roadbed.

Physical characterization and local soil classification in the category LA', according to Nogami and Villibor (2009), indicate that this material presents ease of compaction and adequate cohesion. Bernucci et al. (2006) highlighted the importance of sand in the soil stabilization cost with the use of cement, since a very high percentage of clay in the soil may require considerable increase of the cement content to be used and increased process costs.

Soil compaction and soil-cement mixture

The effect by adding cement in the soil caused slight

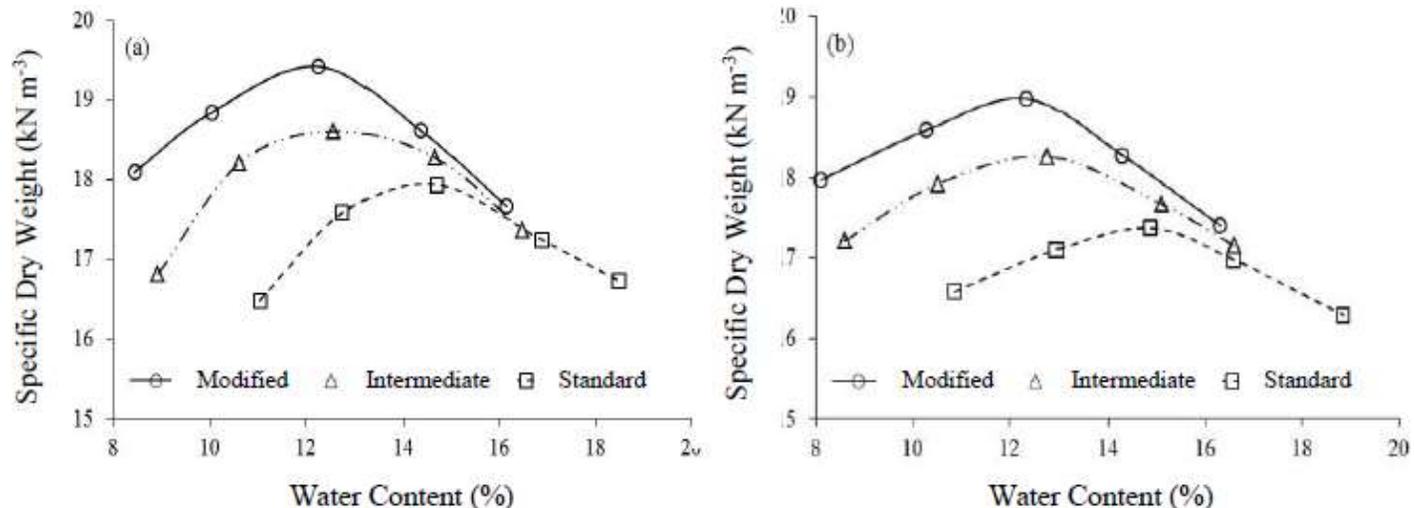


Figure 1. Compaction curves for the local soil (a) and soil-cement mixture; (b) compaction energies were normal (6 kg cm^{-2}), intermediate (13 kg cm^{-2}), and modified (29 kg cm^{-2}).

downward shift of all soil compaction curves (Figure 1). Trindade (2006) noted that the effect of the addition of chemical stabilizers to a soil followed by compaction process can cause changes in the format of its compaction curve, shifting it to the right or to the left, causing changes in its optimal parameters (w_{opt} and γ_{dmax}). According to Specht et al. (2002) the optimum moisture content (w_{opt}) leads to maximum density values in soil-cement mixtures, which reflects in full mechanical strength. The addition of 2% cement produced a less expansive material with higher bearing capacity, improving thus the geotechnical parameters of local soil. It happened because pozzolanic reaction with the surface of particles, increasing adhesive, cohesion and hardness while filling the pores between soil aggregation. The pozzolanic reaction happens even in low cement contents added to the soil as 1 to 4% mixture, increasing durability and water resistance (Baleato and Amorena, 1968; Kézdi and Rétháti, 1988).

