African Journal of Agricultural Research

Volume 11 Number 2 14 January 2016
ISSN 1991-637X

Academic Journals

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African Journal of Agricultural Research

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Vol. 11(2), pp. 57-64, 14 January, 2016
DOI: 10.5897/AJAR12.0675
Article Number: 23B0A7656649
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Growth and yield response of fibre hemp cultivars (*Cannabis sativa* L.) under different N-levels in Eastern Cape Province of South Africa

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Received 7 March, 2012; Accepted 9 December, 2015

Hemp (*Cannabis sativa* L.) has been grown in Southern Africa for medicinal purpose for many centuries, and in the past half century as an illegal drug crop marijuana, "dagga". It was however, noted that hemp has high fibre which can be used in formation of particle boards, textile products, paper and clothing. Field experiments were conducted during 2006/2007 and 2007/2008 seasons at Addo of Eastern Cape Province. The objective of the study was to determine the influence of nitrogen fertilizer rates; thus, 0, 50, 100 and 150 kg ha^{-1} on growth and yield of fibre hemp cultivars, Kompolti, Felina 35 and Novosadska. In 2007/2008, Novosadska was used to replace Felina 35, whose fibre was very short. Trials were laid out in complete randomised block design with four replications and four nitrogen levels and two hemp cultivars. The results revealed that there were significantly higher fresh biomass yields, dry biomass yield, and fibre yield in the fertilized units than in the unfertilized control unit. The highest plant height, fresh and dry biomass yield were obtained with 100 and 150 kg ha^{-1} of N fertilization were applied at Addo during 2006/2007. Higher nitrogen levels tended to produce higher yield and quality in both seasons. Kompolti performed better than Felina 35 and Novosadska. Kompolti had a higher fibre percentage and quantity than other cultivars in both seasons. It was therefore concluded that nitrogen application had a positive effect on growth and yield studied, and the optimum fertilization rate of nitrogen will be 100 and 150 kg ha^{-1}.

Key words: Fibre hemp, cultivars, nitrogen fertilization, yield, quality.

INTRODUCTION

Hemp (*Cannabis sativa* L.) is unknown to South African farmers as an industrial fibre crop. However, it has been grown in Southern Africa for medicinal purposes for many centuries, but cultivated during the past half-century as an illegal drug crop. This plant species was declared illegal in South Africa in 1928 due to its high cannabinoid

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content and potential psycho activity. Although hemp and marijuana are from the same plant family, they have different variations for different uses and physical characteristics (Dippenaar et al., 1996).

Hemp is generally referred to the fibre-producing strain of cannabis (Meijer, 1995). Most soils in Eastern Cape Province are depleted of nitrogen element as a result of mono cropping and leaching, ultimately crop yields and quality decline (Van der Werf, 1995). Brough et al. (2005) postulated that hemp declined yield rapidly when grown continuously in the same soil. Since, soil nutrients decline due to hemp plants uptake. This could be tackled by chemical fertilizers which are always costly and should be used in precise amounts to meet crop needs (Power et al., 1986). The objective of this study was to determine the influence of nitrogen fertilizer rates on growth and yield of fibre hemp cultivars, as well as to determine the best fertilizer rates for optimum yield of hemp cultivars grown in South Africa.

MATERIALS AND METHODS

During 2006/2007 and 2007/2008 cropping seasons, trials were planted at the Agricultural Research Council's Addo experimental station in the Eastern Cape Province, which is situated at 33° 26" 46' S 25° 44" 45' E. The predominant soil type in the sites was a sandy loam classified by the South African classification system as a Hutton series (Soil Classification Working Group, 1991). An auger was used to take soil samples at 30, 60 and 90 cm depths for nutrients analysis.

Trial design and treatments

The trial was laid out in randomized complete block design with four replications. Treatments were four nitrogen levels (0, 50, 100 and 150 kg/ha) and two hemp cultivars (Kompolti and Felina 35). The plot size was 4 m long and 1.5 m wide with an inter-row spacing of 0.25 m. There were six rows per plot in each trial. Along each row, a furrow of about 5 to 10 cm deep was made by hand and the seeds were drilled and covered by hand with soil. Hemp was planted at 600 000 plants/ha. The application of nitrogen fertilizer was split into three intervals, 50% of the required N was applied four weeks after planting since a crop requires more of N on early stages of growth, and 30% 8 weeks after planting and 20% after twelve weeks. However, in 2007/2008 Novosadska was used to replace Felina 35 whose height was rather too short and only produced a lot of seed.

Data collection and analysis

The following data were collected from for two cropping seasons: wet biomass (kg/ha), dry biomass yield, stem diameter (mm), plant height (cm) and fibre percentage (%). Data collected was statistically analyzed using GenStat 5 for windows (7th edition) programme 2003 to develop analysis of variance (ANOVA). Means were separated and compared using a Tukey multiple regressions (Van Ark, 1995).

Wet biomass (kg ha^{-1})

All plants in the two middle rows of each plot were harvested (cut at about 20 cm above ground level). All the freshly harvested plants (one bundle) were weighed using a scale to achieve wet biomass in the field.

Dry stem yield

From the plants harvested in the two middle rows, ten plants were selected at random and weighed for fresh biomass yield. Then, stem samples were oven dried at 60°C for 24 h. The weight obtained was converted to kg ha^{-1}.

Stem diameter (mm)

The ten plants sampled earlier for dry stem yield were used to estimate stem diameter. Each plant was measure for stem diameter using Digital Caliper on the middle of the stem. The ten values obtained were averaged and recorded as stem diameter per plot.

Plant height (m)

The mean height of the ten randomly selected plants was recorded as the plant height per plot. A meter stick was used to measure the plant heights.

Fibre percentage

The following procedure was used to determine fibre percentage and fibre yield. Stem samples were oven dried at 60°C for 24 h. The dry mass of the samples was boiled in 2% NaOH in 1 L of water for 1 h. Bast fibre was manually removed after being dried for 60°C for 24 h. The weight (mass) of bast fibre and woody core was calculated to estimate the bast fibre and fibre yield.

Method of conducting soil analysis in the laboratory

Particle size distribution and soil texture

According to the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990), fifty grams soil was weight in a 500 ml glass beaker and 20 ml Calgon was dispensed in the beaker. 100 ml deionised water was added and it was stirred with a glass rod. The mixture was allowed to stand for 15 min and was transferred into a shake beaker of a high speed electrical mixing machine and was placed on the machine for 5 min. It was transferred into a 1000 ml glass cylinder and was allowed to stand overnight in a 22°C constant room. The following day as early as possible the samples were shaken by hand before taking the readings. The samples were read with the hydrometer exactly 5 min after being shaken. The readings acquired for sand, silt and clay were reported as percentages.

pH (H_2O)

According to the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990), soil pH was determined on a 1:2.5 soil:H_2O ratio suspension with a glass electrode pH Meter. Ten grams of dried soil (2 mm) was placed in a 50 ml glass beaker. 25-cm^3 de-ionized water was added and the mixture was stirred rapidly for 5 s and was allowed to stand for 10 min.

pH was determined after 30 s with the electrode positioned in the supernatant. The pH meter was calibrated with commercially available buffer solutions at pH of 4.0, 7.0 and 8.0.

Table 1. Soil analysis results for hemp trial at Addo Experimental farm during 2006/2007.

Description	0-30 (cm)	30-60 (cm)	60-90 (cm)
pH	7.87	7.92	7.89
Elements mg kg ha^{-1}			
N	2	2	3
P	255	125	140
K	455	291	525
Ca	1770	1830	4190
Mg	350	405	630
Na	143	213	378
Soil texture			
% Sand	60	66	70
% Silt	16	12	18
% Clay	24	22	12

Extractable inorganic nitrogen

Extractable inorganic N in soils is defined as NH^{4+}, NO^{3}- and NO^{2-} extractable at room temperature with a 1.0 mol dm^{-3} KCL solution (Handbook of Standard Soil Testing Methods for Advisory Purposes, 1990). A 30 g soil sample was weight in a 250 ml wide mouth extraction bottle. 150 ml potassium sulphate was dispensed into the extraction bottle. The sample was shaken for 30 min in a reciprocal shaker at 180 oscillations per minute. The sample was allowed to stand for 30 min. It was filtered with a 2V filter paper until there was enough extract for analysis. The analysis was done with an Auto Analyzer.

Extractable phosphorus

According to the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990), the extraction of P by this procedure is based on the solubilization effect of H+ on soil P and the ability of F+ to lower the activity of Al^{3+} and to lesser extent that of Ca^{2+} and Fe^{3+} in the extraction system. An 8 g of soil was placed in an Erlenmeyer flask. 60 ml Bray-2 solution (20°C) was added into the sample. The sample was shaken for 40 s by hand and was filtered immediately through a Whatman no. 2 filter paper into an extraction bottle. One-gram phosphorus free charcoal was added and the mixture was shaken by hand for 40 s. Two drops of flocculant was added and the extract was filtered through a Whatman no. 40 paper. P was determined within 24 h. The analysis was done with a continuous flow analyzer (e.g. Auto Analyzer).

Extractable cations (K, Ca, Mg and Na)

According to the Handbook of Standard Soil Testing Methods for Advisory Purposes (1990), this method is used to determine extractable cations Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} in soils, reflecting the nutrient status. 0.05 g air dried, ≤2 mm soil was placed in a 100 ml extraction bottle. 50 ml NH_4OAc solution cooled to 20±2°C was added to the extraction bottle and was shaken horizontally on a reciprocating shaker at 180 oscillations per minute for 30 min. The extract was rapidly filtered through a Buchner funnel and collect filtrate. The elements K, Ca, Mg, and Na were determined on an atomic absorption spectrophotometer.

RESULTS

Rainfall distribution and temperatures readings

Rainfall distribution (Figure 1) and temperatures were recorded during the growing season, thus from October to July. At Addo, rain was evenly distributed in 2006/2007 and 2007/2008 compared to long-term average rainfall. Addo received the higher rainfall of 92 mm during the 2006/2007 season on March when compared with 2007/2008 and long term averages (Figure 1).

Soil analysis

The results of the soil analysis taken before planting for 2006/2007 and 2007/2008 cropping season are presented in Table 1. The soil at Addo had a high pH and reasonably high potassium and low nitrogen content. Soils at 30 cm depth indicate high percentage of sand. Slight differences were found in soil texture for the three depths analyzed. Limited land in this experimental station dictated that the trial be planted in the same piece of land for 2006/2007 and 2007/08 growing seasons.

Wet biomass yield

Wet biomass yield was significantly different between cultivar and nitrogen levels at P≤0.05 during 2006/2007 season. However, the Cultivar × Nitrogen interaction

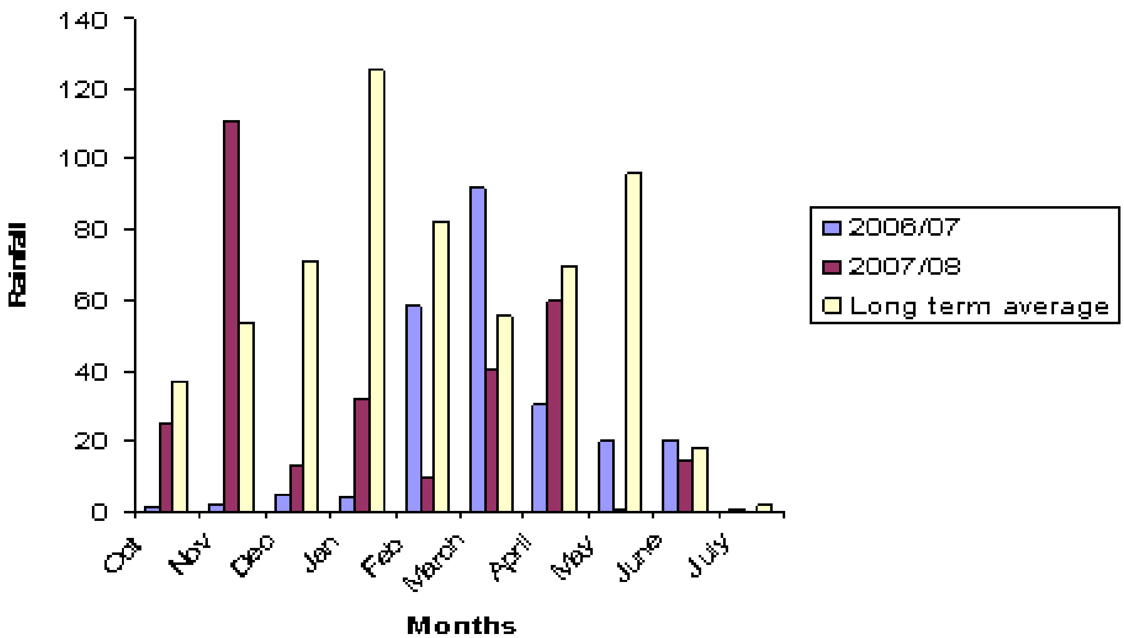

Figure 1. Monthly rainfall distribution (mm) from October to July during 2006/2007 and 2007/2008 growing seasons compared to long term averages at Addo.

Table 2. Wet biomass yield (kg ha^{-1}) for hemp cultivars grown at four different nitrogen fertilizer levels at Addo in 2006/2007 and 2007/2008 season.

Nitrogen rates	2006/2007 season		Average	2007/2008 season		Average
	Kompolti	Felina		Kompolti	Novosadska	
0 kg ha-1	33375 cd	15638 e	25506	4111 b	3031 b	3571
50 kg ha^{-1}	52725 ab	26963 de	39844	6816 a	6121 a	6469
100 kg ha^{-1}	68550 a	33375 d	50963	7850 a	7034 a	7442
150 kg ha^{-1}	56175 ab	40238 bcd	48206	8712 a	8691 a	8702
Average	53206	29053	41130	6872	6219	6546
CV%		16.05			22.5	
LSD (0.05) Cultivars(C)		12449.2			2544	
LSD (0.05) Nitrogen (N)		16950.			3597	
LSD (0.05) C × N		8451.1			1798	

Means within two cultivar columns followed by different letters are significantly different at LSD (0.05) Nitrogen.

component was not significant in the same season (Table 2). Kompolti produced higher wet biomass yield in both seasons than the other cultivars. Kompolti obtain high wet biomass at 100 kg ha^{-1} N fertilization level when compared to other fertilization levels (Table 2). In 2007/2008, significant differences were found between cultivars and nitrogen fertilization levels with 0 kg ha^{-1} having the lowest biomass weight. Although, from 50 to 150 kg ha^{-1} N fertilization levels, the yield means did not vary between cultivars, a pattern of biomass increasing with N application rates was revealed (Table 2). Cultivar by nitrogen interaction was also not significant during 2007/2008 season.

Dry biomass yield

In 2006/2007, mean dry biomass calculations were not done at Addo. Table 3 presents the means for dry biomass at Addo for 2007/2008. At 100 and 150 kg ha^{-1} N fertilization levels, Kompolti gave the highest yield while cultivar by N fertilization interaction had no significant effect on dry biomass. However, the results showed evidence that there were significant differences between

Table 3. Mean dry biomass (kg ha^{-1}) for two hemp cultivars grown at four different nitrogen fertilizer levels at Addo in 2007/2008 season.

Cultivar	Dry Biomass yield				
	0 kg N ha^{-1}	50 kg N ha^{-1}	100 kg N ha^{-1}	150 kg N ha^{-1}	Average
Kompolti	3222^{cd}	6029^{ab}	7314^{a}	7000^{a}	5891
Novosadska	2415^{d}	4271^{bcd}	5202^{ab}	6359^{a}	4562
Average	2819	5150	6258	6680	-
	CV% = 10.5				
	LSD (0.05) Cultivar = 1805				
	LSD (0.05) Nitrogen = 2553				
	LSD (0.05) Cultivar × Nitrogen = 1277				

Means within two cultivar row followed by different letters are significantly different at LSD (≤0.05) nitrogen.

Table 4. Stem diameters (mm) for hemp cultivars grown at four different nitrogen fertilizer levels at Addo in 2006/2007 and 2007/2008.

Nitrogen rates	2006/2007 season		Average	2007/2008 season		Average
	Kompolti	Felina 35		Kompolti	Novosadska	
0 kg ha-1	11.27^{a}	5.70^{c}	8.49	12.25^{ab}	11.00^{a}	11.63
50 kg ha^{-1}	10.56^{ab}	5.70^{c}	8.13	13.50^{a}	8.83^{a}	11.17
100 kg ha^{-1}	12.26^{a}	6.65^{bc}	9.46	14.25^{a}	9.00^{a}	11.63
150 kg ha^{-1}	11.72^{a}	8.56^{abc}	10.14	9.75^{a}	11.75^{a}	10.75
Average	11.45	6.65	9.05	12.44	10.15	11.30
CV%		21.2			13.9	
LSD (0.05) Cultivars (C)		2.84			2.27	
LSD (0.05) Nitrogen (N)		4.02			3.21	
LSD (0.05) C × N		2.01			1.23	

Means within two cultivar columns followed by different letters are significantly different at LSD (0.05) nitrogen.

N levels within cultivars. The 100 and 150 kg N ha^{-1} tended to have higher dry biomass than the other levels.

Stem diameter

During 2006/2007, only the cultivar effect was significant in stem diameter. Kompolti had significantly higher stem diameter compared to Felina 35 (Table 4). However, in 2007/2008, there were significant effects between cultivar by nitrogen level interaction (Table 4). As in 2006/2007, Kompolti showed a better performance in stem diameter as kg N ha^{-1} level were applied. At 100 kg ha^{-1} N fertilization, Kompolti gave the highest stem diameter than in other levels. Felina 35 showed no response to N levels applications. Also, Novosadska stem diameters means did not vary between N levels. However, at 150 kg ha^{-1} stem diameter was at 11.75 mm, while the overall average for Novosadska was 10.15 mm during the 2007/2008 season; this was higher than that of Felina 35 which was 6.65 mm during 2006/2007. This is attributed for 53% higher than that of Felina 35's diameter.

Plant height

In 2006/2007 growing season, there were significant differences in plant height between cultivars and N level (Table 5). The nitrogen levels and cultivar components had no significant interaction effect. In 2007/2008, there was a significant effect on cultivars only (Table 5). Furthermore, the cultivar by nitrogen levels interaction components also had no significant effect on plant height. In 2007/2008, both Kompolti and Novosadska (which replaced Felina 35) showed no response to nitrogen levels. However, it was noticed that Kompolti had slightly higher averages than Novosadska. During 2006/2007 season, there was no significant difference between N-

Table 5. Plant height (cm) for hemp cultivars grown at four different nitrogen fertilizer levels, at Addo in 2006/2007 and 2007/2008.

Nitrogen rates	2006/2007 season		Average	2007/2008 season		Average
	Kompolti	Felina 35		Kompolti	Novosadska	
0 kg ha^{-1}	167.0^{abcd}	104.2^{d}	168.4	171.5^{a}	165.8^{a}	168.4
50 kg ha^{-1}	190.8^{a}	117.0^{cd}	176.8	181.4^{a}	172.3^{a}	176.8
100 kg ha^{-1}	203.3^{a}	131.0^{bcd}	170.5	167.8^{a}	173.3^{a}	170.5
150 kg ha^{-1}	225.3^{a}	178.0^{abcd}	165.3	165.9^{a}	163.^{a}	165.3
Average	196.6	132.6	170.4	171.5	169.3	170.4
CV%		23.4			5.8	
LSD (0.05) Cultivars (C)		57.0			13.80	
LSD (0.05) Nitrogen (N)		80.6			19.52	
LSD (0.05) C×N		40.3			9.75	

Means within two cultivar columns followed by different letters are significantly different at LSD (≤0.05) Nitrogen.

Table 6. Fibre yield percentage (%) for hemp cultivars grown at four different nitrogen fertilizer levels at Addo in 2006/2007 and 2007/2008.

Nitrogen rates	2006/2007 season		Average	2007/2008 season		Average
	Kompolti	Felina 35		Kompolti	Novosadska	
0 kg ha^{-1}	41.50^{a}	33.25^{abc}	37.38	29.00^{a}	28.50^{a}	28.75
50 kg ha^{-1}	41.25^{a}	27.50^{c}	34.38	28.00^{a}	29.75^{a}	28.87
100 kg ha^{-1}	39.75^{ab}	30.00^{abc}	34.88	29.50^{a}	27.25^{a}	27.25
150 kg ha^{-1}	38.75^{abc}	27.75^{bc}	33.55	29.25^{a}	28.75^{a}	29.00
Average	40.31	29.62	34.97	29.03	29.00	29.02
CV%		14.7			16.3	
LSD (0.05) Cultivars(C)		7.62			6.94	
LSD (0.05) Nitrogen (N)		10.78			9.82	
LSD (0.05) C×N		5.39			4.91	

Means within a column followed by different letters are significantly different at P≤0.05.

levels and N-level × cultivar interaction components. Plant height only varied between cultivars: Kompolti and Felina 35 obtained their highest mean at 150 kg ha^{-1} N fertilization, which were 225 and 178 cm, respectively. Generally, in 2006/2007, Kompolti and Felina 35 showed linear response; as nitrogen levels increased, the plant height also increased.

Fibre percentage

In fibre percentage, the two cultivars were significantly different during 2006/2007 season (Table 6). In Table 7, Kompolti had a significantly higher mean fibre percentage when compared with Felina 35. In 2007/2008 growing season, no significant effect was observed for any of the variance components (Table 6). Also the nitrogen fertilizer levels and cultivar by nitrogen fertilizer interaction components had no significant effect on fibre yield. Means within N levels under both cultivars did not show any response to N levels. In 2006/2007, the two cultivars differed significantly of fibre percentage. However, in 2007/2008, when Felina 35 was replaced by Novosadska, there was no significant difference in fibre percentage (Tables 6).