The addition of cement to the soil changed w_{opt} to lower values than natural soil when the same compaction energy was used. Also, the increase in compaction energy led to the reduction of w_{opt} for both the soil and the soil-cement mixture. The maximum soil dry weight (γ_{dmax}) reached on compaction curves was reduced by the addition of cement over 3.1, 1.9 and 2.3% for normal, intermediate, and modified energies, respectively. The increase in compaction energy provided greater γ_{dmax} values for both the soil and the soil-cement mixture.

Pereira (2012) found no significant differences in w_{opt} and γ_{dmax} values when adding 3% of cement to a soil classified as A-4 typical from the Parnamirim-RN, located in northwestern of Brazil. This soil had the following granulometric distribution: (a) Clay, 22%; (b) Silt, 39%; (c) Sand, 36%; and (d) Gravel, 3%.

Soil mechanical resistance and soil-cement mixture

By observing the stress-strain curves of USC shown in Figure 2, the increase in compaction energy has improved the compressive strength of both natural soil and mixture. The compaction energy also resulted in sharper apex of curves where the proofs broke, especially for soil-cement mixture. This behavior indicated stronger particle aggregation structure of the mixture compared to the natural soil, which provided stronger cohesion among aggregated particles by chemical reaction. When the highest point of supported strength was reached, soil-cement mixture proofs ruptured more abrupt than natural soil. In other words, natural soil deformation was higher (4%) than the mixture (3%) at the time of breaking point or deflection in relation to its height.

The chemical reaction acting between cement and soil can also be represented in the overall longitudinal rupture and sharp drop of UCS curves, which meant higher degree of soil particles aggregation (Figure 2). Compacting over modified energy, this soil-cement mixture in energy would resist about 675 kPa after 28 days of curing without traffic, deforming 3% in thickness; higher energies than modified can cause disruption and degradation of the pavement. The local soil in its best response, that is, compaction in modified energy, would withstand 385 kPa with 4% deformation.

The mean values of highest UCS have been increased as compaction energies grows for natural soil and soil-cement mixture. Compaction energies did not affect the deformation at highest stress point in local soil. For the mixture, deformation has been changed with compaction energies without comprehensive relationship between stress and strain (Figure 2). Therefore, increase in