Fibre yield

In 2007/2008, there were variations in fibre between cultivar and cultivar by N fertilization interaction effects (Table 7). Also, there were significant differences among the fertilizer levels. In both cultivars there was a tendency for the fibre yield to increase as the nitrogen level increased (Table 7). Kompolti had significantly higher fibre yield (kg ha^{-1}) as compared to Novosadska across different nitrogen fertilizer levels. Kompolti accounted for 4392 kg ha^{-1} average yield which was 69%

Table 7. Fibre yields (kg ha^{-1}) for two hemp cultivars grown at four different nitrogen fertilizer levels at Addo in 2007/2008.

Cultivar	0 kg N ha^{-1}	50 kg N ha^{-1}	100 kg N ha^{-1}	150 kg N ha^{-1}	Average
Kompolti	2082^{f}	3630^{d}	5003^{b}	6853^{a}	4392
Novosadska	906^{h}	1065^{g}	3606^{e}	4838^{c}	2604
Average	1494	2348	4748	5673	
CV% = 67.9					
LSD (0.05) Cultivar = 30.43					
LSD (0.05) Nitrogen = 43.03					
LSD (0.05) Cultivar x Nitrogen= 21.52					

Means within nitrogen columns followed by different letters are significantly different at P≤0.05.

higher than that of Novosadska.

DISCUSSION

Wet biomass yield and dry biomass

Comparison between two seasons showed that Kompolti had better yields than Felina 35 or Novosadska. This could have been due to variation in cultivar responsiveness to N fertilization and rain distribution in each season. Nitrogen fertilization had a significant effect on wet biomass production. An additional increase in nitrogen level tended to increase biomass yield. In this and related studies, higher nitrogen levels tend to be associated with higher wet biomass in hemp production (Van der Werf, 1991, Van Der Werf et al., 1994). For Addo, the nitrogen levels did not have any significant effect on the dry mass of both cultivars. This might have been due to the replacement of Felina 35 by Novosasdska. It is conceivable that both cultivars behaved in a similar manner in terms of dry biomass yield.

Stem diameter and plant height

Stem diameter is one of the important traits that determine good yield in hemp production (Van der Werf, 1991). Generally, the greater the stem diameter, the higher the expected amount of fibre. It was noted that high stem diameter was produced at nitrogen level of 100 kg ha^{-1} N fertilization. Since both Kompolti and Felina 35 had more or less the same stem diameter at 100 and 150 Kg N ha^{-1}, any of them could be planted at Addo, provided adequate nitrogen is applied. For high fibre yield, a combination of stem diameter and plant height is necessary. In this study, at both seasons and for both cultivars, plant height was associated with an increase in nitrogen level. General observation was that Kompolti grew taller than Felina 35. If the objective is to increase fibre yield, a better choice is Kompolti. Good stand of two hemp cultivars planted at Addo in 2006/2007 was observed at 100 kg/ha nitrogen fertilization. Kompolti was the taller cultivar and the shorter one was Felina 35. Good stand of hemp plants influences biomass yield and quality of fibre (Dewey, 2000). This is consistent with Meijer et al. (1995).

In N fertilization, the planting date is an important factor in successful hemp fibre production. The hemp plant is sensitive to day-length and the vegetative growth period must therefore, be prolonged. An early planting date (October to November in the Eastern Cape Province) resulted in taller plants with higher fibre yield. Later planting will result in early flowering and, therefore, poor fibre production (Van der Werf, 1991). The planting date for dry land hemp is determined by the availability of soil moisture. If irrigation water is inadequate, germination is limited (Meijer et al., 1995).

Fibre percentage and fibre yield

In hemp production, a very important objective is high yield and quality fibre production. In both seasons, Kompolti out-performed Felina 35 in terms of fibre percentage. When no fertilizer had been applied, low and poor yields of fibre were obtained, especially if there is no added nitrogen fertilizer in some instances (Van der Werf et al., 1995). With hemp, the ultimate objective is to adopt the management practices that will eventually lead to high fibre yield. Such management practices will include appropriate choice of cultivars, fertilizer levels, and irrigation methods, planting depth, plant population, etc. In both seasons and cultivars, a high fibre yield tended to be associated with nitrogen levels. The optimum nitrogen fertilization level appeared to be between 100 and 150 kg ha^{-1}.

Conclusions

Application of N fertilizer improved growth and fibre yield of hemp cultivars during 2006/2007 and 2007/2008 seasons at Addo Research Station. Nitrogen fertilizer rates at 100 and 150 kg N ha^{-1} produced the best fibre yield in both growing seasons, and therefore, selected as the best optimum application rates. However, compared with the other examined cultivars, Kompolti showed higher potential as good cultivar for hemp fibre production. Kompolti was found to be well adapted to South African climatic conditions. Usage of correct recommended rates of fertilization could lead to better growth and fibre yields.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(2), pp. 65-73, 14 January, 2016
DOI: 10.5897/AJAR2015.10296
Article Number: 2C547DE56654
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Supplementation of selenium and vitamin E in diets for pacu (*Piaractus mesopotamicus*): Effect on performance, body yields and lipid stability

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Received 14 August, 2015; Accepted 15 October, 2015

Current study evaluates the supplementation of selenium and vitamin E in the diet on performance, body yields and lipid stability of pacu. Seven hundred and twenty juveniles were distributed in 36 cages installed in a masonry tank. During 100 days, the animals were fed on diets supplemented with four selenium levels (0, 1, 2 and 4 mg/kg), combined with three levels of vitamin E (0, 100 and 200 mg/kg) in a 4x3 factorial arrangement. Selenium combined with vitamin E levels did not affect (P>0.05) growth and body yield of pacu. The interaction between levels of selenium and vitamin E did not influence (P>0.05) the lipid stability of main trunks. When the levels of vitamin E only are evaluated, 200 mg vitamin E/kg diet decreases lipid oxidation in 90 and 120 days of storage. The combination of 0 Se + 0 vitamin E / kg diet was influenced by storage time, and the highest rate of lipid oxidation was observed with 120 days of storage. Level 1 mg selenium + 200 mg vitamin E/kg in the diet of the pacu decreased meat lipid oxidation after 90 days of storage under freezing, coupled to the maintenance of performance and body yields.

Key words: Aquaculture, dietary antioxidants, lipid oxidation.

INTRODUCTION

In Brazilian aquaculture, the pacu *Piaractus mesopotamicus* (Holmberg, 1887) stands out for its high growth rate, easy adaptation to aquaculture systems and high fecundity (Sampaio et al., 2008), coupled to other factors such as its meat quality, with good acceptance by consumers (Jomori et al., 2003). Small quantities of vitamins and minerals are required for the animals normal growth, reproduction, health and metabolism (Lovell, 1998). Vitamin E is the most important metabolic antioxidant in the cell membrane, protecting it from oxidation by fatty acids and cholesterol, and reducing or inhibiting the production and action of free radicals

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Table 1. Percentage composition and nutritional requirements of the basal diet used.

Food[1]	(%)	Calculated composition	(%)
Corn grain	40.59	Starch	31.24
Wheat bran	20.00	Total arginine	1.79
Soybean meal	20.00	Calcium	0.98
Poultry viscera meal	13.10	Digestible energy (kcal / kg)	3000.00
Commercial fish meal	5.00	Phenylalanine	1.20
Vitamin and mineral supplement[2]	0.50	Crude fiber	3.99
Salt	0.30	total phosphorus	0.93
DL-Methionine	0.18	Fat	4.40
Soybean oil[4]	0.17	Histidine	0.64
Antifungal (calcium propionate)	0.10	Isoleucine	1.09
L-Lysine HCl	0.04	Leucine	2.13
Antioxidant (B H T)	0.02	Linoleic acid	1.18
TOTAL	100.00	Total lysine	1.43
		Methionine + total Cystine	1.07
		Total methionine	0.65
		Crude protein	26.00
		Total threonine	1.03
		Total tryptophan	0.29
		Total valine	1.29

[1]Available nutrients based on Abimorad and Carneiro (2004). [2]Supplementation levels per kg feed: 12 000 IU vitamin A, vitamin D3 3000 IU, Vitamin K3 MNB 15 mg / kg, 20 mg vitamin B1 / kg, 20 mg vitamin B2 / kg vitamin B6, 18 mg / kg vitamin B12 0.04 mg / kg, vitamin C 300 mg / kg, niacin 100 mg / kg, Calcium Pantothenate 50 mg / kg; biotin 1 mg / kg, 6 mg Folic Acid / kg Inositol 150 mg / kg, Choline chloride 500 mg / kg, copper sulfate pentahydrate, 18 mg / kg, iron sulfate monohydrate 80 mg / kg, Manganese Sulfate 50 mg / kg, Zinc Sulfate 120 mg / kg; calcium iodate 0.8 mg / kg, cobalt sulfate 0.6 mg / kg, 12 combinations of selenium and vitamin E, selenium 0, 1, 2 and 4 mg / kg vitamin E and 0, 100 and 200 mg / kg.

(Sampaio et al., 2004). Besides being a component of several selenoproteins, Selenium is a co-factor and an integral part of the glutathione peroxidase enzyme (GPx) (Rotruck et al., 1973).

Vitamin E is included in the non-enzymatic antioxidant system of the animal organism, while selenium, as member of GPx, integrates the enzymatic system for antioxidant protection. Selenium and vitamin E act in synergy, since selenium cannot protect the cell or tissue components that have low GPx concentrations (Rotruck et al., 1973). However, these cell or tissue components may be protected by vitamin E, which acts as antioxidant by different mechanisms. Combined selenium and vitamin E are the main antioxidants in the organism.

Antioxidants from the diet do not have only important functions in living organisms but they may decrease the lipid oxidation in tissues after death. Studies on vitamin E supplementation in diets for fish revealed its antioxidant action in *in vivo* and in the reduction of oxidation after death, with an improvement in conservation during storage (Onibi et al., 1996; Pirini et al. 2000; Shiau and Shiau, 2001; Ruff et al., 2002; Hamre et al., 2004; Fogaça and Santana, 2007).

However, the literature reports no studies involving the relationship between selenium and vitamin E for pacu, both in performance and lipid oxidation after death. Current analysis evaluates the antioxidant effects of selenium and vitamin E supplementation in the diet on the pacu´s productive performance, body parameters and lipid stability.

MATERIALS AND METHODS

The experiment was conducted at the Institute of Research in Environmental Aquaculture (InPAA), Universidade Estadual do Oeste do Paraná (UNIOESTE), Toledo campus, Toledo PR Brazil, from March to July 2011, for 100 days. Seven hundred and twenty pacu juveniles (*P. mesopotamicus*), retrieved from a commercial fish farm, with initial average weight = 43.52±1.03 g and total length = 12.33±1.65 cm, were used in current assay. Fish were distributed in 36 cages and installed in a 200m² masonry tank. The 1 m³ cages were made of polyester coated with flexible PVC and with a 5 mm mesh. The experimental unit was a tank with 20 juveniles. Fish were kept in the experimental structures for seven days previously to adapt themselves to the experimental conditions.

The experiment comprised twelve treatments with three replicates per diet. The experimental design was completely randomized, in a 4x3 factorial arrangement, with four selenium inclusion levels (0, 1, 2 and 4 mg / kg) and three vitamin E inclusion levels (0, 100 and 200 mg/kg diet).

The experimental diets were formulated to obtain 26% crude protein and 3000 kcal digestible energy/kg (Table 1), where composition of feed was calculated with software SuperCrac (SUPERCRAC, 2004). DL-α-tocopherol was the additional source of vitamin E, with activity of 50% vitamin E, while sodium selenite with 45% selenium availability was employed for selenium supplementation.

The ingredients for the processing of rations were initially ground in a hammer-type mill with a 0.5 mm sieve; they were then milled, weighed and mixed for the preparation of the experimental diets. Different levels of vitamin and mineral supplements were added to the mixture. The mixtures were humidified (28% water) and extruded through a EX-MICRO® mill with 10 kg/h production capacity. The diets were dried in a forced-air oven for 12 h, at 55°C, resulting in a product with approximately 10% moisture. Feeding was carried out twice a day, at 10:00 am and 5:00 pm, by apparent satiation and the amount of diet provided was weighed to estimate apparent feed conversion.

The water tank temperature was measured daily at 10:00 am and 5:00 pm and the water physical and chemical parameters (pH, dissolved oxygen and electrical conductivity) were measured once a week at 6:00 am and 4:00 pm with portable meters (YSI Model 55 Dissolved oxygen, YSI Incorporated, Yellow Springs, USA and Alkafit pHmeter AT315 SP, Alkafit, Florianópolis, Brazil). At the end of the experiment, fish were fasted for 24 h to empty the digestive tract. The fish were then removed from the experimental units and anesthetized in benzocaine at 250mg /l (euthanasia), packed in ice inside a cooler and transported to the Fish Technology Laboratory (UNIOESTE), campus Toledo, Toledo PR Brazil.

Individual measures of final weight, total and standard lengths were taken for weight gain calculation (WG = final weight - initial weight), apparent feed conversion (FCR = feed intake / weight gain) and fish survival in each experimental unit. Afterwards, the animals were then opened at the ventral abdominal cavity, from the urogenital hole up to the jaw bones, followed by careful viscera removal to prevent contamination of meat with fecal material. The head was cut and the fish, without head and viscera, were washed in chlorinated water, removing fins and skin, leaving only the trunk. Intraperitoneal fat and liver were manually removed from the viscera, and weighed. Data was used to calculate the hepatosomatic index [HSI = (liver weight / fish weight) x (100)] and intraperitoneal fat index [IGI = (intraperitoneal fat weight / fish weight) x (100)].

The main trunks were placed in plastic bags and stored in a freezer (18±2°C) for 30 days, when chemical analyzes were performed (moisture, protein, lipids and ash), according to methodology proposed by AOAC (2005).

Lipid oxidation was analyzed by the thiobarbituric acid reactive substances method (TBARS) (Vyncke, 1970) to investigate the lipid stability of meat after 60, 90 and 120 days of storage. The amount of malondialdehyde (MDA), the main substance formed during the oxidation and which reacts with thiobarbituric acid, was calculated by the standard curve equation: $y = 73.689 + 0.0223\ x$ ($r^2 = 0.9968$). Results were expressed as mg MDA per kg of sample.

Data underwent analysis of variance (ANOVA) at 5% significance in a factorial arrangement, verifying the interaction between selenium and vitamin E. When significant interaction was reported, Duncan test´s was applied at 5% significance to compare means. Data were also evaluated for homogeneity of variances (Levene´s test) with statistic program SAEG 9.1 (UFV, 2007).

RESULTS AND DISCUSSION

Water temperature in the tanks averaged 20.8±2.62°C during the experiment. There was a linear temperature decrease throughout the experimental days ($y = -0.074 + 23.291\ x$ $r^2 = 0.7325$) since the experiment began in March and ended in July (period of low temperatures). The physical and chemical parameters of the tank water were 4.67±0.75 mg $O_2D \cdot L{-1}$; 7.26±0.64 and 40.08±2.63 mS cm^{-1} for dissolved oxygen, pH and electrical conductivity, respectively. Selenium and vitamin E levels did not affect (P>0.05) the parameters total and standard length, weight gain, feed conversion and survival (Table 2).

There was no significant interaction (P> 0.05) between selenium and vitamin E on performance and survival, and selenium and vitamin E separately did not affect productive performance.

Similar results in current study were reported for tilapia (*Oreochromis niloticus*) fed on different levels of organic selenium (0, 0.25, 0.50, 1.0, and 1.5 mg Se / kg) in the diet, with no significant difference in performance (weight gain and apparent feed conversion) (Gomes, 2008). Further, in a study on juvenile *Sparus aurata* evaluating the effect of supplementation of 250 mg vitamin E/kg diet in a system of high stocking density (40 kg/m^3), Montero et al. (1999) did not report any effect (P> 0.05) on weight gain and survival. There was no difference in growth for the Atlantic halibut (*Hippoglossus hippoglossus*) between treatments with 189 and 613 mg vitamin E / kg in the diet (Ruff et al., 2002).

Current study confirms results by Sampaio (2003) who evaluated levels of selenium (0, 0.25, 0.50 and 1.00 mg / kg diet) and vitamin E (0,100, 200 and 300 mg / kg) for the tilapia (*O. niloticus*). The author failed to register any effect of the interaction of these nutrients on weight gain, feed conversion and survival rate. Studying largemouth bass (*Micropterus salmoide*) fed on graded levels of vitamin E (160, 280, and 400 mg/kg) associated with either 1.2 or 1.8 mg/kg selenium (Se), Chen et al. (2013) reported that vitamin E and selenium inclusion could protect largemouth bass from the oxidative damage challenged by dietary oil oxidation, although none could enhance growth and feed utilization.

The absence of any significant effect of diets on production performance differs from results by Cavichiolo et al. (2002), who evaluated the effect of vitamins C and E on Nile tilapia larvae (*O. niloticus*). They reported that treatment with 300 mg vitamin E per kg diet provided increased weight and final length of larvae, and reduced the occurrence of the ectoparasite *Trichodina* sp in tilapia larvae. In the case of hybrid tilapia (*O. niloticus* x *O. aureus*) fed on increasing levels of vitamin E, Huang and Huang (2004) found greater weight gain in tilapia fed on 62.5 IU vitamin E / kg diet. Likewise, Gonçalves et al. (2010) registered that supplementation with 400 mg / kg vitamin E improved the standard length and weight gain of tambacus (*Colossoma macropomum* × *P. mesopotamicus*).

Rainbow-trout (*Oncorhynchus mykiss*) fed on 50 ppm de vitamin E and on 0.35 ppm selenium improved gain weight and food conversion. There was also an improvement of the fillet´s functional quality when compared to control (Rodríguez and Rojas, 2014).

The highest selenium level in current experiment (4 mg/kg diet) did not cause any damage on fish performance.

Table 2. Performance and survival data of pacu *P. mesopotamicus* fed on diets supplemented with different levels of selenium and vitamin E.

Selenium (mg/kg)	Vitamin E (mg/kg)	TL[1]	SL[2]	WG[3]	AFC[4]	Survival (%)
	0	14.07±0.18	11.63±0.08	34.15±4.85	3.30±0.60	71.67±14.43
0	100	13.80±0.10	11.40±0.21	28.31±2.02	4.17±0.39	63.33±22.55
	200	14.17±0.22	11.69±0.20	38.00±4.21	2.78±0.22	83.33±7.64
	0	14.05±0.25	11.61±0.08	31.81±4.18	2.98±0.25	71.67±20.21
1	100	13.92±0.60	11.37±0.49	30.50±8.38	3.45±0.86	80.00±8.66
	200	13.95±0.17	11.51±0.27	30.87±7.78	3.71±0.63	78.33±10.41
	0	14.11±0.28	11.55±0.24	30.18±3.77	3.79±0.62	80.00±20.00
2	100	13.88±0.51	11.53±0.44	30.86±7.42	3.91±0.64	73.33±7.64
	200	14.05±0.25	11.66±0.25	32.94±4.37	3.21±0.48	83.33±5.77
	0	13.83±0.50	11.30±0.33	27.84±7.00	3.56±1.06	86.67±15.28
4	100	14.12±0.58	11.67±0.50	32.57±8.26	3.43±1.05	76.67±16.07
	200	14.02±0.40	11.66±0.21	33.54±4.78	3.34±0.51	76.67±10.41
Selenium (mg/kg)						
	0	14.01±0.22	11.57±0.20	33.49±5.40	3.42±16.41	72.78±16.41
	1	13.97±0.34	11.50±0.30	31.06±6.12	3.38±0.63	76.67±12.75
	2	14.01±0.33	11.58±0.29	31.32±4.86	3.64±0.60	78.89±11.93
	4	13.99±0.45	11.54±0.36	31.36±6.49	3.44±0.79	80.00±13.23
Vitamin E (mg/kg)						
	0	14.01±0.30	11.52±0.23	31.00±4.97	3.41±0.67	77.5±16.45
	100	13.93±0.44	11.49±0.38	30.56±6.20	3.74±0.74	73.33±14.35
	200	14.05±0.25	11.63±0.21	33.87±5.41	3.26±0.54	80.42±8.11
Selenium x Vitamin E		ns	ns	ns	ns	ns
Selenium effect		ns	ns	ns	ns	ns
Vitamin E effect		ns	ns	ns	ns	ns

[1]Total length (cm), [2]Standard length (cm), [3]Weight gain (g), [4]Apparent feed conversion. ns= not significant (P>0.05). Data expressed as mean ± standard deviation.

In a study with *Pogonichthys macrolepidotus* juveniles fed on 0.4, 0.7, 1.4, 2.7, 6.6, 12.6, 26.0 and 57.6 mg selenomethionine / kg diet levels, Teh et al. (2004) observed a histopathologic effect only when concentrations were greater than or equal to 6.6 mg Se / kg diet. The selenium poisoning occurred in the rainbow-trout when diets contained selenium levels exceeding 13 mg / kg (Hilton et al., 1980). Thus, the level of 4 mg selenium / kg diet for pacu (*P. mesopotamicus*) does not seem to cause poisoning and deterioration in fish performance. However, Lin and Shiau (2005) emphasized that the minimum selenium level required by fish diet differed according to the species, or rather, between 0.25 and 0.80 mg / kg.

Although selenium supplementation in the diet of pacu did not cause any damage on fish performance, the concentration of 1.5 mg Se / kg diet improved growth and increased antioxidant defenses of matrinxã (*Brycon cephalus*) by increasing the activity of the enzymes glutathione peroxidase and increased the level of reduced glutathione (Monteiro et al., 2007). Trunk yield, intraperitoneal fat index and hepatosomatic index of pacu fed on diets with different selenium and vitamin E levels did not differ significantly (P>0.05) when treatments were assessed (Table 3). Moreover, selenium and vitamin E separately did not affect (P>0.05) main trunk yields, hepatosomatic index and intraperitoneal fat.

Signor et al. (2010) reported approximately a 60% main trunk yield, or rather, above the average value of 50.60% found in current analysis. An increase in fish size provided a higher main trunk yield.

The intraperitoneal fat index was lower than that reported by Bittencourt et al. (2010) and Signor et al. (2010), although similar to that observed by Hilbig et al.,(2012) for the pacu. Change in the intraperitoneal fat index occurred if fish required fat to obtain energy, a

Table 3. Main trunk yield (%), intraperitoneal fat index (% IGI) and hepatosomatic index (HSI%) of pacu *P. mesopotamicus* fed on different levels of selenium and vitamin E.

Selenium (mg/kg)[1]	Vitamin E (mg/kg)	% Main trunk	IGI	IHS
	0	49.22±0.69	4.10±2.21	2.77±0.23
0	100	49.34±4.44	4.62±2.03	2.79±0.23
	200	46.91±4.96	4.99±0.70	2.60±0.82
	0	51.14±5.49	4.46±0.10	2.33±0.26
1	100	52.82±6.16	5.84±3.30	2.85±0.53
	200	52.84±2.68	5.58±1.42	2.90±0.23
	0	46.32±3.40	4.24±0.61	3.34±0.15
2	100	52.30±1.94	5.31±1.47	2.96±0.59
	200	52.23±0.98	4.99±0.57	2.55±0.25
	0	52.55±3.94	5.05±2.02	2.46±0.32
4	100	52.41±6.60	4.54±1.23	2.67±0.71
	200	49.17±0.28	5.72±1.02	2.72±0.12
Selenium (mg/kg)				
0		48.49±3.55	4.57±1.59	2.72±0.45
1		52.27±4.42	5.29±1.91	2.69±0.42
2		50.28±3.59	4.85±0.97	2.95±0.47
4		51.38±4.19	5.10±1.39	2.62±0.41
Vitamin E (mg/kg)				
0		49.81±4.05	4.46±1.35	2.72±0.46
100		51.72±4.60	5.08±1.93	2.82±0.48
200		50.28±3.50	5.32±0.91	2.69±0.41
Selenium × Vitamin E			ns	ns
Selenium Effect			ns	ns
Vitamin E effect			ns	ns

ns = not significant (P>0.05). Data expressed as mean ± standard deviation.

fact that occurred during long fasting (Hilbig et al., 2012). However, since this experiment had an apparent satiation food supply, the fish probably did not need to mobilize lipids for energy production.

Despite the lack of significant difference (P> 0.05) in the hepatosomatic index of pacu fed on different levels of selenium and vitamin E, fish with selenium accumulation in tissues tended to have a higher hepatosomatic index (Pyle et al. 2005). However, the latter was not observed in this experiment, since the hepatosomatic index was equal for the minimum selenium level and for a greater inclusion of dietary selenium. Checking the above, Gomes (2008), who evaluating levels of organic selenium (0, 0.25, 0.50, 1.0, and 1.5 mg Se/kg) in the diet of tilapia (*O. niloticus*), reported no significant difference in the hepatosomatic index. For *P. mesopotamicus* with average weight of 377g, Hilbig et al. (2012) found a 0.77% average hepatosomatic index, a lower rate than that in that in current assay, which averaged 2.75%.

Moisture, protein, lipids and ash contents of pacus were not significantly different (P>0.05) among supplementations with selenium and vitamin E (Table 4), neither was there any difference when selenium and vitamin E levels were evaluated separately. In fact, results corroborated those by Otani (2009) who did not register any significant difference in moisture, protein and ash contents in fillets of tilapia fed on 0 and 100 mg α-tocopherol per kg diet. However, the same author observed increased lipid contents in these fillets when compared to diet without antioxidant addition, or rather, the use of antioxidants in the diet may protect the lipids of fillets from lipid oxidation, during freezing.

In a study evaluating supplementation with organic selenium (0.0, 0.25, 0.50, 0.75 and 1.0 mg / kg) in the diet of Nile tilapia matrices, Pereira et al. (2009) did not report any significant differences in moisture, protein and

Table 4. Proximate composition of pacu *P. mesopotamicus* fed on different levels of selenium and vitamin E.

Selenium (mg/kg)	Vitamin E (mg/kg)	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
	0	52.38±20.91	33.41±13.90	12.23±5.56	4.22±1.68
0	100	58.89±15.45	28.86±9.84	10.56±4.73	3.71±1.29
	200	53.42±17.91	32.71±12.47	12.32±4.73	3.80±1.21
	0	61.36±12.59	27.46±7.97	10.10±4.04	3.60±0.89
1	100	66.22±12.51	22.62±8.02	9.43±3.01	2.91±1.26
	200	59.91±15.53	28.97±10.82	9.18±4.73	3.70±1.33
	0	63.16±18.71	26.75±12.61	10.89±5.09	3.20±1.46
2	100	66.54±16.17	24.31±10.31	8.24±4.11	2.93±1.20
	200	68.91±8.97	21.96±6.95	7.86±2.04	2.71±0.82
	0	56.19±19.16	30.78±13.17	10.58±4.84	4.13±1.85
4	100	66.19±13.86	24.81±11.37	8.15±2.84	3.09±1.66
	200	62.57±12.81	27.84±8.76	8.08±3.63	3.55±1.18
Selenium (mg/kg)					
0		54.90±16.08	31.66±10.76	11.71±4.43	3.91±1.24
1		62.50±12.14	26.35±8.34	9.57±3.48	3.40±1.08
2		66.20±13.39	24.34±9.09	9.00±3.71	2.94±1.05
4		61.65±14.14	27.81±10.08	8.94±3.56	3.59±1.45
Vitamin E (mg/kg)					
0		58.27±16.08	29.60±10.72	10.95±4.27	3.79±1.36
100		64.46±12.87	25.15±8.82	9.10±3.36	3.16±1.21
200		61.20±13.43	27.87±9.41	9.36±3.84	3.44±1.08
Selenium × Vitamin E		ns		ns	ns
Selenium Effect		ns		ns	ns
Vitamin E effect		ns		ns	ns

ns = not significant (P>0.05). Data expressed as mean ± standard deviation.

lipid contents of fish fed on different selenium levels. Results corroborated those in current study.

Current assay revealed there was no difference in the proximate composition of pacu for different levels of vitamin E. However, Sau et al. (2004) obtained significant differences (P<0.05) in protein rates of rohu carcass (*Labeo rohita*) at different levels of vitamin E supplementation in the diets. In this study, carcasses of the group fed on diets with 200 mg / kg had lower average percentage of crude protein (57.17%) when compared to groups with 100 mg/kg (58.34%) and 0 mg/kg (58.34%). There were no differences in proximate composition with means 70.9 and 71.2% moisture, 9.7 and 9.5% lipids, 18.4% crude protein and 1.5% ash, respectively, for groups 300 and 1500 mg / kg, in rainbow-trout fed on 300 and 1500 mg vitamin E/kg diet (Chaiyapechara et al., 2003). Results of lipid oxidation for the main trunks of pacus fed on different levels of selenium and vitamin E are shown in Table 5.

In the case of lipid oxidation in different evaluation times, only level 0 Se + 0 vit. E / kg diet was affected by storage time, and the highest oxidation rate was observed for 120 days. However, when selenium levels were evaluated separately, levels 0 and 4 of Se / kg diet were significantly influenced by storage time, and the lipid oxidation was higher after 120 days (P<0.05) when compared to that after 60 and 90 days. Vitamin E supplementations (0, 100 and 200 mg vit. E / kg diet) were also affected by different storage periods, with significant increase (P<0.05) of TBARS overtime, as shown in Table 5.

This observation was also made by Otani (2009) for tilapia fillets and by Weber et al. (2008) for silver catfish fillets, perhaps due to an increase of compounds formed by lipid oxidation which reacts with thiobarbituric acid, thereby increasing TBARS rates. However, these rates tended to decrease overtime since there was a reduction of the substrates which reacted with thiobarbituric acid, as reported by Otani (2009).

The combination of selenium and vitamin E did not affect (P>0.05) lipid oxidation of fillets, neither did selenium levels separately affect this parameter.

Table 5. Mean rates of lipid oxidation by the formation of thiobarbituric acid reactive substances (mg malonaldehyde / kg of main trunk) of pacus supplemented with different concentrations of selenium and vitamin E in the diet during storage under freezing (-18±2°C).

Selenium (mg/kg)	Vitamin E (mg/kg)	Time (days)			Time effect
		60	90	120	
	0	0.31±0.10 ^{b}	0.48±0.16 ^{b}	1.19±0.59 ^{a}	P<0.05†
0	100	0.28±0.02	0.67±0.27	1.00±0.58	ns
	200	0.23±0.08	0.34±0.16	0.54±0.24	ns
	0	0.37±0.12	0.72±0.53	0.89±0.69	ns
1	100	0.37±0.26	0.52±0.42	0.88±0.62	ns
	200	0.25±0.05	0.34±0.09	0.51±0.21	ns
	0	0.61±0.32	0.75±0.28	0.94±0.29	ns
2	100	0.38±0.15	0.63±0.16	0.93±0.40	ns
	200	0.34±0.31	0.40±0.35	0.47±0.35	ns
	0	0.39±0.03	0.55±0.28	0.88±0.64	ns
4	100	0.26±0.21	0.67±0.39	0.98±0.45	ns
	200	0.20±0.08	0.31±0.11	0.52±0.32	ns
Selenium (mg/kg)					
0		0.27±0.07 ^{b}	0.49±0.23 ^{b}	0.91±0.52 ^{a}	P<0.05†
1		0.33±0.16	0.53±0.38	0.76±0.51	ns
2		0.44±0.25	0.59±0.23	0.78±0.32	ns
4		0.28±0.20 ^{b}	0.51±0.33 ^{ab}	0.79±0.51 ^{a}	P<0.05†
Vitamin E (mg/kg)					
0		0.42±0.21 ^{b}	0.63±0.32 ^{bA}	0.98±0.50 ^{aA}	P<0.05†
100		0.32±0.17 ^{c}	0.62±0.29 ^{bA}	0.95±0.45 ^{cA}	P<0.05†
200		0.26±0.16 ^{b}	0.35±0.18 ^{abB}	0.51±0.25 ^{aB}	P<0.05†
Selenium × Vitamin E[2]		ns	ns	ns	
Selenium effect[2]		ns	ns	ns	
Vitamin E effect		ns	P<0.05††	P<0.05††	

[1]mg/kg of diet; [2]ns = not significant. † different lowercase letters on the same line indicate significant difference (P<0.05) between different storage times; †† different uppercase letters in the same column indicate significant difference (P<0.05) between the different levels of vitamin E. Data expressed as mean ± standard deviation.

However, when the levels of vitamin E were assessed separately (Figure 1), levels of vitamin E influenced lipid oxidation after 90 and 120 days of storage and the level of 200 mg vitamin E / kg diet provided a lower rate (P<0.05). This finding showed the postmortem anti-oxidant effect of adding vitamin E on the diet of pacu (*P. mesopotamicus*). Cheah et al. (1995) claim that vitamin E is effective in fish conservation during processing and storage, since it inhibits the degradation of lipids by oxidation.

The antioxidant properties of vitamin E were also observed by Huang and Huang (2004), who found that dietary supplementation of vitamin E for hybrid tilapia (*O. niloticus x O. aureus*) decreases lipid peroxidation of postmortem tissue, whereas increase of dietary vitamin E causes decrease in muscle TBARS. The above authors observed that tilapia fed on 300 IU vitamin E / kg diet had the lowest TBARS rates in the muscle: 1.47 nmol MDA / mg. Moreover, Huang and Huang (2004) also found that vitamin E supplementation increased the glutathione level in the liver.

This is probably due to the contents of vitamin E stored in the muscle, which are greater in fish fed on higher doses of vitamin E. Vitamin E is actually a potent biological antioxidant: its high levels in tissues inhibit lipid peroxidation and decrease the formation of malondialdehyde. This trend has also been reported in the Atlantic salmon (Onibi et al., 1996) and sea bass (Gatta et al., 2000).

Results similar to current study were provided by Fogaça and Santana (2007) with tilapia fed on 0, 100 and 200 vit.E mg / kg diet and after 63 days processed in burgers. The authors noted that the burgers in the control group (without the addition of vitamin E) had higher TBARS rates than treatments with vitamin E supplementation. In fact, vit.E 200 mg / kg was the best to decrease

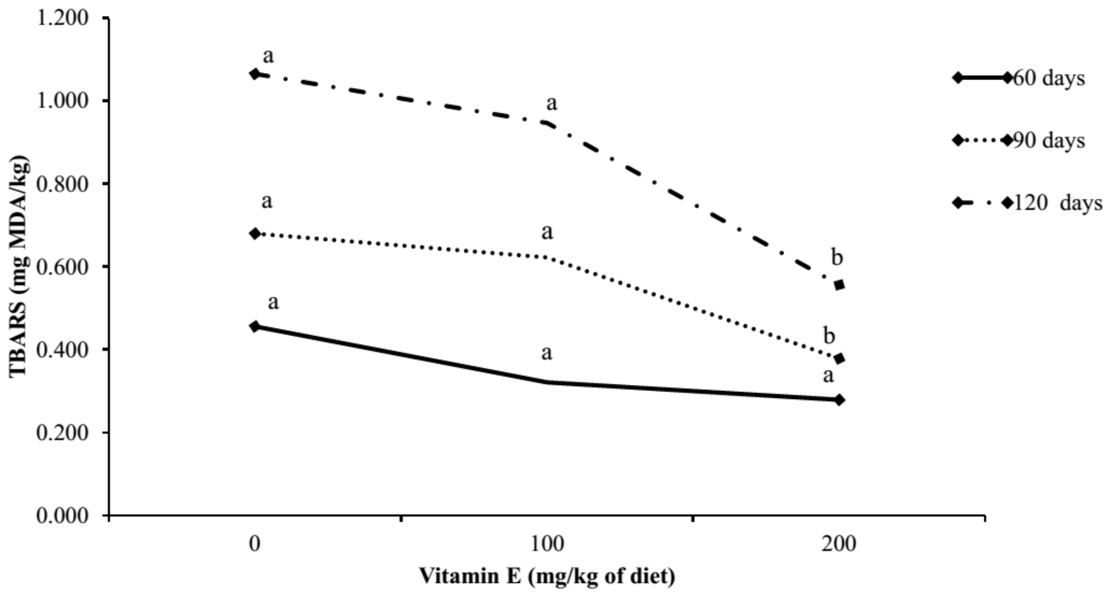

Figure 1. Relationships between vitamin E levels and the formation of thiobarbituric acid reactive substances at different storage times. Different letters indicate significant difference (P <0.05) between the different levels of vitamin E.

to decrease lipid oxidation since it indicated Vitamin E´s antioxidant activity.

Thus, the addition of 200 mg vitamin E / kg diet in pacus is ideal to reduce lipid oxidation after 90 and 120 days of storage, under freezing. This is due to the fact that vitamin E supplementation in the diet makes it incorporated into lipid membranes, protecting the tissue from any post-mortem oxidation.

Level 1 mg selenium + 200 mg vitamin E / kg in the diet of juvenile pacu (*P. mesopotamicus*) decreases lipid oxidation of meat after 90 days of storage, under freezing, and maintains performance and body yields.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(2), pp. 74-86, 14 January, 2016
DOI: 10.5897/AJAR2015.10401
Article Number: 0807A4B56658
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Spatial variability of selected physico-chemical properties of soils under vegetable cultivation in urban and peri-urban wetland gardens of Bamenda municipality, Cameroon

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Received 17 September, 2015; Accepted 20 November, 2015

Urban wetlands in Bamenda municipality play a vital role in vegetable production, but variability in the physico-chemical properties of soils is of dire concern. Twenty-one surface soil samples were analyzed for their physico-chemical properties using standard methods to examine the fertility status of the soils, evaluate their variability and constraints, and provide adequate data for planning sustainable land management. The coefficient of variation was used as an index of soil variability, while sources of soil variation and subsequent grouping into management units were identified using linear correlation, linear regression and principal component analyses. The results indicated that the soil pH was slightly variable while organic matter varied considerably. Correlation coefficients between some of the soil parameters were highly significant (p<0.01) ranging from - 0.950 to 0.999, but most of them had correlation values less than 0.500. Regressions depicted multiplicity of sources of different variables. Six principal components: base status, organic matter, weathering, acidity, sodium (Na) dispersal, and neo-synthesis, grouped the soils in management units and explained 96.24% of the variations observed. For a proper management of the soils, individual management units should be established for the municipality.

Key words: Soil variability, physico-chemical properties, correlation and regression, principal component analysis.

INTRODUCTION

Vegetables are an important component of a healthy diet in the world (Obuobie et al., 2006). They have been hailed for their nutritional and non-nutrient bioactive ingredients (Smith and Eyzaguirre, 2007). If consumed daily in sufficient amounts, they would help prevent major diseases such as cardio-vascular diseases and certain cancers. The low intake of fruit and vegetables is responsible for about 31% of ischaemic heart diseases and 11% of strokes (WHO, 2002). Each year, over 2.7 million lives would be saved if fruit and vegetables consumption are sufficiently increased. It has been recommended that a minimum of 400 g of fruit and vegetables should be consumed/day/individual (FAO/WHO, 2003).

However, the increase in soil degradation has posed a serious threat to agricultural productivity (Vanlauwe et al., 2002). Some of the major factors of soil degradation are the decline in soil fertility as a result of the lack of nutrient inputs (Tening et al., 1995; Hartemink, 2010), the use of urban wastes (Alloway, 1995), inappropriate land use, poor management, erosion, salinization (Bationo et al., 2006) and climatic constraints.

Tropical soils have low soil organic carbon, pH, CEC and are mainly composed of low activity clays and sesquioxides (Yerima and Van Ranst, 2005a). Under such conditions, crop yield could only be increased by adequate application of fertilizers and organic manure. This will require that baseline fertility status of these soils and how they vary in space are known. In urban environments and wetlands in particular, soil management practices greatly affect soil properties (Taboada-Castro et al., 2009). These also affect soil reactions and the availability of different nutrient elements (Abd-Rahim et al., 2011). The soils of the wetlands of Bamenda municipality are frequently being disturbed; anthropogenic disturbed soils have peculiar properties (Ibrahim et al., 2012). As such, soil degradation and the depletion of nutrients are perceived to increase and would become a serious threat to productivity and health of soils. Unfortunately, and too frequently, vegetable farmers in the wetland gardens usually handle the soils in a similar manner believing that the soils are the same. Soil properties exhibit a great spatial and temporal variability (Yerima and Van Ranst, 2005a). Studies on soil variability have relevance in sampling (Tabi and Ogunkunle, 2007), in site specific soil fertility management (UNEP, 2006), in definition of land management units (Tittonell et al., 2008; Salami et al., 2011) and in explaining variation in crop growth and yield (Kosaki and Juo, 1989; Tittonell et al., 2008). The management pattern of soil physico-chemical properties variability has implications for variable rates of applications and design of soil sampling strategies in alluvial flood plain soils.

In the Bamenda municipality, urban and peri-urban wetland soils are increasingly being used for the cultivation of vegetables. Too often, both commercial and resource-poor vegetable farmers in this municipality have incurred huge losses of their investment due to poor crop yield which could partly be emanating from the fertility status of the soils.

Some preliminary studies revealing these constraints have been carried out on the soils of this municipality (Kometa, 2013; Asongwe et al., 2014). However, a comprehensive work on the soils of this sub-region is yet to be done. This will assist in exposing more properties of these soils to satisfy the needs of a larger spectrum of resource users. It is thus hypothesized that soil variability in the area is high and needs more than one management regime.

The objectives of the study will be to: a) evaluate fertility status of the soils, b) Identify variations and the current utilization constraints and, c) group soils in management regimes to provide data for use by stakeholders in planning sustainable land management in the Bamenda urban Municipality.

MATERIALS AND METHODS

Description of the study area

The area covered by this study includes urban and peri-urban wetlands in the Bamenda City Council of the North West Region of Cameroon (Figure 1). It is part of the Bamenda escarpment and located between latitudes 5° 55'' N and 6° 30'' N and longitudes 10° 25'' E and 10° 67'' E. The town shows an altitudinal range of 1200 - 1700 m, and is divided into two parts by escarpments; a low lying gently undulating part with altitudes ranging from 1200 to 1400 m, with many flat areas that are usually inundated for most parts of the year, and an elevated part at 1400 to 1700 m altitude that forms the crest from which creeks, and streams, supplying the low lying parts take their rise.

This area has two seasons; a long rainy season, which runs from mid-March to mid-October and a short dry season that spans from mid-October to mid-March. The area lies within the thermic and hyperthemic temperature regimes. Mean annual temperatures stand at 19.9°C. January and February are the hottest months with mean monthly temperatures of 29.1°C and 29.7°C, respectively. This area is dominated by the Ustic and Udic moisture regimes with the Udic extending to the south (Yerima and Van Ranst, 2005b). Annual rainfall ranges from 1300 - 3000 mm (Ndenecho, 2005). The area has a rich hydrographical network with intense human activities and a dense population along different water courses in the watershed. The area is bounded on the West, North and East by the Cameroon Volcanic Line (made up of basalts, trachytes, rhyolites and numerous salt springs). The geologic history of this area–originates from the Precambrian era where there was vast formation of geosynclinal complexes, which became filled by clay-calcareous, and sandstone sediments (Yerima and Van Ranst, 2005b). These materials, crossed by intrusions of crystalline rocks, were folded in a generally NE-SW direction and underwent variable metamorphism (Yerima and Van Ranst, 2005a). The Rocks in the area are thus of igneous (granitic and volcanic) and metamorphic (migmatites) origin (Kips et al., 1987), which give rise to ferralitic soils (GP- DERUDEP, 2006).