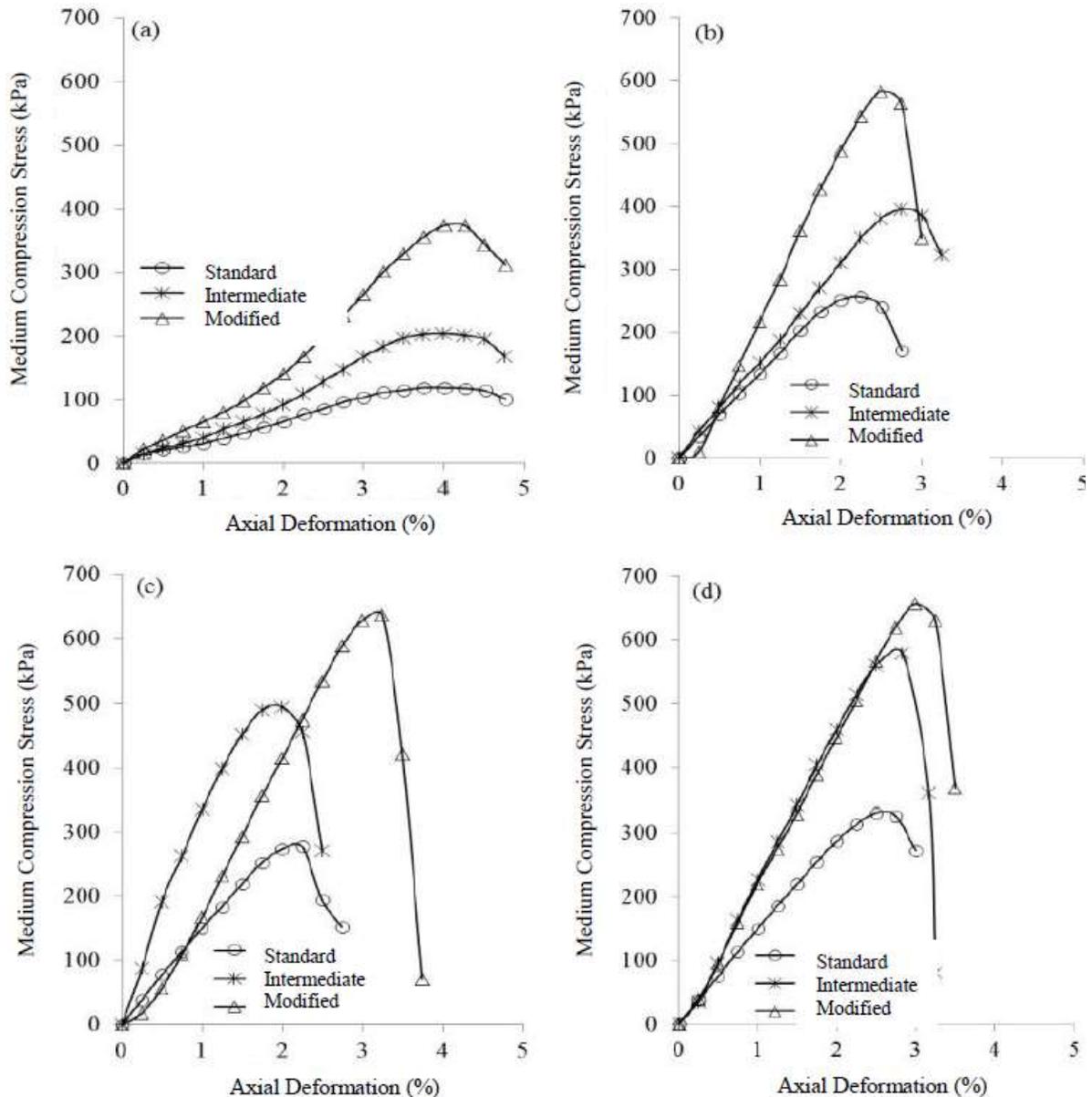


Figure 2. Mean stress-strain curves of unconfined compressive strength (UCS) for compaction energies and curing periods: Local soil (a); soil-cement mixture at 1 day curing (b); soil-cement mixture at 7 days curing; soil-cement mixture at 28 days curing.

compaction energy produced a positive effect in terms of UCS gains in local soil, which meant higher strength for supporting the traffic of forest transportation. It can be improved by adding 2% of cement and leaving 28 days curing before starting the traffic. Using soil-cement mixture, higher values of UCS can be found compacting on intermediate energy, which produced statistical similar result to modified energy ($p < 0.05$) (Table 1).

The coefficient of variation (CV) of the variance analysis was low (Table 1), considering the classification proposed by Gomes (2000), which demonstrated high experimental control for compaction proceedings. It was

observed that all energy levels were significantly different when compared to the mixtures ($p < 0.01$). The best UCS responses for the standard and intermediate compaction energies were found after 28 days of curing. For modified compaction energy, UCS at 7 and 28 days were statistically equal, at 1% probability by the Tukey test, thus demonstrating that in the 7-day curing period, the soil-cement mixture has reached maximum value of mechanical resistance.

The unconfined compression strength test (UCS) has been used routinely in many experimental programs described in literature, when the objective is to verify the

Table 1. UCS values for compaction energies and curing periods and statistical results from two-way ANOVA and Tukey test.