The main human activity in and around this area is agriculture, which according to Grassfield Particpatory-Decentralised and Rural Development Project (GP-DERUDEP, 2006) involves over 70% of the population that use rudimentary tools. More than 81.7% of the active agricultural population is involved in farming, 11.6% in fishing and 6.5% in grazing (GP- DERUDEP, 2006). Farming

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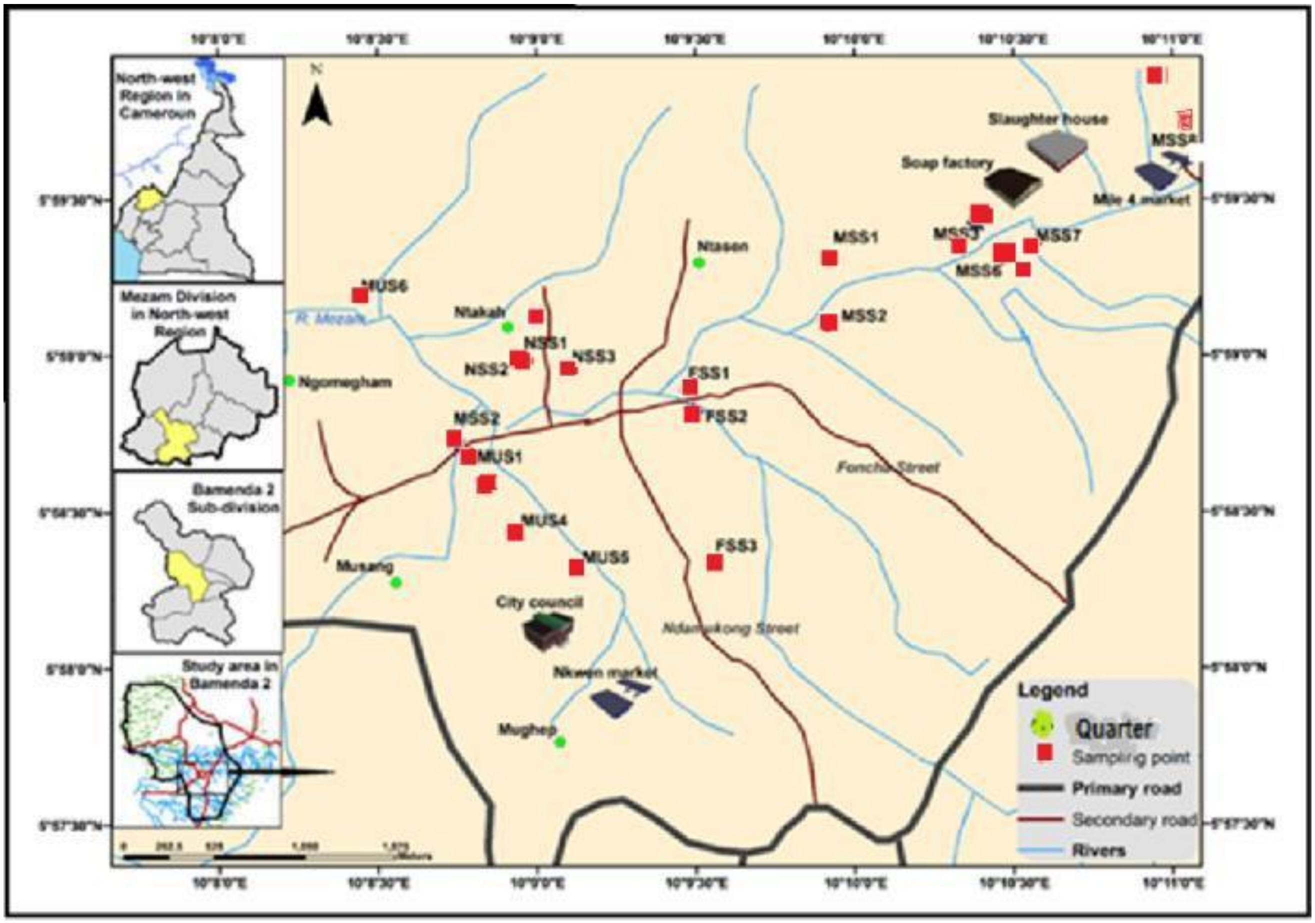

Figure 1. Soil sampling areas in the wetlands of Bamenda Municipality, Cameroon.

and grazing involves the use of fertilizers that is a potential source of pollution. The area equally harbours the commercial centre that has factories ranging from soap production, and mechanic workshops to metallurgy, which may be potential sources of pollutants. An important vegetation type in this area is the raffia palm (*Raffia farinifera)* bush, which is largely limited to the wetlands (Valleys and depressions). *R. farinifera* provides raffia wine, a vital economic resource to the inhabitants who are fighting against the cultivation of these wetlands by vegetable farmers.

Soil sampling and analysis

Twenty-one soil samples (0 - 25 cm) were randomly collected within the wetlands (Figure 1) and taken to the laboratory in black plastic bags. The soil samples were air-dried and screened through a 2-mm sieve. They were analyzed for routine parameters in the Environmental and Analytical Chemistry Laboratory of the University of Dschang, Cameroon. Particle size distribution, cation exchange capacity (CEC), exchangeable bases, electrical conductivity (EC) and pH were determined by standard procedures (Pauwels et al., 1992). Soil pH was measured both in water and KCl (1:2.5 soil/water mixture) using a glass electrode pH meter. Part of the soil was ball-milled for organic carbon (OC) (Walky and Black method) and Kjeldahl-N as largely described by Pauwels et al. (1992). Available P was determined by Bray II method. Exchangeable cations were extracted using 1 N ammonium acetate at pH 7. Potassium (K) and sodium (Na) in the extract were determined using flame photometer and magnesium (Mg) and calcium (Ca) were determined by complexiometric titration. Exchangeable acidity was extracted with 1 M KCl followed by quantification of Al and H by titration (Pauwels et al., 1992). Effective cation exchange capacity (ECEC) was determined as sum of bases and exchanged acidity. Apparent CEC (CEC at pH7) was determined directly as outlined by Pauwels et al. (1992). Based on critical values of nutrients established for vegetables, nutrients were declared sufficient or deficient.

Statistical analysis

The data were subjected to statistical analysis using Microsoft Excel 2007 and SPSS statistical package 20.0. Soil properties were assessed for their variability using coefficient of variation (CV) and compared with variability classes (Tables 1 and 2, respectively).

$$CV\% = \frac{Sd\, X100}{\overline{X}}$$

Where: Sd = standard deviation; = $\overline{X}$ arithmetic mean of soil properties.

Table 1. Grouping coefficient of variation into variability classes.

CV (%)	Variability grouping (class)
< 15	Slightly variable
15-35	Moderately variable
>35	Highly variable

Table 2. Critical values of nutrients and soil properties.

Properties	Critical values				
	Very low	Low	Medium	High	Very high
OM (%)	<1	1-2	2-4.2	4.2-6	>6
Total N (%)	< 0.5	0.5-1.25	1.25-2.25	2.25-3.0	>3.0
C/N	<10 = good, 10-14 = medium and >14 = poor				
Ca (cmol/kg)	<2	2-5	5-10	10-20	>20
Mg (cmol/kg)	<0.5	0.5-1.5	1.5-3	3-8	>8
K (cmol/kg)	<0.1	0.1-0.3	0.3-0.6	0.6-1.2	>1.2
Na (cmol/kg)	<0.1	0.1-0.3	0.3-0.7	0.7-2.0	>2
Bray 2–P (mg/kg)	<7	< 7-16	16-46	>46	
pH	5.3 - 6.0 moderately acid, 6.0-7.0 = slightly acid, 7.0-8.5 = moderately alkaline				
ESP (%)		<2	2-8	8-15	15-27
CEC7 (cmol/kg)	0-20	21-40	41-60	61-80	81-100

Adapted from Beernaert and Bitondo (1992).

In order to identify the factors causing variation in the soil properties, principal component analysis (PCA) were applied. Varimax rotation was applied to do away with the problem of autocorrelation and to reduce the contributing soil factors to orthogonal principal components (Phil-Eze, 2010). The coefficients of the principal components are the Eigen values. Analysis of correlation coefficients and coefficient of variations were also used to identify soil factors that correlate significantly and differ, respectively.

RESULTS AND DISCUSSION

Physico-chemical properties of surface soil samples collected from the wetland gardens of Bamenda municipality

The results of the physical and chemical properties of the soils vary considerably (Tables 3 and 4). Ninety percent of the soils in the wetlands have a sandy loam texture while 10% are of the sandy clay-loam textural class (Table 3). The clay contents of the soils ranged from 10 to 21% with an average of 16.7% (Table 5). According to Mengel and Kirkby (1987), sites with high percentage of clay and silt are recommended for agricultural practices as they are capable of providing good aeration and retention and therefore supply nutrients and water. However, these soils were poor in such parameters, predicting low agronomic potentials. The soils of the area vary from acidic, through moderately acidic to slightly acidic. Average soil pH (H_2O) was 5.3 and 4.6 for pH (KCl). Generally, pH (KCl) ranged from 4.0 to 5.4 and was lower than those of pH water which ranged from 4.3 to 6.3. The variation of ∆pH (pH (KCl) – pH (H_2O)) was negative throughout. This indicates that the net charge on the exchange complex is negative, and thus exhibit cation exchange capacity. However, according to Yerima and Van Ranst (2005a), some tropical soils due to intensive rainfalls and weathering are dominated by positive charges with anion exchange capacity predominant. Percent organic carbon ranged from 3.21 (Mile 4 market area) to 13.63% (Mulang 4 near houses) with an average value of 8.19 % in the entire area. The organic matter, according to critical values by Beernaert and Bitondo (1992) (Table 2), varied from high to very high values, with a range from 5.67 to 23.50%. It had a weak positive correlation ($r = 0.218$; $p>0.05$) with the clay fraction of soil. This is an indication that the distribution of organic matter in the soil is not influenced by clay. This variation might be attributed to the constant addition of organic matter from varying anthropogenic activities such as the application of poultry manure, and municipal wastes, which showed significant differences of physico-chemical parameters to those of soils at the 1% probability level (Table 4) and varying levels of stratification due to seasonal flooding. In the tropics, soil organic matter is central to sustaining soil fertility on smallholder farms (Swift and Woomer, 1993; Woomer et al., 1994). In low-input agricultural

Table 3. Physical properties of surface soil samples within the wetlands of the Bamenda Municipality.

S/N	Site	Code	Latitude	Longitude	Sand (%)	Silt (%)	Clay (%)	Texture
1	Fuwambi Near Ntasen	MSS1	5 59 18.497N	10 9 53.924E	56	24	20	Sandy loam
2	Fuwambi near GTTC	MSS2	5 59 19.308N	10 9 55.389E	58	24	18	Sandy loam
3	Slap 1	MSS3	5 59 21.014N	10 10 20.174E	58	21	21	Sandy loam
4	Slap 2	MSS4	5 59 258.273N	10 10 22.948E	57	22	21	Sandy clay loam
5	Slap 3	MSS5	5 59 19.239 N	10 10 27.781E	58	20	20	Sandy loam
6	Slap 4	MSS6	5 59 17.017N	10 10 31.386E	55	26	19	Sandy loam
7	Slap 5	MSS7	5 59 20.336N	10 10 32.531E	56	24	20	Sandy loam
8	Mile 4 market	MSS8	5 59 39.144N	10 11 3,953E	57	23	20	Sandy loam
9	Foncha right of road	FSS1	5 58 53.253N	10 9 27.820E	63	25	12	Sandy loam
10	Foncha left of road	FSS2	5 58 49.082N	10 9 29.438E	64	26	10	Sandy loam
11	Ndamukong	FSS3	5 58 20.420N	10 9 34.060E	63	26	11	Sandy loam
12	Ntahkah inn	NSS1	5 58 59.017N	10 8 56.579E	63	18	19	Sandy loam
13	Ntahkah out	NSS2	5 58 59.010N	10 8 58.270E	61	19	20	Sandy clay loam
14	Ntahkah before bridge	NSS3	5 58 57.143N	10 9 5.063E	62	18	20	Sandy loam
15	Mulang council junction	MUS1	5 58 43.706N	10 8 44.482E	79	10	11	Sandy loam
16	Mulang left of road	MUS2	5 58 10.771N	10 8 46.882E	68	17	15	Sandy loam
17	Mulang middle	MUS3	5 58 35.424N	10 8 50.221E	80	10	10	Sandy loam
18	Mulang 4 near houses	MUS4	5 58 26.165N	10 8 56.023E	62	20	18	Sandy loam
19	Army Rescue	MUS5	5 58 19.239N	10 9 7.228E	62	18	20	Sandy loam
20	Ngomegham	MUS6	5 59 10.965N	10 8 26.001E	80	10	10	Sandy loam
21	Mbelewa	MBSS	6 0 33.580N	10 10.250E	67	18	15	Sandy loam

systems in the tropics, it helps retain mineral nutrients (N, S, micronutrients) in the soil and make them available to plants in small amounts over many years as it is mineralized. In addition, soil organic carbon increases soil flora and fauna (associated with soil aggregation, improved infiltration of water and reduced soil erosion), complexes toxic Al and manganese (Mn) ions (leading to better rooting), increases the buffering capacity on low-activity clay soils, and increases water-holding capacity (Woomer et al., 1994). Continuous cropping, with its associated tillage practices, provokes an initial rapid decline in soil organic matter, which then stabilizes at a low level (Woomer et al., 1994).

Total N ranged from 0.3 to 0.8% (Table 5). Landon (1991) reported that for tropical soils, total nitrogen content of 0.13% is sufficient. Nitrogen is highly mobile and easily lost and vegetables need high quantities. This necessitates high application of nitrogen fertilizers to maintain the production of vegetables in the wetlands which are already vulnerable given that they are dominated by the sandy fraction. The C/N ratio varying from 5.58 to 36.03 indicated that, the soils range from good to poor. Despite the fact that the soil are rich in organic carbon, the very high C/N ratio witnessed in some areas indicate difficulties in mineralization which could be ascribed to water stagnation. Likewise, farm specific practices might have also influenced the inconsistent pattern in mineralization. Areas characterized by rapid mineralization would result in high nitrogen losses which necessitate high nitrogen fertilization, a constraint to peasant farmers caused by poverty. Generally, a majority of the soils had C/N ratios less than 25. According to Mengel and Kirkby (1987), soils with C/N ratios less than 25 ameliorate soil properties and the organic materials of the mull type.

Available P was associated with organic carbon. It ranged from 8 to 76 mg/kg. Available P concentrations lower than 16 mg/kg in soils are considered low to ensure adequate phosphate supply to most plants (Landon, 1991). The availability of phosphorus might also be limited due to the nature of the parent material that is generally granitic (Kometa, 2013), high phosphorus sorption (Coulombe et al., 1996; Tening et al., 2013), and low pH values observed in these soils.

Calcium and Mg dominate the exchange complex but their concentrations were low ranging from 0.20 to 0.54 cmol/kg for Ca (with a variance of 0.015) and 0.37 to 0.68 cmol/kg for Mg (with a variance of 0.011). Accordingto Landon (1991), deficiencies of Ca are normal in soils with pH ≤ 5.5 which have been obtained in some sites of this study. Continues cultivation without returning residues to soil depletes these nutrients. Major sources of Mg in soils include amphiboles, olivine, pyroxene, dolomites and phyllosilicate clay minerals (Todd, 1980). The low values of Mg in the soils of the study area could be an indication that the aforementioned minerals are not present in substantial amounts. Calcium and Mg showed a highly significant positive correlation

Table 4. Chemical properties of surface soil samples, poultry manure and municipal waste within the wetlands of the Bamenda Municipality.

Code	pH (1:2.5)		EC (uS/cm)	OM	Tot. N	C/N()	Av. P (mg/kg)	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	ΣBases	Exch. Acid	Al^{3+}	H^{+}	CECsoil	CECclay	ECEC	B.S.ECEC (%)
	H_2O	KCl		%				cmol(+)/kg					cmol(+)/kg			cmol(-)/kg			
MSS1	4.6	4.3	235	10.13	0.8	7.34	18	0.31	0.56	0.03	0.12	1.02	0.45	0.04	0.41	32.30	60.21	1.47	69.39
MSS2	5.6	4.7	212	12.96	0.6	12.53	8	0.20	0.37	0.04	0.13	0.73	0.09	ND	ND	33.93	44.44	0.82	89.02
MSS3	4.5	4.3	230	18.23	0.6	17.63	14	0.31	0.46	0.05	0.12	0.94	0.4	ND	ND	37.62	5.49	1.34	74.15
MSS4	6.3	5.2	197	16.21	0.7	13.43	12	0.20	0.56	0.04	0.13	0.92	0.01	ND	ND	35.98	16.98	0.93	98.92
MSS5	5.3	4.0	237	18.64	0.3	36.03	22	0.20	0.52	0.04	0.13	0.88	0.4	ND	ND	38.04	3.81	1.26	69.84
MSS6	4.5	4.1	247	16.21	0.6	15.67	33	0.31	0.37	0.06	0.15	0.89	0.47	0.05	0.42	34.34	10.14	1.36	65.44
MSS7	4.3	4.0	248	12.96	0.3	25.07	17	0.54	0.6	0.04	0.14	1.32	0.49	0.05	0.44	35.77	49.21	1.89	69.84
MSS8	5.2	4.7	233	5.67	0.5	6.58	19	0.31	0.56	0.04	0.13	1.04	0.1	0.04	0.06	32.60	106.26	1.14	91.23
FSS1	6.3	4.7	113	14.18	0.3	27.42	22	0.54	0.68	0.04	0.13	1.39	0.01	ND	ND	34.44	50.67	1.40	99.29
FSS2	5.7	4.5	105	17.42	0.4	25.26	17	0.20	0.37	0.03	0.13	0.72	0.06	ND	ND	35.46	6.22	0.78	92.31
FSS3	6.0	4.8	167	8.10	0.7	6.71	13	0.31	0.56	0.04	0.13	1.04	0.09	ND	ND	33.93	161.11	1.13	89.38
NSS1	5.7	4.5	78	14.18	0.6	13.71	13	0.54	0.46	0.04	0.13	1.17	0.06	ND	ND	34.13	30.38	1.23	95.12
NSS2	5.6	4.8	127	7.70	0.7	6.38	11	0.31	0.37	0.04	0.13	0.85	0.09	ND	ND	34.34	94.71	0.94	90.43
NSS3	5.1	4.5	130	18.23	0.3	35.25	20	0.54	0.68	0.04	0.13	1.39	0.3	0.03	0.27	37.74	6.38	1.42	97.89
MUS1	5.4	5.0	458	11.34	0.4	16.45	76	0.31	0.44	0.04	0.13	0.92	0.09	0.02	0.07	34.54	107.77	1.01	91.09
MUS2	5.8	5.4	279	16.21	0.6	15.67	25	0.20	0.56	0.02	0.13	0.90	0.05	ND	ND	34.24	12.16	0.95	94.74
MUS3	4.9	4.3	437	7.70	0.8	5.58	17	0.54	0.46	0.04	0.13	1.17	0.3	0.03	0.27	32.39	169.96	1.47	79.59
MUS4	4.6	4.1	273	23.50	0.6	22.72	16	0.31	0.66	0.04	0.13	1.14	0.4	0.04	0.36	48.99	11.05	1.54	74.03
MUS5	5.4	4.9	269	15.80	0.6	15.28	8	0.31	0.56	0.05	0.12	1.04	0.10	0.03	0.07	39.06	10.61	1.14	91.23
MUS6	6.0	5.1	140	11.75	0.5	13.63	20	0.31	0.37	0.04	0.12	0.84	0.01	ND	ND	47.00	116.59	0.85	98.92
MBSS	4.5	4.2	57	19.45	0.7	16.11	12	0.31	0.4	0.07	0.12	0.87	0.40	0.04	0.36	29.96	1.72	1.27	68.50
Poultry manure	7.4	7.1	1500	40.51	29.00	0.81	130	24960.00	8043.3	13442.00	1329.00	47774.3	0.002	ND	ND	50.32	ND	47774.302	100.00
Municipal waste	7.6	5.5	5201	82.24	17.20	2.77	112	21961.00	7698.0	13711.00	14213.00	57583.0	0.004	ND	ND	44.43	ND	57583.00	100.00

(r = 0.869 and r = 0.780) with exchangeable bases at the 1% probability level (Table 6). The Al concentration ranged from 0.02 to 0.05 cmol/kg. In the exchangeable acidity, it concentrations were significantly (r = 0.710) lower than that of H, at the 5% probability level. This is an indication that the sources of charges on the exchange colloids displaced by neutral salts are pH dependent. The CEC of the soil according to the critical values of soil nutrients varied from low to medium, ranging from 29.96 to 48.99 cmol/kg of soil with a standard deviation of 4.523. These soils could thus have a limited amount of weatherable minerals warranting nutrient application when extensively cultivated. It was reported that, ECEC values of 4 meq/100g of soil marks the minimum limits (FAO, 2006). These soils thus have moderate potential to hold nutrients against leaching losses. It is thus imperative to raise the pH of the soils in areas of low pH in order to increase this potential.

Base saturation ECEC for the soils ranged from 65.44% at Slap 4 to 99.29% at the right side of the road of Foncha Street. This parameter is a good indicator of soil fertility in terms of the availability of nutrient elements. However, an ideal soil should have the exchange complex saturated in the ratio of 76/18/6 by the elements Ca/Mg/K, respectively (Yerima and Van Ranst, 2005a). In this study, Ca, Mg, and K occupied,

Table 5. Descriptive statistics of surface soil samples from the Wetland gardens of Bamenda Municipality.

Variable	Descriptive statistics							
	N	Range	Minimum	Maximum	Mean	Std. Error	Std. deviation	Variance
Sand (%)	21	25.00	55.00	80.00	63.2857	1.68345	7.71455	59.514
Silt (%)	21	16.00	10.00	26.00	19.9524	1.11372	5.10369	26.048
Clay (%)	21	11.00	10.00	21.00	16.6667	0.91894	4.21110	17.733
pH H_2O	21	2.00	4.30	6.30	5.3000	0.13680	0.62690	0.393
pH KCl	21	1.40	4.00	5.40	4.5952	0.08521	0.39048	0.152
EC (uS/cm)	21	401.00	57.00	458.00	212.9524	22.44756	102.86762	10581.748
OM (%)	21	17.83	5.67	23.50	14.1224	0.99936	4.57965	20.973
N (%)	21	0.50	0.30	0.80	0.5524	0.03560	0.16315	0.027
CN	21	30.45	5.58	36.03	16.8786	1.94166	8.89779	79.171
Av.P (mg/kg)	21	68.00	8.00	76.00	19.6667	3.09172	14.16804	200.733
Ca^{2+} (cmol(+)/kg)	21	0.34	0.20	0.54	0.3386	0.02707	0.12407	0.015
Mg^{2+} (cmol(+)/kg)	21	0.31	0.37	0.68	0.5033	0.02315	0.10608	0.011
K^{+} (cmol(+)/kg)	21	0.05	0.02	0.07	0.0414	0.00221	0.01014	0.000
Na^{+} (cmol(+)/kg)	21	0.03	0.12	0.15	0.1290	0.00153	0.00700	0.000
ΣBases (cmol(+)/kg)	21	0.67	0.72	1.39	1.0086	0.04245	0.19453	0.038
Acidity (cmol(+)/kg)	21	0.48	0.01	0.49	0.2081	0.03893	0.17840	0.032
Al^{3+} (cmol(+)/kg)	10	0.03	0.02	0.05	0.0370	0.00300	0.00949	0.000
H^{+}	10	0.38	0.06	0.44	0.2730	0.04844	0.15319	0.023
CECsol (cmol(+)/kg)	21	19.03	29.96	48.99	36.0381	0.98710	4.52347	20.462
CECclay (cmol(+)/kg)	21	168.24	1.72	169.96	51.2319	11.71137	53.66824	2880.280
ECEC (cmol(+)/kg)	21	19.03	30.36	49.39	36.2462	0.98819	4.52846	20.507
BSECEC (cmol(+)/kg)	21	23.85	65.44	99.29	85.25	0.12999	0.59570	0.355
Valid N (listwise)	10							

respectively 38.33, 56.98 and 4.68% on the absorption complex, necessitating amendments with Ca 1.7894 cmol/kg, Mg 0 cmol/kg and K, 0.1266 cmol/kg. CEC clay was not directly determined but was calculated from CEC soil. It ranged from 3.2 to 7.5 cmol/kg. This indicates that the mineralogy of the soil is dominated by low activity clays of the 1:1 type and sesquioxides transported from the weathering granitic uplands. The Na content was fairly constant throughout the area with 1.3 value.

Variability of surface soil properties in the wetlands of Bamenda municipality

Table 7 shows the mean physico-chemical properties of surface soil in the three major sites assessed. These soils were evaluated for their variability using coefficient of variation (CV %). Tabi and Ogunkule (2007) have similarly used the thresholds of CV presented in Table 1 to group variability in soil properties. According to Ogunkule (1993), Tabi and Ogunkunle (2007), Tabi et al. (2012), CV values ranging from 0-15% are considered slightly variable, 15-35% moderately variable, while > 35% they are considered highly variable (Table 8). Soil pH (H2O or KCl), percent sand, CEC soil, and Na were consistently slightly variable. The variability of soil pH is similar to those reported by Tabi and Ogunkunle (2007) for Alfisols in Southern Nigeria, Tabi et al. (2012) for vertisols under rice cultivation in the Logone flood plain of Nothern Cameroon and Ndukwu et al. (2013) for soils affected by animal wastes in Uyo, Akwa Ibom State of Nigeria. Soil pH is a very important parameter that influences many physico-chemical properties of soils including the availability of nutrients. Despite the fact that the variability reported for pH is small, minor changes in pH units have significant effects on nutrient availability. Silt, clays, EC, ECEC, Al^{3+}, were slightly moderately variable. However, they were dominated by moderately variable class. This could be attributed to variation in levels of alluvial materials received. In addition, variation in chrono sequences of materials that have been subjected to different intensities of weathering could have a significant effect on these physical parameters. All these factors have significant implications on water and nutrient management of the wetlands of Bamenda municipality. In a majority of the farms, the exchangeable bases were moderately variable. The moderate variability of these essential nutrients could be linked to variation in soils amendment activities by farmers.

Table 6. Pearson correlation coefficients for some physico-chemical parameters of soil profiles in wetlands of Bamenda Municipality.

	Sand	Silt	Clay	pH H_2O	pH KCl	EC	OM	N	CN	AV.P	Ca	Mg	K	Na	ΣBases	Acidity	Al	H	CECsol	CECclay	ECEC	BSCEC	BSECEC
Salt	1																						
Silt	-0.850**	1																					
Clay	-0.785**	0.346	1																				
pH H_2O	0.227	-0.042	-0.366	1																			
pH KCl	0.361	-0.299	-0.263	0.743**	1																		
EC	0.336	-0.418	-0.114	-0.266	0.056	1																	
OM	-0.200	0.102	0.219	-0.226	-0.204	-0.207	1																
N	0.051	-0.081	0.041	-0.098	0.138	0.101	-0.258	1															
CN	-0.170	0.125	0.108	-0.074	-0.320	-0.187	**0.687****	**-0.819****	1														
Av.P	0.428	-0.378	-0.330	-0.028	0.142	**0.557****	-0.083	-0.332	0.116	1													
Ca	0.157	-0.167	-0.058	-0.185	-0.325	-0.038	-0.173	-0.218	0.144	-0.016	1												
Mg	-0.262	0.197	0.238	0.008	0.039	0.063	0.184	-0.297	0.383	-0.083	0.375	1											
K	-0.095	0.011	0.164	-0.425	-0.427	-0.179	0.242	0.134	-0.011	-0.070	0.097	-0.279	1										
Na	-0.282	0.348	0.090	-0.125	-0.258	0.164	-0.027	-0.260	0.162	0.254	0.165	-0.002	0.020	1									
ΣBases	-0.058	0.013	0.107	-0.139	-0.215	0.020	-0.013	-0.308	0.301	-0.044	**0.869****	**0.780****	-0.050	0.149	1								
Acidity	-0.329	0.157	0.387	-0.919**	-0.834**	0.185	0.331	0.028	0.254	-0.026	0.169	0.108	0.388	0.195	0.192	1							
Al	-0.768**	0.866**	0.565	-0.827**	-0.789**	-0.468	0.149	0.051	0.020	-0.458	-0.024	-0.025	0.248	0.497	0.007	0.739*	1						
H	-0.038	0.488	0.204	-0.950**	-0.913**	-0.363	0.375	0.190	0.228	-0.321	0.242	-0.015	0.132	0.338	0.160	0.999**	0.710*	1					
CECsol	0.154	0-.243	0.002	0.033	0.159	0.025	0.432	-0.228	0.321	-0.054	-0.092	0.192	-0.099	-0.149	0.047	0.008	-0.019	0.030	1				
CECclay	0.518*	0-.334	-0.523*	0.213	0.208	0.338	**-0.852****	0.266	**-0.606****	0.178	0.230	-0.130	-0.186	-0.030	0.080	-0.277	-0.373	-0.355	-0.142	1			
ECEC	0.141	-0.236	0.017	-0.003	0.126	0.032	0.445*	-0.226	0.331	-0.055	-0.086	0.196	-0.084	-0.141	0.054	0.048	0.003	0.059	0.999**	-0.153	1		
BSCEC	-0.085	0.102	0.053	-0.136	-0.269	0.003	-0.262	-0.135	0.070	-0.018	**0.834****	**0.584****	0.009	0.179	0.866**	0.161	0.042	0.154	-0.450*	0.190	-0.443*	1	
BS ECEC	-0.080	0.100	0.046	-0.114	-0.251	-0.005	-0.266	-0.140	0.069	-0.018	0.833**	0.585**	0.003	0.177	0.866**	0.138	0.026	0.127	-0.449*	0.193	-0.443*	1.000**	1

*, **Significant at 5 and 1% level, respectively.

Table 7. Physico-chemical properties of surface soil properties at the Mile Four, Foncha and Mulang sites of the Bamenda Municipality.

Site	Sand	Silt	Clay	H_2O	KCl	ECuS/cm	OM	Tot N	C/N	Av. P	Ca^{2+}	Mg^{2+}	K^+	Na^+	ΣBases	Exch. Acid.	Al^{3+}	H^+	CECsoil	CECclay	ECEC	B.S. ECEC
		%		pH (1:2.5)			%			mg/Kg			cmol(+)/Kg				cmol(+)/Kg			_cmol(+)/Kg		%
Mile Four site	57	23	20	5.04	4.41	230	13.88	0.55	16.8	18	0.30	0.50	0.04	0.1305	0.97	0.3013	0.045	0.333	35.07	37.07	1.28	76.56
Foncha site	63	22	15	5.73	4.63	120	13.30	0.50	19.1	16	0.41	0.52	0.04	0.1265	1.09	0.1017	0.030	0.270	35.01	58.25	1.19	91.60
Mulang site	72	14	14	5.35	4.87	309	14.38	0.58	14.9	27	0.33	0.51	0.04	0.1239	1.00	0.1583	0.030	0.193	39.37	71.36	1.15	86.96

The moderate variability of these bases implies that, for proper management of the farms, a unique policy for the area is insufficient to meet the desires of the farmers such as the application of organic and/or inorganic fertilizers. Organic matter, available P, exchange acidity, CEC clay, BSECEC, H^+, Tot. N, were moderately to highly variable. Most of these parameters owe a lot of their origin to organic materials. Natural denudation inclusive, urban swept off, farming activities such as residue management, crop species and land preparation/ management practices could have influenced the nature and management of soil properties of the area. The available phosphorus and base saturation which is equally slightly variable reflects pH variability.

Sources of variation of soil properties

The correlation coefficients (Table 6) were used to group various soil properties at each site. The coefficients ranged between -0.950 and 0.999. Even though some ofthe correlations were highly significant (p<0.01), most of them had correlation values less than 0.500. The highest positive

Table 8. Variability classes of soil properties for the different sites.

Site	Least variable (CV< 15%)	Moderately variable (15 < CV≤ 35%	Highly variable CV> 35%)
Mile Four site	Sand, silt, clay pH H_2O, pHKCl, EC, Na^+, Al^{3+}, CECsoil, ECEC	OM, Tot.N, Ca^{2+}, Mg^{2+}, K^+, ΣBases, BSECEC.	C/N, Av. P, Exch.acodity, H^+, CECclay.
Foncha site	Sand, pH H_2O, pH KCl, K^+, Na^+, CECsoil, ECEC.	Silt, clay, EC, OM, Av.P, Mg^{2+}, ΣBases, BSECEC.	Tot. N, C/N, Ca^{2+}, Exch. Acidity, ECECclay
Mulang site	Sand, Ph H_2O, pHKCl, Na^+	Silt, Clay, Tot. N, Ca^{2+}, Mg^{2+}, K^+, Na^+, ΣBases, Al^{3+}, CEC sol, B	EC, OM, C/N, Av.P, Exch. Acidity, H^+, CECclay, BSCEC

correlation coefficients were observed between ECEC and CEC soil (0.999), exchangeable acidity and H^+ (0.999), exchangeable acidity and Al (0.739), BSCEC and exchangeable bases (0.866), Ca and BSCEC (0.834), Mg and exchangeable bases (0.585), OM and CN (0.687) all at the ninety-nine percent confidence interval. The highest negative correlation coefficients were obtained between pH, H_2O and H^+ (-0.950), pH H_2O and Al (-0.827), and pH H_2O and acidity (-0.919) which was significant at the 1% probability level. Similarly at the 1% probability level, sand equally had a negative correlation with Al content of the soils (r = -0.768). The negative correlation could be attributed to the fact that as the soil becomes sandier, very few colloids are left in place for retention of Al which is toxic to the plants.

Simple linear regressions establish functional relationships between variables. In the wetlands of Bamenda municipality, except for organic matter and CEC that showed a definite functional relationship, despite the correlations, the clay content and CEC, total N and organic matter, and total N and available P did not show a definite functional linear relationships (Figure 2). This is an indication that the high correlations witnessed amongst these variables are influenced by a multiplicity of factors. These results strongly contrast those of Yerima et al. (2009) on inceptisols of the Bale Mountain area of Ethopia. These differences could be attributed to the fact that soils of the wetlands of Bamenda municipality are fluvisols with short-range variability. This is in agreement with reports by FAO-ISRIC-ISSS (1998) and Yerima and Van Ranst (2005b) who have suggested the irregular distribution of organic matter and other parameters in fluvisols. The inexistence of definite trends in these urban and peri-urban soils could be ascribed to the continuous excavation activities and/or seasonal deposition of run-off sediments and urban wastes. The data of 22 variables considered in this study were also subjected to R-mode factorial analysis using the six-factor model, which accounted for 96.24% of the total data variance. The resulting varimax is summarized in Table 9. The computations were performed with the SPSS computer software package version 20.0. Only variables with loadings > 0.5 were considered significant. Contributions of the various principal components (PCI) were 20.9 (PC1), 18.7 (PC2), 17.1 (PC3), 16.6 (PC4), 14.98 (PC5) and 8.02% (PC6). The 6 PCs identified constitute the minimum dataset required to group fluvisols for vegetable cultivation in the wetlands of Bamenda municipality. The factors (PCI) extracted from Table 9 are as follows (Table 10):

Factor 1 is made up of pH H_2O, pH KCl, exchangeable acidity, Al and H. This factor is an acidity factor because of the high negative loading (- 0.949) for pH water and (- 0.949) pH KCl, and high positive loading of exchangeable acidity (0.945), H^+ (0.942) and Al^{3+} (0.738). Acidity of this environment is highly influenced by pH dependent charges as opposed to charges from isomorphous—substitution. The source of this acidity would be anthropogenic from the urban area.

Factor 2 constitutes, BSECEC, ΣBases, Ca and Mg. The principal component was named the base status factor because it had a high positive loading on base saturation ECEC (0.933), sum of bases (0.871), exchangeable Ca (0.916), and moderate loading of Mg (0.508).

This is consistent with observations for soils in a similar environment of the Logone and Chari plain in the northern region of Cameroon under rice cultivation (Tabi et al., 2012).

Factor 3 is composed of sand, silt, clay, Al and EC. This component was termed weathering and associated moisture retention factor because of a high positive loading of clay (0.950) greater than silt (0.901), moderate positive loading of Al (0.616) and a moderate negative loading of EC (-0.579).

Factor 4 grouped EC, OC, OM, C/N, K and CEC clay: the factor was ascribed an organic matter factor because of a high (same) positive

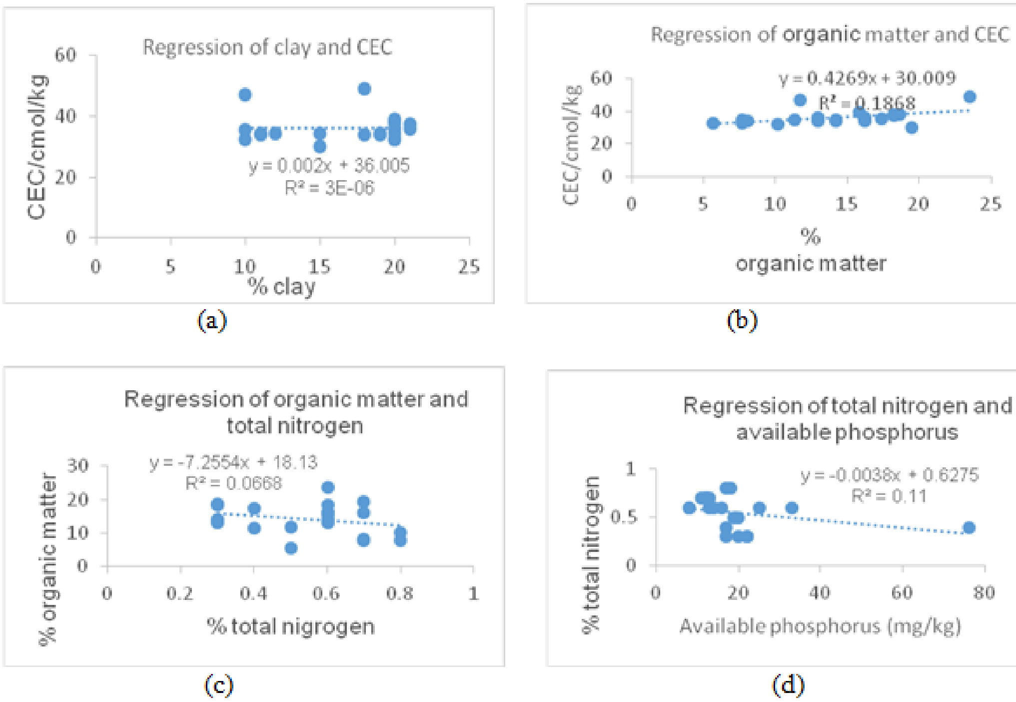

Figure 2. Simple linear regressions between (a) clay content of soils and CEC, (b) organic matter and CEC, (c) total nitrogen, organic matter, available phosphorus and total nitrogen of soils from the wetland gardens of Bamenda Municipality Cameroon.

Table 9. Total Variance explained from Physico-chemical properties of wetland soils in the wetland gardens of Bamenda Municipality.

Component	Total	Initial Eigenvalues		Extraction sums of squared loadings			Rotation sums of squared loadings		
		% of variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.501	31.254	31.254	7.501	31.254	31.254	5.015	20.896	20.896
2	4.933	20.553	51.807	4.933	20.553	51.807	4.477	18.655	39.550
3	4.437	18.486	70.293	4.437	18.486	70.293	4.110	17.125	56.675
4	2.536	10.567	80.860	2.536	10.567	80.860	3.975	16.562	73.237
5	1.921	8.004	88.864	1.921	8.004	88.864	3.596	14.983	88.220
6	1.770	7.377	96.240	1.770	7.377	96.240	1.925	8.020	96.240
7	0.198	0.827	99.663						
8	0.081	0.337	100.000						
9	6.649E-016	2.770E-015	100.000						
10	4.939E-016	2.058E-015	100.000						
11	3.993E-016	1.664E-015	100.000						
12	3.097E-016	1.290E-015	100.000						
13	2.069E-016	8.621E-016	100.000						
14	1.530E-016	6.376E-016	100.000						
15	5.699E-017	2.375E-016	100.000						
16	4.010E-017	1.671E-016	100.000						
17	-5.979E-017	-2.491E-016	100.000						
18	-1.267E-016	-5.281E-016	100.000						
29	-1.565E-016	-6.519E-016	100.000						
20	-1.663E-016	-6.930E-016	100.000						
21	-2.446E-016	-1.019E-015	100.000						
22	-6.116E-016	-2.548E-015	100.000						

Extraction method: Principal Component Analysis Rotation method: Varimax with Kaiser normalization, Rotation converged in 10 interactions.

Table 10. Varimax Rotated of Factor Matrix (Six-Factor Model) of some physico-chemical properties of surface soil samples in the wetland gardens of the Bamenda Municipality.

	Component					
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**
Sand (%)	-0.263	0.031	-0.954	-0.080	-0.092	-0.031
Silt (%)	0.409	-0.116	0.901	0.017	0.008	0.078
Clay	0.033	0.092	0.950	0.162	0.204	-0.038
pH H2O	-0.949	-0.013	-0.181	-0.126	0.026	0.015
pH KCl	-0.931	-0.238	-0.155	-0.069	0.217	0.006
EC (uS/cm)	-0.172	-0.129	-0.579	-0.638	0.255	0.310
OM (%)	0.224	-0.210	-0.003	0.862	0.401	-0.040
Tot. N (%)	0.329	-0.452	-0.224	-0.296	-0.150	-0.690
CN	-0.002	0.428	0.098	0.732	0.359	0.357
Av. P(mg/kg)	-0.306	-0.258	-0.418	-0.141	-0.093	0.690
Ca^{2+} cmol(+)/kg)	0.223	0.916	-0.232	-0.019	0.059	0.119
Mg^{2+} (cmol(+)/kg)	-0.191	0.508	0.367	0.073	0.718	-0.166
K^{+} (cmol(+)/kg)	0.179	-0.390	-0.041	0.626	-0.540	-0.010
Na^{+} (cmol(+)/kg)	0.435	0.019	0.115	-0.078	0.014	0.818
ΣBases (cmol(+)/kg)	0.051	0.871	0.088	0.040	0.468	0.038
Exch. ACid(cmol(+)/kg)	0.945	0.092	0.148	0.178	0.047	-0.012
Al^{3+} (cmol(+)/kg)	0.738	-0.074	0.616	0.011	-0.076	0.099
H^{+} (cmol(+)/kg)	0.942	0.101	0.117	0.185	0.053	-0.018
CECsoil (cmol(+)/kg)	-0.031	-0.131	0.046	0.277	0.937	0.057
CECclay (cmol(+)/kg)	-0.141	0.193	-0.477	-0.811	-0.151	0.032
ECEC (cmol(+)/kg)	-0.003	-0.128	0.050	0.282	0.937	0.057
BSECEC (cmol(+)/kg)	0.088	0.933	0.032	-0.224	-0.259	-0.057

loading for organic matter content (0.862) and organic matter quality (C/N) (0.732), moderate positive loading from K (0.626); but a high negative loading from CECclay (-0.811). The major source of this component would be the decomposition of natural vegetation material in the area.