Treatments/curing	UCS (kPa)		
	SE (6 kg cm ⁻²)	IE (13 kg cm ⁻²)	ME (29 kg cm ⁻²)
Local soil/ No curing	120.42±0.77 ^{Cc}	205.91±9.53 ^{Db}	385.10±78.06 ^{Ca}
Soil-cement mixture/ 1 day	256.19±10.83 ^{Bc}	376.45±54.33 ^{Cb}	585.75±42.00 ^{Ba}
Soil-cement mixture/ 7 days	290.75±30.59 ^{Bc}	496.95±17.57 ^{Bb}	650.52±40.60 ^{Aa}
Soil-cement mixture/ 28 days	334.73±27.72 ^{Ac}	578.59±35.70 ^{Ab}	675.45±11.47 ^{Aa}

Compaction energies: standard (SE); intermediate (IE); modified (ME). Statistical significance for two-way ANOVA and Tukey was 1% probability. Means followed by distinct uppercase letters (vertical) compare the effect of the curing for soil-cement mixture; means followed by distinct lowercase letters (horizontal) compare the effect of compaction energy and differ significantly at 1% probability by the Tukey test.

Table 2. Average values of CBR and expansion for the local soil (LS) and soil-cement mixture (Mix SC) on intermediate (IE) and modified (ME) compaction energies at 28 curing days, and framing as road construction material.

Material	IE (13 kg cm ⁻²)		ME (29 kg cm ⁻²)		Flexible pavement for highway indication (DNIT, 2006)
	CBR (%)	Exp (%)	CBR (%)	Exp (%)	
LS	13.5±2.3	0.03±0.00	8.0±2.8	0.06±0.04	Subgrade and subgrade reinforcement
CS mix	44.1±10.5	0.02±0.00	41.7±5.8	0.02±0.00	Sub-base

CBR = California bearing ratio; Exp = Expansion.

effect of cement in soil stabilization, because it is a simple test, of rapid execution, low cost and it is reliable (Consoli et al., 2007). Jaritngam et al. (2012) mentioned that the cement mixture to a laterite soil is able to produce a material with higher mechanical strength when compared with a soil in its natural state. Bagui (2012) mentioned that the consumption of aggregate material is lower for the case of a base stabilized with cement compared to the conventional method. This feature is extremely important because of the granular materials shortage. Jaritngam et al. (2014) also reported that adding 3% of cement to a laterite soil resulted in a soil-cement mixture with mechanical resistance as high as that of granular materials, demonstrating that the laterite soils reinforced with cement are a viable replacement for aggregate in road pavement construction.

Classification of soil-cement mixture as road materials

CBR trials and expansion of soil-cement mixture were performed for the best results of UCS trials, that is, compaction in intermediate and modified energies, and 28 curing days (Table 2).

The addition of cement to local soil followed by compaction brought increased bearing capacity to the soil, a fact evidenced by the increase of the CSI value of 226% in intermediate energy and 422% in modified energy. By increasing energy compaction on soil-cement

mixture, CBR has not been increased and it probably can be explained by the breakdown of soil particles when compacted with higher energy, causing slight deformation and a rearrangement of particles which resulted in a lower CSI value. The CBR behavior seems to be related to particle-sized distribution and mineral composition of laterite soil rather than cement reaction itself. More studies on the physical and chemical properties of this soil must be performed. For the expansion, cement reduced the values relative to the local soil in numerical terms. However, the compaction energy did not affect the expansion values in the soil-cement mixture. As for the classification of the soil-cement mixture as a material to be used in conventional road paving, according to the DNIT (2006), the local soil can be used in sub-roadbed, while the soil-cement mixture has its use in the sub-base. Therefore, soil-cement mixtures with low stabilizer content, acting as modifier of soil properties, might represent an efficient and economical solution for flexible pavements projects (Sariosseiri and Muhunthan, 2009; Solanski et al., 2009).

Conclusions

The local soil had its engineering properties modified by treatment with cement as stabilizer, producing a material with geotechnical characteristics desirable for forest road construction. The soil-cement mixture showed higher mechanical strength and lower expansion when

compared to natural-state, a lateritic soil. This improvement enables its use as construction material of base course in forest roads.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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A background image of a cow with brown and white patches grazing in a green field. The image is framed with rounded corners.

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