Factor 5 was composed of Mg, CECsoil, ECEC and K. The factor had high positive loading from CECsoil (0.937), ECEC (0.937), moderate loading from Mg (0.719) and a moderate negative loading from K (-0.540). This factor was described as a mineral neo-synthesis related component derived mainly from the deposition of Mg eroded from uplands and deposited in the wetland which gradually replaces K in interlayers of micaceous minerals in the wetlands to form expanding minerals such as vermiculites.

Factor 6 grouped N, available P and Na. This is a dispersal-mineralization factor influenced by anthropogenic activities of the International soap factory (high sodium released) and the cow slaughter house (high organic matter containing nitrogen which is rapidly mineralized due to dispersion caused by Na). The factor had high positive loading from Na (0.818), moderate loading from available phosphorus (0.690) but a negative yet moderate loading from total N (- 0.690). Potentially mineralizable nitrogen is an important measure of N supplying capacity in wetland soils, which is most usually not calculated. The findings of this component are in conformity with the reports of Mengel and Kirkby (1987) and Tabi et al. (2012) that nitrogen is one of the most important factors controlling potential mineralizable N in wetland soils. Generally, the results reported here agree with those of other authors. For example, Salami et al. (2011) identified potential fertility, available phosphorus, organic matter, acidity and sand- silt as factors responsible for soil fertility variation in the northern guinea savanna agro-ecological zone of Nigeria. Kosaki and Juo (1989) identified inherent fertility status (represented by Mg, Ca, K, sand, silt and clay) and available phosphorus as major factors causing soil variation in wetland fields in southwestern Nigeria. In fact, no sampling site was completely homogeneous in relation to soil management units

Conclusion

The fluvisols of the Wetlands of Bamenda municipality have been highly influenced by human activities. Ninety percent of the soils have a sandy loam texture while 10%

are of the sandy clay-loam textural class. The surface soils were slightly acidic to neutral with an average pH (H2O) of 5.3 and 4.6 for pH (KCl). The organic matter content varied from high to very high values, ranging from 5.67 to 23.50% but with a fast mineralization rate. Most of the soil properties were positively correlated but did not show definite linear relationships (except organic matter and CEC), depicting multiplicity of sources. The pH was slightly variable, while other soil properties were moderately to highly variable. Six principal components (PCs): base status, organic matter, weathering, acidity, Na dispersal and mineral neo-synthesis factors, explained 96.24% of the variations observed in the soil properties. They constitute a minimum dataset required to group soils in the Bamenda wetland gardens. It is recommended that a detailed mapping of soil properties be carried out for the establishment of a soil fertility map for Bamenda municipality; fertilizer recommendations should be established for the different soil management units and farming systems which favor buildup of soil organic matter and use of animal manure should be encouraged.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work is part of an ongoing Ph.D programme. The authors express their gratitude to Prof. Mbomi Elizabeth Saillieh for her contributions to the statistical analysis and the authorities of the Soil Science Department, University of Dschang for making this feasible.

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Vol. 11(2), pp. 87-99, 14 January, 2016
DOI: 10.5897/AJAR2015.9632
Article Number: 48E65FD56662
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effects of applications of lime, wood ash, manure and mineral P fertilizer on the inorganic P fractions and other selected soil chemical properties on acid soil of Farta District, Northwestern highland of Ethiopia

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Received 18 February, 2015; Accepted 9 October, 2015

An incubation study was conducted for two months to evaluate the effects of different treatments on the inorganic P fractions and other chemical properties on an acidic soil. Four rates of lime and wood ash (3.5, 7.0, 9.2, 11.2 tons $CaCO_3$ equivalent ha^{-1}) were separately applied to obtain target soil pH values of 5.5, 6.0, 6.5 and a lower pH obtained with half of the lime requirement needed to bring the pH to 5.5. Three rates of mineral P fertilizer and manure P (32.5, 65, 130 kg P ha^{-1}) were separately applied into the whole soil volume. Additionaly, wood ash (7 tons $CaCO_3$ ha^{-1}) and lime (7 tons $CaCO_3$ ha^{-1}) were combined separately with each of the manure and mineral P rates. The experiment was laid down in a completely randomized design with two replications. Lime and wood ash treatments showed a significant effect on the pH and exchangeable acidity compared to manure. The highest lime and wood ash rates (11.2 tons $CaCO_3$ ha^{-1}) increased the pH from 4.89 to 6.03 and 5.93; decreased the exchangeable acidity from 2.22 to 0.14 and 0.16 $cmol_c$ kg^{-1}, respectively. The maximum increment in Olsen P by 132%, aluminum bound P (Al-P) by 160%, iron bound P (Fe-P) by 22% and oxalate extractable P (P_{ox}) by 33% were observed by the application of mineral P at 130 kg P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}). Therefore, lime and wood ash applications in acidic soils can effectively ameliorate H^+ and Al^{3+} toxicity and P deficiency.

Key words: Acidity, incubation, lime requirement, P form.

INTRODUCTION

Acid soils limit crop production on 30 to 40% of the world's cultivated land and up to 70% of the world's potentially arable land (Haug, 1983). Different reports have also indicated that there is significant soil acidity coverage in Ethiopia (Wassie and Shiferaw, 2009). Hence, it is a serious threat to crop production in most

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highlands of Ethiopia and a major crop production constraint in the small scale farmers of the country.

The two fundamental factors limiting the fertility of acid soils are nutrient deficiencies such as P, Mo, Ca, Mg and K and the presence of phytotoxic substances such as soluble Al and Mn. The low P status of highly weathered acid soils is a particular problem because large amounts of P need to be applied in order to raise concentration of available soil P to an adequate level (Sanchez and Uehara, 1980). Low P status is partially attributable to continuously negative P balances and partially to inherent soil properties. These soils contain large quantities of Al and Fe hydrous oxides which have the ability to adsorb P onto their surfaces. Thus, much of the added P is fixed and is not readily available for crop use (Srivastava et al., 1969; Sharma et al., 1980; Tomar et al., 1984). Tekalign and Haque (1987), Lupwayi and Haque (1996) and Shiferaw (2004) indicated that most of the highland soils of Ethiopia are P deficient.

Soil P occurs as inorganic and organic forms and their relative distribution varies with climate, vegetation, parent materials and soil management practices (Fairhurst et al., 1999). Inorganic P forms are divided into two groups such as active forms which include Al-P, Fe-P and calcium bound P (Ca-P) and inactive forms which include occluded aluminium-iron bound P (occl-Al-Fe-P) and reductant soluble Fe bound P (reds-Fe-P) (Walker and Syers, 1976). The active inorganic P fractions are the most available forms to plants, with the degree of availability increasing in the order of Ca-P, Fe-P and Al-P under well drained conditions (Walker and Syers, 1976). Soil drainage condition is one of the factors that affect available soil P. For example, P availability will be higher in flooded soil because the flooding results in reducing conditions which change the previously insoluble ferric phosphate to more soluble ferrous phosphate (Fageria, 2009).

There are considerable evidence in literature suggesting that application of lime, wood ash, manure and mineral P fertilizers can be used for the control of acidity related problems and P deficiency in acid soils. Liming and application of phosphate fertilizers as organic or inorganic P forms have been suggested for the control of P deficiency problems in acid soils (Opara-Nadi, 1988).

In acid soils with high levels of exchangeable Al, organic matter (OM) plays a significant role in the reduction of P adsorption and increased P availability due to cumulative effects of several mechanisms (Erich et al., 2002; Opala et al., 2010). These include release of inorganic P from decaying residues, blockage of P adsorption sites by organic molecules released from the residues, a rise in soil pH and complexation of soluble Al and Fe by organic molecules (Iyamuremye and Dick, 1996).

Lime and wood ash applications increased pH of acidic soils and resulted in a decrease in P adsorption. This may be due to the increased concentration of hydroxyl ion (OH^-) that increase surface negative charge and leading to competition with P ions for adsorption sites and increased P availability (Fageria, 2007; Mweta et al., 2007; Kisinyo et al., 2013). In addition to its liming effect, wood ash contains P and can be used to alleviate P deficiencies better than lime (Adetunji, 1997; Nkana et al., 2002; Saarsalmi et al., 2006; Awodun et al., 2007; Insam et al., 2009).

Although much of the Ethiopian highlands have a high potential for crop production, almost no detailed work has been done on effect of different amendments on the status, forms and dynamics of P in the soils. Therefore, the objective of this study was to evaluate the effects of applications of lime, wood ash, manure and mineral P fertilizers on available P, inorganic P forms, oxalate extractable P (P_{ox}), degree of P saturation and other selected soil chemical properties on acid soil of Farta District, Northwestern Highlands of Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Gassay area of Farta District in South Gondar Zone of the ANRS (Figure 1). The District lies between 11° 32’ and 12° 03’ N latitude and 37° 31’ and 38° 43’ E longitude, and covers an estimated area of 1118 km^2 within the altitude range of 1900 to 4035 m above sea level (masl). In terms of topography, 45% of the total area is gentle slope, while flat and steep slope lands account for 29 and 26%, respectively.

According to the Regional Office of Planning for Northwestern Ethiopia (1985), geologically the study area is covered with thick trap series volcanic rocks which were erupted from fissures during the early and middle Tertiary and from Choke Shield volcanic mountain center during the Miocene and Pliocene. The trap volcanic series consists mainly of weathered and jointed basalt. The soils of the study area are developed from the parent materials of volcanic origin, predominantly Tertiary basalt. The majority of the soils in Farta District are Luvisols (FAO, 1981). The soils of the study area have not been classified according to U.S. Soil Taxonomy, but tentatively many of them are likely to fall in the Alfisols order. Luvisols are potentially suitable for a wide range of agricultural uses because of their favorable physical characteristics and moderate chemical fertility.

Considering land use, an estimated 64.7% of the area is cultivated for different annual and perennial crops, while areas under grazing and/or browsing, forests and shrubs, settlements, and wastelands account for about 10.2, 0.6, 7.8 and 16.7%, respectively. The natural vegetation in the study area consists of some tree species that are remnants of a once dense evergreen forest occurring on slopes and sparse grass complex in various areas. The dominant tree species in the area include *Juniperus procera, Olea africana and Hajenia abyssinica.* Currently, refilling or replantation strategy is being implemented in the study area (WOARD, 2005). The rural households are engaged primarily in crop-livestock mixed farming systems. Barley, wheat, teff, sorghum, maize, faba beans, peas and potatoes are dominant crops while chickpeas and some oil crops are also grown.

The average minimum, maximum and mean temperatures are 9.3, 22.3 and 15.8°C, respectively (Figure 2). The rainfall pattern is unimodal, stretching from May to September. The mean annual

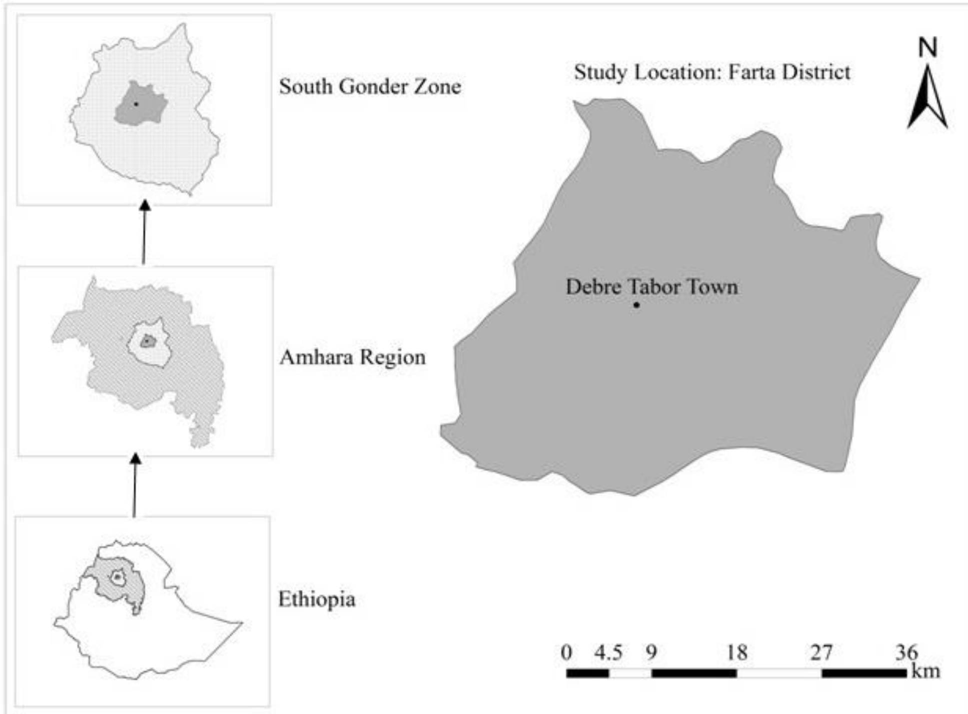

Figure 1. Location map of the study area.

rainfall is 1445 mm (NWRMSA, 2010).

Soil sample collection and analysis

A bulk soil sample was taken from the top soil (0 to 20 cm) from the very strong acidic soil of Gassay area in Farta District, Northwestern Highlands of Ethiopia. The soil was air dried, ground and passed through a 2 mm sieve and analyzed for selected chemical and physical properties according to standard laboratory procedures.

Analysis of soil physicochemical properties

Soil particle size distribution was analyzed by the Bouyoucos hydrometer method as described by Day (1965). Soil bulk density was measured from three undisturbed soil samples collected using a core sampler (2.5 cm radius and 5.0 cm height) as per the procedures described by Jamison et al. (1950).

Soil pH (1:2.5) was measured potentiometrically in water solutions using a combined glass electrode pH meter (Chopra and Kanwar, 1976). The potential CEC of the soil was determined from soil samples saturated with NH_4^+ at pH 7.0, subsequently replaced by K^+ from a percolated 1 M KCl solution. The excess salt was removed by washing with ethanol and the NH_4^+ that was displaced by K^+ was measured using the micro-Kjeldahl procedure (Chapman, 1965) and reported as CEC. Total exchangeable acidity was determined by saturating the soil samples with 1 M KCl solution and titrated with 0.02 M NaOH as described by Rowell (1994). From the same extract, exchangeable Al in the soil samples was determined by application of 1 M NaF which form a complex with Al and released NaOH and then NaOH was back titrated with a standard solution of 0.02 M HCl.

Total carbon, all assumed to be OC, and total N contents of the soil sample was determined using the Vario MAX elemental analyzer by the dry oxidation method. Available P was determined both by Olsen and Bray I methods. The analysis of P by Olsen method was carried out by shaking the soil samples with 0.5 M $NaHCO_3$ at nearly constant pH of 8.5 in 1:20 of soil to solution ratio for half an hour as described by Olsen et al. (1954). The Bray I P analysis was carried out by shaking the soil samples with an extracting solution of 0.03 M NH_4F in 0.1 M HCl for 1 min as described by Bray and Kurtz (1945). The inorganic P fractions were successively extracted with 1 M NH_4Cl, 0.5 M NH_4F, 0.1 M NaOH, 0.25 M H_2SO_4, 0.3 M Na-dithionite and Na-citrate bicarbonate solution, and 0.1 M NaOH to estimate easily soluble P, Al-P, Fe-P, Ca-P, reds-Fe-P and occl-Al-Fe-P, respectively, according to the procedure by Chang and Jackson (1957). The oxalate extractable P, Al and Fe (P_{ox}, Al_{ox} and Fe_{ox}) were extracted with 0.05 M ammonium oxalate ($(NH_4)_2C_2O_4.2H_2O$, pH 3.3) for two hours in the dark (Niskanen, 1989). The suspension was centrifuged and filtered through a Whatman No. 42 filter paper to get a clear solution. The extracts were analyzed for P, Al and Fe by inductively coupled plasma optical emission spectroscopy (ICP-OES). Dithionite citrate bicarbonate-extractable Fe and Al (Fe_d and Al_d) were determined by the method of Mehra and Jackson (1960). The extracts were analyzed for Al and Fe by ICP-OES.

The P sorption capacity (PSC, mmol kg^{-1}) of the soil was calculated as the sum of the concentrations of oxalate-extractable metals (Fe_{ox} and Al_{ox}, mmol kg^{-1}) according to Hartikainen et al. (2010) as:

$$PSC = Al_{ox} + Fe_{ox}$$

The degree of P saturation (DPS, expressed as %) was determined as the percentage of the ratio of the oxalate extractable P (P_{ox}, mmol kg^{-1}) to the sorption capacity as follows:

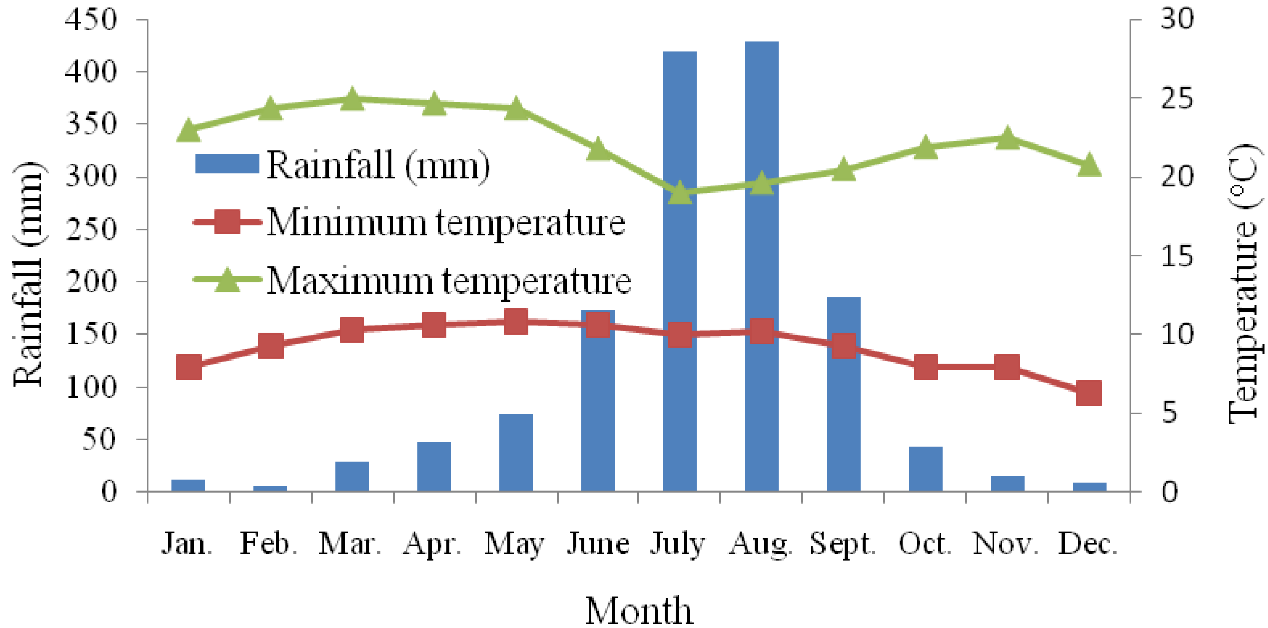

Figure 2. Mean monthly rainfall and mean monthly maximum and minimum temperatures of the study area.

$DPS = (P_{ox}/PSC) \times 100\%$

DPS was also calculated separately for Al_{ox} and Fe_{ox} as:

$DPS\ Al_{ox}\text{-}P = (Al\text{-}P/Al_{ox}) \times 100\%$

$DPS\ Fe_{ox}\text{-}P = (Fe\text{-}P/Fe_{ox}) \times 100\%$

Analysis of the composition of manure, wood ash and lime

Total contents of P, Ca, Mg, K, Na, Al and zinc (Zn) in the cow dung manure and wood ash from the wood of *Eucalyptus* tree were measured in suspensions obtained after ashing at 500°C, dissolution with 0.2 M HCl and digestion on a hot plate. Phosphorus was determined by the vanado-molybdate colorometric procedure using spectrophotometer at 460 ηm wavelength based on standard laboratory procedure. Calcium, Mg, K, Na, Al and Zn were determined with ICP-OES. The total N contents of the manure and the wood ash samples were determined by the micro-Kjeldhal method as described by Jackson (1967).

The pH and EC of the manure and wood ash were measured in water (sample: water ratio of 1:5) using a pH meter and conductivity meter, respectively. The calcium carbonate equivalents (CCE) of the wood ash and the Dejen lime were determined by dissolving the wood ash and the lime using excess of standard 0.5 M HCl and followed by gentle boiling. After filtration, the excess HCl was back titrated with standard 0.1 M NaOH solution using phenolphthalein as an indicator. From the amount of NaOH used to neutralize the excess acid of the blank and the filtrate, the CCE values of both the wood ash and the lime were calculated.

Incubation study

The incubation study was conducted for two months as described below. The composite soil sample was air dried ground and passed with 2 mm sieve and then 0.5 kg soil was placed in plastic pot and mixed with different treatments in a greenhouse. Four rates of lime and wood ash (3.5, 7.0, 9.2 and 11.2 tons $CaCO_3$ equivalent ha^{-1}) were separately applied to obtain target pH values of 5.5, 6.0, 6.5 and a lower pH obtained with half of the lime requirement needed to bring the pH to 5.5. The lime and the wood ash used for this particular study had a CCE of 94.6 and 71.7%, respectively. Hence, the actual field application rates of lime were 0, 3.69, 7.37, 9.69 and 12.22 tons lime ha^{-1}; the wood ash rates were 0, 4.88, 9.67, 12.83 and 16.18 tons wood ash ha^{-1}. The rates of wood ash expressed based on its P content were 61, 121, 159 and 194 kg P ha^{-1} (23, 46, 61 and 74 mg P kg^{-1} soil).

Three rates of manure P (12.5, 25.0 and 50.0 mg P kg^{-1}) or (32.5, 65.0 and 130 kg P ha^{-1}), based on its total P content, were applied uniformly to the whole soil volume. The actual field application rates of manure were 3.414, 6.828 and 13.655 tons manure ha^{-1}. Three rates of mineral P fertilizer (12.5, 25 and 50 mg P kg^{-1} soil) or (32.5, 65.0 and 130 kg P ha^{-1}), as triple superphosphate (TSP), were separately applied uniformly to the whole soil volume. The manure was ground and passed through a 0.25 mm sieve. Wood ash (7 tons $CaCO_3$ ha^{-1}) and lime (7 tons $CaCO_3$ ha^{-1}) were combined separately with each of the manure P and mineral P fertilizer rates as additional treatments. A control treatment with no soil amendments was used for the incubation experiment. A total of 54 pots were used for the incubation experiment. The experiment was laid down in a completely randomized design with two replications. The units of the treatments were converted into hectare bases by assuming that the plough depth is 20 cm and the bulk density of the soil is 1.3 g cm^{-3}. All pots were subjected to wetting and drying cycles during the incubation period.

Statistical analysis

Analysis of variance was carried out on the effect of treatments on the inorganic P fractions and selected chemical properties of the soil using the generalized linear model (GLM) procedure of the Statistical Analysis System (SAS) software version 9.00 (SAS Institute, 2004). Duncan's Multiple Range Test was employed to

Table 1. Selected physical and chemical properties of the experimental soil.

Parameter	Value
Sand (%)	26
Silt (%)	31
Clay (%)	43
Bulk density (g cm^{-3})	1.3
pH (H_2O)	4.88
Cation exchange capacity ($cmol_c$ kg^{-1})	24.7
Exchangeable acidity ($cmol_c$ kg^{-1})	2.31
Exchangeable Al ($cmol_c$ kg^{-1})	1.50
Organic carbon (%)	1.96
Total nitrogen (%)	0.17
Bray I P (mg kg^{-1})	5.7
Olsen P (mg kg^{-1})	7.0

Table 2. Chemical characterization of manure and wood ash.

Amendment	pH-H_2O (1:5)	EC (dS m^{-1})	CCE	N	P	Ca	K	Mg	Na	Al	Zn
			%							mg kg^{-1}	
Manure	8.0	6.01	-	1.69	0.475	1.20	1.45	0.63	0.19	16675	105
Wood ash	12.3	9.33	72	0.05	1.240	15.9	2.23	2.64	0.22	155	3

EC = electrical conductivity; CCE = calcium carbonate equivalent.

test the significance difference between means of treatments.

RESULTS AND DISCUSSION

Initial soil properties, manure and wood ash compositions

The soil used for the incubation experiment was clayey in texture (Table 1). The value of the Olsen extractable P was 7.0 mg kg^{-1} which was in the low range of P content (Cottenie, 1980) and the pH was 4.88 which was in the very strongly acidic soil reaction range (Jones, 2003) (Table 1). The soil had initially a potential CEC of 25 $cmol_c$ kg^{-1} which qualified the moderate CEC range (Hazelton and Murphy, 2007). The soil had also relatively high content of exchangeable acidity (2.41 $cmol_c$ kg^{-1}) and Al (1.81 $cmol_c$ kg^{-1}). The percentage of acid saturation and Al saturation were 9.4 and 6.1%, respectively.

The wood ash had CCE and pH values of 71.7% and 12.3, respectively (Table 2). Because of its alkalinity and elevated contents of alkali and alkaline earth elements, wood ash can be utilized to raise the pH of acid soils (Demeyer et al., 2001). The wood ash has relatively higher content of total P compared to the manure. Therefore, wood ash could be used as an alternative liming material and source of P for acidic soils. Compared to the wood ash, the manure had higher content of total N. This is because the wood lost its N content by the combustion process as the N in the wood is converted to gaseous products.

Effects of the treatments on pH, exchangeable acidity and Al, CEC and available P

The effects of the treatments on the pH, exchangeable acidity, exchangeable Al and available P were very highly significant ($P \leq 0.001$) (Table 3). The soil pH varied from 4.88 to 6.03; exchangeable acidity from 2.22 to 0.12 $cmol_c$ kg^{-1}; exchangeable Al from 1.28 to 0.06 $cmol_c$ kg^{-1}. The Olsen extractable P of the soil varied from 7 to 16.3 mg kg^{-1} and the Bray I P from 5.7 to 14.0 mg kg^{-1} (Table 3). The output of the correlation matrix (Table 6) indicated that the pH of the soils was negatively and strongly correlated to the exchangeable acidity ($r = 0.91$, $P \leq 0.01$), exchangeable Al ($r = 0.82$, $P \leq 0.01$) and dithionite citrate bicarbonate extractable Al ($r = 0.89$, $P \leq 0.01$). This result verified that these soil parameters are responsible for the pH change in acid soils. The Olsen extractable P was positively and strongly correlated with Bray I P ($r = 0.97$, $P \leq 0.01$), DPS ($r = 0.87$, $P \leq 0.01$), P_{ox} ($r = 0.88$, $P \leq 0.01$), Al-P ($r = 0.95$, $P \leq 0.01$) and Fe-P (r

Table 3. Effects of applications of the treatments on selected chemical properties.

Treatment	Rate	pH	Ex. Ac	Ex. Al	CEC	Bray I P	OlsenP	PAcs	PAls
			$cmol_c$ kg^{-1}			mg kg^{-1}		%	
Control	-	4.89^{i}	2.22^{a}	1.28^{a}	25.7	5.7^{o}	7.0^{p}	8.6^{a}	5.0^{a}
Lime (tons $CaCO_3$ ha^{-1})	3.5	5.15^{f}	0.85^{f}	0.13^{e}	25.8	5.8^{o}	7.4^{op}	3.3^{f}	0.5^{e}
	7.0	5.43^{e}	0.35^{h}	0.09^{e}	26.1	6.3^{n}	7.7^{no}	1.3^{g}	0.3^{e}
	9.2	5.63^{bcd}	0.23^{jkl}	0.07^{e}	26.4	6.5^{m}	8.1^{n}	0.9^{i-l}	0.3^{e}
	11.2	6.03^{a}	0.14^{no}	0.07^{e}	26.4	6.8^{l}	8.7^{m}	0.5^{m}	0.3^{e}
Wood ash (tons $CaCO_3$ ha^{-1})	3.5	5.16^{fg}	0.78^{g}	0.12^{e}	25.9	9.0^{h}	9.6^{kl}	3.0^{f}	0.5e
	7.0	5.42^{e}	0.35^{h}	0.10^{e}	26.1	9.4^{g}	10.5^{j}	1.3^{g}	0.4^{e}
	9.2	5.66^{bc}	0.19^{k-o}	0.08^{e}	26.2	9.8^{f}	12.2^{fgh}	0.7^{j-m}	0.3^{e}
	11.2	5.93^{a}	0.16^{mno}	0.06^{e}	26.3	10.2^{e}	13.8^{d}	0.6^{lm}	0.2^{e}
Manure P (kg ha^{-1})	32.5	4.97^{hi}	1.90^{c}	0.90^{b}	26.0	7.1^{k}	8.7^{m}	7.3^{c}	3.5^{b}
	65.0	4.98^{hi}	1.74^{d}	0.72^{c}	26.2	7.8^{j}	9.2^{lm}	6.6^{d}	2.7^{c}
	130.0	5.03^{gh}	1.32^{e}	0.45^{d}	26.1	9.9^{f}	11.4^{i}	5.1^{e}	1.7^{d}
Mineral P (kg ha^{-1})	32.5	4.88^{i}	2.22^{a}	1.27^{a}	25.9	7.0^{k}	8.7^{m}	8.6^{a}	4.9^{a}
	65.0	4.90^{i}	2.17^{ab}	1.28^{a}	26.2	8.0^{i}	9.2^{lm}	8.3^{b}	4.9^{a}
	130.0	4.96^{hi}	2.13^{b}	1.25^{a}	26.6	10.7^{d}	11.6^{ghi}	8.0^{b}	4.7^{a}
Mineral P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	5.58^{cd}	0.27^{ij}	0.09^{e}	25.8	7.1^{k}	9.0^{m}	1.0^{g-j}	0.3^{e}
	65.0	5.67^{bc}	0.23^{j-m}	0.08^{e}	25.7	8.1^{i}	10.1^{jk}	0.9^{i-l}	0.3^{e}
	130.0	5.61^{bcd}	0.22^{j-m}	0.09^{e}	25.8	10.2^{d}	13.1^{e}	0.9^{jkl}	0.3^{e}
Manure P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	5.57^{cd}	0.31^{hi}	0.09^{e}	26.4	7.0^{k}	8.9^{m}	1.2^{ghi}	0.3^{e}
	65.0	5.64^{bcd}	0.20^{j-n}	0.10^{e}	26.7	7.6^{j}	9.7^{kl}	0.8^{j-m}	0.4^{e}
	130.0	5.71^{b}	0.12^{o}	0.09^{e}	26.5	8.9^{h}	11.6^{hi}	0.5^{m}	0.3^{e}
Mineral P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	5.53^{de}	0.31^{hi}	0.08^{e}	25.9	10.6^{d}	12.2^{fg}	1.2^{gh}	0.3^{e}
	65.0	5.56^{cd}	0.24^{jk}	0.09^{e}	25.9	11.6^{c}	15.0^{c}	0.9^{h-k}	0.3^{e}
	130.0	5.52^{de}	0.22^{j-m}	0.08^{e}	26.0	14.0^{a}	16.3^{a}	0.8^{j-m}	0.3^{e}
Manure P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	5.62^{bcd}	0.32^{hi}	0.07^{e}	25.7	10.3^{e}	12.5^{f}	1.2^{g}	0.3^{e}
	65.0	5.64^{bcd}	0.20^{jkl}	0.09^{e}	25.8	11.4^{c}	14.4^{c}	0.9^{h-l}	0.3^{e}
	130.0	5.62^{bcd}	0.15^{l-o}	0.07^{e}	26.4	13.4^{b}	15.6^{b}	0.6^{klm}	0.3^{e}
F-test		***	***	***	ns	***	***	***	***
CV (%)		0.95	4.26	13.73	1.54	12.04	1.00	4.90	13.41

Means followed by the same letter within a column are not significantly different at P > 0.001; *** = significant at P ≤ 0.001 using the Duncan's multiple range test; ns = non-significant at P > 0.05; CV = coefficient of variation of treatments; Ex. Ac = exchangeable acidity; Ex. Al = exchangeable Al; PAcs = percentage of acid saturation; PAls = percentage of Al saturation.

= 0.90, P ≤ 0.01). This result indicated that Al-P and the Fe-P are the most important sources of easily soluble P which are important for plant growth by supplying P to the soil solution. However, Hartikainen (1982) stated that Al-P has a dominant influence over Fe-P in maintaining P concentration in the soil solution in the first place. The contribution of Fe-P to maintain P concentration in the soil solution can be expected to become source of available P with decreasing Al-P content.

The lime and wood ash at each respective application levels had significant (P ≤ 0.001) and similar effects on the soil pH, exchangeable acidity and exchangeable Al (Table 3). This was because both of the lime and the wood ash were applied based on adjusted CCE to have the same $CaCO_3$ equivalent. Wood ash could thus be highly effective in neutralizing acidified soils. The strong alkalinity of wood ash indicated that it can be an alternative to lime either by itself or as a mixture of lime and wood ash for the management of acidity.

Both the lime and wood ash contained cations such as Ca^{2+} and Mg^{2+} to exchange and/or replace H^{+} on the exchange sites and anions such as CO_3^{2-} and OH^{-} to neutralize the H^+ released from the exchange sites and hydrolyzing Al species to the soil solution (Fageria, 2007). The highest lime rate (11.2 tons $CaCO_3$ ha^{-1}) significantly ($P \leq 0.001$) increased the pH from 4.89 to 6.03 and reduced both the exchangeable acidity from 2.22 to 0.14 $cmol_c$ kg^{-1} and exchangeable Al from 1.28 to 0.07 $cmol_c$ kg^{-1}. Ayeni et al. (2008) also reported increased soil pH relative to non-ash treated soils. This verified the known truth that applications of liming agents are very effective to alleviate the Al^{3+} and H^+ ions toxicity of acidic soils. The lime rate to raise the pH to 5.5 was highly effective in alleviating Al toxicity problem totally for the soil used for the incubation experiment.

The application of the highest wood ash and lime rates (11.2 tons $CaCO_3$ ha^{-1}) significantly ($P \leq 0.001$) increased the Bray I extractable P by 79 and 19% and the Olsen extractable P by 97 and 24%, respectively, over the control. The highest available P content associated with the wood ash treated soil was attributed to the fact that wood ash was a source of P in addition to its liming effect (Table 2). An increased available P has been observed previously by the application of ash by different studies (Adetunji, 1997; Nkana et al., 2002; Saarsalmi et al., 2006; Awodun et al., 2007; Insam et al., 2009).

Manure was more effective in the reduction of exchangeable acidity and exchangeable Al compared to its effect in increasing the pH of the soil (Table 3). The reductions in exchangeable Al observed due to application of manure can partially be attributed to its ability to increase the soil pH. This is consistent with other studies (Narambuye and Haynes, 2006; Tang et al., 2007; Opala et al., 2010) which reported an increase in soil pH with a concomitant decrease in exchangeable Al during decomposition of organic residues in soils. An increase in soil pH apparently results in precipitation of exchangeable and soluble Al as insoluble Al hydroxides thus reducing concentration of Al in soil solution.

The application of manure P at 130 kg P ha^{-1} significantly ($P \leq 0.001$) increased the Bray I extractable P and the Olsen extractable P to 9.9 and 11.4 mg kg^{-1}, respectively. The mineral P fertilizer application had non-significant effect on the pH, CEC, exchangeable acidity and exchangeable Al compared to the control. The application of mineral P on acidic soils did not have a significant effect on the pH and exchangeable acidity on acidic soils of western Kenya (Opala et al., 2010).

However, the effect of mineral P applications on the Bray I extractable and the Olsen extractable P was significantly ($P \leq 0.001$) higher than that of the control treatment. The application of mineral P at 130 kg P ha^{-1} significantly ($P \leq 0.001$) increased the Bray I and Olsen P to 10.7 and 11.6 mg kg^{-1}, respectively. The high available P contents associated with the mineral P treated soils were attributed to the fact that mineral P applied in the form of TSP is a source of soluble P. At the end of the incubation period, the percentage recovery of the applied manure P and mineral P as Bray I extractable P varied from 8 to15% and Olsen extractable P from 9 to 16%. A similar result has also been found on the effects of applications of mineral P and manure on the available P contents of acidic soils of western Kenya (Opala et al., 2010).

The application of each manure rate with lime (7.0 tons $CaCO_3$ ha^{-1}) and wood ash (7.0 tons $CaCO_3$ ha^{-1}) separately significantly ($P \leq 0.001$) increased the pH and reduced the exchangeable acidity compared to the separate application of the manure, lime and wood ash due to the synergetic effect of the manure and the liming materials to reduce exchangeable acidity and exchangeable Al and increase the pH of acidic soil.

Though the application of manure plus lime did not have significant difference in available P compared with separate application of manure, the application of manure P plus wood ash increased available P compared to the separate manure application. Application of 130 kg manure P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) increased the Bray I P by 135, 36 and 43% and Olsen extractable P by 122, 37 and 48% over the control, 130 kg manure P ha^{-1} and wood ash (7.0 tons $CaCO_3$ ha^{-1}) treatments, respectively, due to the synergetic effects of the manure and wood ash as sources of soluble P to the soil. Both the application of mineral P fertilizer plus lime and wood ash separately (7.0 tons $CaCO_3$ ha^{-1}) had a non significant difference in terms of increasing pH, reducing exchangeable Al and exchangeable acidity compared to the lime and wood ash rate (7.0 tons $CaCO_3$ ha^{-1}).

However, the application of mineral P plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) gave the highest available P compared to the combined application of mineral P with lime (7.0 tons $CaCO_3$ ha^{-1}). This is due to the fact that both the mineral P fertilizer and the wood ash were sources of P and made the available P of the incubated soil to be higher. In general, the separate application of the highest manure P and mineral P rates and their combination with 7.0 tons $CaCO_3$ equivalent ha^{-1} lime, all the mineral P and manure P rates plus 7.0 tons $CaCO_3$ equivalent of either lime or wood ash and all the wood ash rates except the lower rate increased the available P content from the lower range to the medium one. None of the treatments had a significant effect on the CEC of the incubated soil compared to the control.

Effects of the treatments on the inorganic P fractions

The forms and distribution of the various inorganic P fractions (Al-P, Fe-P and Ca-P, reds-Fe-P and occl-Al-Fe-P) of the control treatment used for the incubation experiment are shown in Table 4. The distributions of the P fractions were in the following orders: Fe-P > reds-Fe-P > occl-Al-Fe-P > Al-P > Ca-P. The Fe-P was the most abundant active P form contributing more than 50% of the active P fractions. The abundance of the Fe-P correlated to the high content of free iron oxides in the soil (Table 5). According to Piccolo and Gobena (1986), the low levels of Ca-P compared to Al-P seem to be a consequence of the weathering process of the soils; soil under well drained conditions resulting in the dissolution of the native Ca-P. The high content of Fe-P and Al-P compared to the Ca-P also indicated the advanced stage of weathering of the soil resulting in the dissolution of native Ca-P in acid soils. The degree of P fixation with Al, Fe and Ca was directly related to the intensity of weathering in that when Al and Fe fixation dominated in the soil system, the soil is highly weathered and vice versa (Piccolo and Gobena, 1986). Hence, the present soil is highly weathered with high content of Fe-P active inorganic P fraction.

Previous studies on the different Ethiopian surface soils showed that the active inorganic P fractions were found in the order Ca-P > Fe-P > Al-P (Desta, 1982). Piccolo and Gobena (1986), working on seven Ethiopian soils found that the relative abundance of the inorganic P forms in the profiles was in the order: Fe-P > Al-P > Ca-P > reds-Fe-P. Wakene and Heluf (2003) working on Nitosol under different land use system such as research, farmers and virgin fields found that Fe-P > Al-P > Ca-P in the research field which received high P fertilizer but the order in the other land use system were Fe-P > Ca-P > Al-P.

The effects of the treatments on the inorganic P fractions are shown in Table 4. The result indicated that application of different lime rates had an effect on the Al-P and the Fe-P fractions. The highest lime rate (11.2 tons $CaCO_3$ ha^{-1}), significantly ($P \leq 0.001$) increased the Al-P from 25 to 39 mg kg^{-1} and decreased the Fe-P from 157 to 144 mg kg^{-1}. However, application of lime did not have a significant effect on the value of easily soluble P, Ca-P, occluded Al-Fe-P and reds-Fe-P compared to the control treatment except the highest rate which significantly increased the occl-Al-Fe-P. The increase in the Al-P content of the lime treated soils may be due to the release P content from Fe-P pools.

Application of wood ash significantly ($P \leq 0.001$) increased the easily soluble P, Ca-P, Fe-P and occl-Al-Fe-P fractions. The highest wood ash rate (11.2 tons $CaCO_3$ ha^{-1}) increased the easily soluble P by 389%, Al-P by 132%, Fe-P by 11%, Ca-P by 85% and occl-Al-Fe-P by 16% over the control treatment. This might be due to the phosphate ion released from the wood ash followed by adsorption by the Al and Fe oxides and precipitation as Ca-P. Wood ash did not give significant difference in the value of the reds-Fe-P. In general, wood ash amendment of acid soil was more effective in increasing the value of the active P fractions compared to lime amendment.

It is shown that application of manure P and mineral P fertilizer at 130 kg P ha^{-1} significantly increased the easily soluble P by 94 and 94%; Al-P by 40 and 54%; Fe-P by 7 and 8% over the control, respectively. However, the manure and mineral P fertilizer application had no significant effect on the Ca-P, occl-Al-Fe-P and reds-Fe-P except the application of 130 kg mineral P ha^{-1} treatment which increased significantly the occl-Al-Fe-P fraction. This showed that most of the added P was found to be converted into active Al-P and Fe-P. Chang and Jackson (1958) also observed that application of phosphatic fertilizers to acid soils increases the fixation and transformation of added phosphate into Fe-P. In a study by Sharma et al. (1980) on the transformation of P added at 0, 50, 100 and 150 mg kg^{-1} to three acid soils of Himachal Pradesh and incubated for 1, 7, 30 and 90 days, it was found that most of the added P was transformed into Al-P, Fe-P and very little to Ca-P fraction at one day interval. The added P which was transformed into Al-P increased (69.5 to 77.8 mg kg^{-1}) up to 7 days and later decreased (77.8 to 65 mg kg^{-1}) slowly with time up to 90 days at all the levels of application. The conversion of added P into Fe-P increased (111.5 to 119.3 mg kg^{-1}) slowly with time up to 90 days and very little (24 to 23 mg kg^{-1}) was changed to Ca-P.

The effect of mineral P application on the different inorganic P fraction was the same as that of the manure treatment in this particular study. Increase in Al-P evident from manure treatment is because, initially, OM forms complex with P fixing metallic cations and reduces fixation of P in soil. The release of P occurs from the reaction products through anion exchange and during later period, due to degradation of organo-metalic complexes, the released P could be precipitated as Al-P. Hence, the action of organic sources is to delay the process of P fixation temporarily and in the long run it favors the precipitation of reaction product (Tomar et al., 1984).

The application of each of the mineral P fertilizers plus lime (7.0 tons $CaCO_3$ ha^{-1}) significantly increased only the Al-P and the Ca-P compared to the separate application of mineral P fertilizer. The increase in Ca-P in the application of mineral P plus lime (7.0 tons $CaCO_3$ ha^{-1}) may be due to the fact that lime increased considerably Ca^{2+} in the soil, which reacted with added P and caused precipitation as calcium phosphates. The increase in the fixation of added P by Ca due to liming also reported by Amarasiri and Olsen (1973). The application of each of the manure rates plus lime (7.0 tons $CaCO_3$ ha^{-1}) increased only the Al-P and occl-Al-Fe-P and decreased the Fe-P compared to the separate manure application. On the other hand, the lower value of Ca-P noticed in the application of manure P plus lime (7.0 tons $CaCO_3$ ha^{-1}) compared to combined application of mineral P plus the same lime rate because the manure appears to be more effective to form a complex with Ca and Mg which reduces the chance of the reaction of the P with the Ca.

Table 4. Effects of the applications of the treatments on the inorganic P fractions.

Treatment	Rate	Easily soluble P	Al-P	Fe-P	Ca-P	Reds – Fe-P	Occl-Al-Fe-P	Sum of fractions
		mg kg^{-1}						
Control	-	0.35^{e}	24.7^{m}	157^{jk}	12.8^{f}	67	30.3^{kl}	292^{lm}
Lime (tons $CaCO_3$ ha^{-1})	3.5	0.35^{e}	25.7^{lm}	151^{l}	12.8^{f}	67	31.2^{g-l}	287^{m}
	7.0	0.35^{e}	27.5^{kl}	148^{lm}	12.8^{f}	67	31.7^{f-l}	288^{m}
	9.2	0.35^{e}	29.0^{jk}	147^{lm}	12.8^{f}	67	31.9^{f-l}	288^{m}
	11.2	0.35^{e}	30.9^{hij}	144^{m}	12.8^{f}	67	32.8^{e-i}	288^{m}
Wood ash (tons $CaCO_3$ ha^{-1})	3.5	0.68^{d}	31.8^{g}	163^{ghi}	13.3^{ef}	66	31.1^{i-l}	308^{j}
	7.0	1.03^{c}	43.9^{e}	168^{ef}	14.7^{e}	67	31.2^{g-l}	325^{h}
	9.2	1.34^{b}	51.7^{d}	170de	18.5^{d}	67	32.1^{f-l}	341^{g}
	11.2	1.71^{a}	57.3^{bc}	174^{cd}	23.7^{b}	67	35.3^{cd}	359^{d}
Manure P (kg ha^{-1})	32.5	0.35^{a}	28.0^{k}	158^{jk}	12.8^{f}	67	30.4^{jkl}	296^{kl}
	65.0	0.52^{de}	29.3^{ijk}	161^{hij}	12.8^{f}	67	30.3^{kl}	301^{k}
	130.0	0.68^{d}	34.8^{g}	168^{efg}	12.8^{f}	67	31.1^{h-l}	314^{i}
Mineral P (kg ha^{-1})	32.5	0.35^{e}	28.7^{jk}	159^{ijk}	12.7^{f}	68	32.0^{f-l}	301^{k}
	65.0	0.52^{de}	31.5^{hi}	164^{ghi}	12.8^{f}	69	32.1^{f-l}	309^{ij}
	130.0	0.68^{d}	38.1^{f}	169^{e}	12.8^{f}	69	33.5^{def}	323^{h}
Mineral P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	0.35^{e}	34.3^{g}	159^{ijk}	18.5^{d}	67	32.5^{e-j}	312^{ij}
	65.0	0.35^{e}	36.9^{f}	164^{fgh}	20.1^{c}	67	32.4^{f-k}	321^{h}
	130.0	0.35^{e}	43.7^{e}	177^{c}	24.1^{ab}	68	33.8^{e-i}	345^{fg}
Manure P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	0.35^{e}	30.7^{hij}	149^{lm}	12.8^{f}	69	32.0^{f-l}	294^{lm}
	65.0	0.52^{de}	32.8^{gh}	150^{lm}	12.8^{f}	68	34.4^{cde}	298^{kl}
	130.0	0.68^{d}	37.3^{f}	157^{k}	12.7^{f}	68	35.8^{bc}	311^{ij}
Mineral P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	1.04^{c}	52.4^{d}	177^{c}	18.5^{d}	68	33.2^{e-h}	350^{ef}
	65.0	1.36^{b}	57.4^{bc}	186^{b}	20.6^{c}	69	35.4^{cd}	369^{c}
	130.0	1.38^{b}	64.1^{a}	192^{a}	24.1^{ab}	67	37.6^{ab}	386^{a}
Manure P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	1.04^{c}	50.9^{d}	178^{c}	20.1^{c}	68	33.3^{efg}	351^{e}
	65.0	1.04^{c}	55.8^{c}	182^{b}	22.7^{b}	67	36.4^{bc}	366^{c}
	130.0	1.22^{bc}	59.3^{b}	187^{b}	25.3^{a}	67	38.9^{a}	379^{b}
F-test		***	***	***	***	ns	***	***
CV (%)		12.04	2.57	1.21	4.21	1.97	2.61	0.72

Means followed by the same letter within a column are not significantly different at P > 0.001; *** = significant at P ≤ 0.001 using Duncan's multiple range test; ns = non-significant at P > 0.05; CV = coefficient of variation; Al-P = Al bound P; Fe-P = Fe bound P; Ca-P = Ca bound P; reds-Fe-P = reductant soluble Fe-P; occl-Al-Fe-P = occluded Al-Fe-P.

Similar results were reported by Srivastava et al. (1969) who observed decrease in content of Ca-P and increasein Al-P with the application of farm yard manure and compost.

Due to the synergetic effect of the manure and wood ash and mineral P fertilizer and wood ash as a source of phosphate ion to be sorbed by Al, Fe and Ca of the soil, manure plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) and mineral P fertilizer with wood ash (7.0 tons $CaCO_3$ ha^{-1}) significantly (P ≤ 0.001) increased the easily soluble P, the active Al-P, Fe-P and Ca-P fractions and the occl-Al-Fe-P compared to the separate application of manure P, mineral P, wood ash (7.0 tons $CaCO_3$ ha^{-1}) and the control treatments. For instance, both the manure and mineral P rates of 130 kg P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) cause a significant increase in easily soluble

Table 5. Effects of applications of the treatments on Al_{ox}, Fe_{ox}, P_{ox}, DPS, Al_d and Fe_d.

Treatment	Rate	Al_{ox}	Fe_{ox}	P_{ox}	PSC	Al_{ox}-P^a	Fe_{ox}-P^b	P_{ox}	Al_d	Fe_d
		(mmol kg^{-1})				(DPS, %)			(mmol kg^{-1})	
Control	-	139.4^{c-f}	85.6^{bc}	7.6^{i}	225.0^{def}	0.57^{q}	5.91^{hij}	3.4^{l}	95^{a}	358^{a}
Lime (tons $CaCO_3$ ha^{-1})	3.5	139.3^{c-f}	84.0^{de}	7.8^{hi}	223.3^{efg}	0.60^{pq}	5.78^{ijk}	3.5^{kl}	92^{cd}	351^{c-h}
	7.0	139.0^{d-h}	83.6^{def}	7.8^{hi}	222.6^{f-i}	0.64^{op}	5.72^{jk}	3.5^{kl}	89^{efg}	343^{jk}
	9.2	138.3^{d-i}	82.4^{ef}	7.8^{hi}	220.7^{h-k}	0.68^{mno}	5.77^{ijk}	3.6^{kl}	88^{fg}	345^{ijk}
	11.2	137.5^{g-k}	82.4^{f}	7.8^{hi}	219.9^{jk}	0.72^{klm}	5.69^{jk}	3.6^{kl}	88^{g}	342^{k}
Wood ash (tons $CaCO_3$ ha^{-1})	3.5	139.3^{c-g}	84.2^{de}	8.2^{ghi}	223.5^{efg}	0.78^{hij}	6.25^{fg}	3.7^{jkl}	93^{bc}	355^{bc}
	7.0	138.5^{d-h}	83.0^{ef}	8.3^{gh}	221.5^{g-j}	1.02^{e}	6.53^{cd}	3.8^{ijk}	90^{de}	346^{h-k}
	9.2	137.9^{e-j}	82.6^{ef}	8.7^{d-g}	221.5^{g-j}	1.21^{d}	6.58^{cd}	4.0^{e-j}	91^{de}	347^{g-k}
	11.2	137.4^{g-k}	81.7^{f}	9.4^{abc}	219.0^{jk}	1.35^{c}	6.86^{b}	4.3^{a-d}	90^{de}	345^{ijk}
Manure P (kg ha^{-1})	32.5	136.4^{i-m}	83.6^{def}	7.8^{hi}	219.0^{jk}	0.66^{no}	6.11^{gh}	3.6^{kl}	94^{ab}	352^{b-e}
	65.0	135.7^{no}	83.4^{def}	8.6^{fg}	219.4^{jk}	0.69^{lmn}	6.21^{g}	3.9^{f-j}	94^{ab}	353^{b-e}
	130.0	134.8^{o}	83.8^{def}	9.1^{c-f}	218.6^{k}	0.83^{gh}	6.45^{def}	4.2^{a-f}	94^{ab}	352^{b-f}
Mineral P (kg ha^{-1})	32.5	138.1^{e-j}	84.1^{de}	8.2^{ghi}	222.2^{fgh}	0.67^{no}	6.06^{gh}	3.7^{jkl}	95^{a}	355^{abc}
	65.0	137.0^{j-m}	82.6^{ef}	8.7^{d-g}	221.2^{g-k}	0.74^{jkl}	6.27^{efg}	4.0^{e-j}	94^{ab}	356^{ab}
	130.0	136.2^{j-m}	83.1^{ef}	9.6^{abc}	219.6^{jk}	0.90^{f}	6.53^{cd}	4.4^{abc}	94^{ab}	352^{b-f}
Mineral P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	138.9^{efg}	89.0^{a}	8.1^{ghi}	227.0^{ef}	0.79^{hi}	5.78^{ijk}	3.6^{kl}	90^{de}	351^{b-f}
	65.0	140.1^{a-c}	88.9^{a}	8.7^{efg}	229.0^{ab}	0.85^{g}	5.97^{hi}	3.8^{g-k}	90^{de}	349^{d-i}
	130.0	141.0^{a}	88.1^{a}	9.2^{c-f}	229.1^{ab}	1.00^{e}	6.47^{de}	4.1^{d-i}	90^{ef}	350^{d-i}
Manure P (kg ha^{-1}) plus lime (7 tons $CaCO_3$ ha^{-1})	32.5	138.8^{e-h}	88.2^{a}	8.3^{gh}	227.0^{a-d}	0.71^{k-n}	5.41^{l}	3.7^{jkl}	90^{de}	353^{bc}
	65.0	140.1^{a-d}	88.6^{a}	9.1^{c-f}	228.6^{ab}	0.76^{ijk}	5.40^{l}	4.0^{e-j}	90^{def}	352^{b-f}
	130.0	140.7^{ab}	89.0^{a}	9.4^{bcd}	229.7^{a}	0.86^{fg}	5.68^{k}	4.1^{b-h}	89^{efg}	351^{c-h}
Mineral P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	139.2^{c-f}	87.2^{ab}	9.3^{b-e}	226.4^{bcd}	1.21^{d}	6.58^{cd}	4.1^{b-g}	90^{def}	347^{e-j}
	65.0	139.8^{b-e}	88.1^{a}	9.7^{abc}	228.0^{abc}	1.32^{c}	6.80^{b}	4.3^{a-e}	90^{de}	348^{f-j}
	130.0	139.8^{b-e}	87.1^{ab}	10.1^{a}	227.0^{bcd}	1.48^{a}	7.10^{a}	4.5^{a}	89^{ef}	346^{h-k}
Manure P (kg ha^{-1}) plus wood ash (7 tons $CaCO_3$ ha^{-1})	32.5	139.0^{d-g}	87.6^{ab}	9.2^{c-f}	227.0^{bcd}	1.18^{d}	6.54^{cd}	4.1^{b-h}	88^{efg}	353^{b-e}
	65.0	137.7^{h-k}	87.8^{ab}	9.5^{abc}	226.6^{cde}	1.31^{c}	6.70^{bc}	4.2^{a-f}	89^{efg}	351^{b-g}
	130.0	136.7^{k-m}	88.5^{a}	9.9^{ab}	225.2^{bcd}	1.40^{b}	6.80^{b}	8.8^{ab}	88^{fg}	346^{h-k}
F-test		***	***	***	***	***	***	***	***	***
CV (%)		0.46	0.52	3.27	0.65	2.55	1.54	3.53	0.81	0.59

Means followed by the same letter within a column are not significantly different at P > 0.001; *** = significant at P ≤ 0.001 using Duncan's multiple range test; CV = coefficient of variation; Al_{ox}, Fe_{ox} and P_{ox} = oxalate extractable Al, Fe and P, respectively; Al_d and Fe_d = dithionite citrate bicarbonate extractable Al and Fe, respectively; PSC = P sorption capacity; [a and b] = DPS of the active Al-P and Fe-P fractions with respect to Al_{ox} and Fe_{ox}, respectively.

P by 248 and 294%; Al-P by 140 and 160%; Fe-P by 19 and 22%; Ca-P by 98 and 88%; occl-Al-Fe-P by 28 and 24%, respectively, compared to the control treatment. The soils used in this study contained free Al and Fe oxides which are responsible for the high contents of occl-Al-Fe-P in the applications of manure P and mineral P rates plus wood ash. The non-significant difference in the reds-Fe-P among the treatments was observed in this study. According to Chang and Jackson (1957), the reds-Fe-P is the Fe-oxide precipitate formed on the surface of Fe and Al-oxides during weathering. This may result in slow transformation between the added P and the native reds-Fe-P. However, application of wood ash to soil must be controlled to maximize its benefit and prevent adverse effects on the environment or future fertility/sustainability of the fields to which they are applied.

The result indicted in Table 4 verified that most of the applied P was converted into the active inorganic P

Table 6. Correlation coefficients (r) among selected soil chemical properties.

Property	BP	OLP	DPS	P_{ox}	Al-P	Fe-P	pH	Ex. Al	Ex. Ac	Alox	Feox	Ald
OLP	0.97**											
DPS	0.87**	0.86**										
P_{ox}	0.88**	0.88**	0.98**									
Al-P	0.92**	0.95**	0.78**	0.80**								
Fe-P	0.92**	0.90**	0.76**	0.77**	0.89**							
pH	0.26	0.40**	0.21**	0.27*	0.49**	0.11						
Ex. Al	-0.27*	-0.35**	-0.14	-0.21	-0.40**	-0.14	-0.82**					
Ex. Ac	-0.32*	-0.42**	-0.20	-0.28*	-0.51**	-0.17	-0.91**	0.97**				
Al_{ox}	-0.04	0.04	-0.15	0.01	0.10	-0.01	0.39**	-0.43**	-0.49**			
Fe_{ox}	0.30*	0.36**	0.22	0.39**	-0.30*	0.38*	0.25	-0.28*	-0.34**	0.52**		
Al_d	-0.27*	-0.36**	-0.17	-0.24	-0.45*	-0.13	-0.89**	0.82**	0.90**	-0.43**	-0.27*	
Fe_d	-0.25	-0.29*	-0.13	-0.12	-0.39**	-0.12	0.64**	0.59**	0.64**	-0.09	0.18	0.68**

*, ** = significant at 0.05 and 0.01 probability levels, respectively; BP = Bray I P; OLP = Olsen P; DPS = degree of P saturation; Ex. Ac and Ex. Al= exchangeable acidity and Al, respectively.

fractions in which the percentage of recovery of applied P as Al-P dominated the Fe-P and Ca-P. The effect of application of mineral P fertilizer on the recovery of applied P into the inorganic P fractions such as Al-P and Fe-P was higher than application of manure P. This showed that application of mineral P fertilizers to highly weathered acid soils with high content of Al and Fe oxides and hydroxides resulted in the strong adsorption of the P into Al-P and Fe-P which in turn resulted in the low content of soluble P available to plant growth. The percentage recovery of the applied manure P as Al-P and Fe-P ranged from 17 to 24%, and 10 to 21%, respectively, whereas the percentage recovery of the applied mineral P as Al-P and Fe-P ranged from 26 to 29% and 16 to 24%, respectively. This can be further verified by the result of the lower percentage recovery of the applied P as easily soluble P due to the applications of the treatments. The percentage recoveries of the available P which were extracted by the Bray I and Olsen P methods were ranging from from 7 to 14% and 8 to 15%, respectively.

Effects of the treatments on the Al_{ox}, Fe_{ox}, P_{ox}, DPS, Al_d and Fe_d

The soil used for the incubation experiment was very rich in oxalate extractable Al and Fe and dithionite citrate bicarbonate extractable Al and Fe (Table 5). Piccolo and Gobena (1986) working on seven Ethiopian soils found that the Fe_d varied between 10 to 525 mmol kg^{-1}. Therefore, the soil used for the incubation is rich in oxides and hydroxides of Fe. The low pH and high content of the Al and Fe oxides and hydroxides of the soil results in a significant P fixation and precipitation of applied and native P of the soil as Al-P and Fe-P.

The data in Table 5 illustrate the difference between the P_{ox}, Al_{ox}, Fe_{ox}, PSC and DPS values of the soils as the result of the treatments used in the incubation experiment. The P_{ox} values of the incubated soils varied from 7.6 to 9.9 mmol kg^{-1}. The sum of the two oxides (Al_{ox} + Feo_x), an important estimate of the PSC of the soil, was significantly influenced by the application of the treatments. The maximum increment (2.1%) in the value of the PSC was recorded at the application of 130 kg manure P ha^{-1} plus lime (7.0 tons $CaCO_3$ ha^{-1}) compared to the control treatment. The DPS was significantly influenced by the different treatments used for the incubation. The DPS values varied from 3.4% in the control to 4.5% in 130 kg mineral P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) treatment.

Lime application did not have any significant effect on the P_{ox} and DPS values in which the maximum increment in P_{ox} (3.0%) and DPS (5.4%) was observed at the highest lime rate (11.2 tons $CaCO_3$ ha^{-1}) compared to the control treatment but the values increased numerically with increasing the lime levels. The DPS of Al_{ox} is higer than that of Fe_{ox} in all lime treated soils. This may be due to the transfer of the phosphate ion from Fe to Al (Table 4). Wood ash significantly increased the P_{ox} and DPS value of the soil compared to the control and the lime treated soils. The maximum increment in P_{ox} (19.8%) and DPS (22.0%) was recorded at the highest wood ash rate (11.2 tons $CaCO_3$ ha^{-1}) compared to the control. The high content of P in the wood ash made the P_{ox} and DPS higher than in lime treated soils. This may be due to the fact the P available in the wood ash was immediately fixed by the amorphous Al_{ox} and Fe_{ox} present in the soil which in turn increased the DPS.

Application of manure P, mineral P, manure P with lime, mineral P with lime, manure plus wood ash and mineral P plus wood ash significantly ($P \leq 0.001$)

increased the values of P_{ox} and DPS values. The maximum increment of P_{ox} (24.9%) and DPS (24.2%) were recorded at the application of 130 kg mineral P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}) compared to the control treatment. The DPS with respect to Al_{ox}-P ranged from 0.57 to 1.48% and Fe_{ox}-P ranged from 5.91 to 7.10% where the lowest and the highest DPS were observed at the control and the application of 130 kg mineral P ha^{-1} plus wood ash (7.0 tons $CaCO_3$ ha^{-1}), respectively. This result showed that more of Fe_{ox} are occupied by P as compared to Al_{ox}. In line with this, the percentage recovery of the applied P as Al-P was greater than Fe-P. This clearly indicated that Al_{ox} had high tendency of P fixation than that of Fe_{ox}. All the treatments resulted in the reduction of the Al_d and Fe_d compared to the control treatment. The reduction for the value of Al_d varied from 0.1 to 7.5% and Fe_d from 0.6 to 4.4%.

The reductions in Al_{ox}, Fe_{ox}, Al_d and Fe_d contents as the result of manure and mineral P application may be due the fixation of Al and Fe in the form of Al-P and Fe-P. Both the amorphous and crystalline Al and Fe are the potential P fixation sites and their role can be explained as follow. Upon hydrolysis, the amorphous and crystalline forms of Al and Fe expose much larger surface area where high affinity for fixation of P takes place on protonated sites. The fixation could also take place by the hydroxyl displacement by $H_2PO_4^-$ ions from the surface of hydrous oxides of Al and Fe. Similar findings were also made in Kaistha et al. (1997). The complex formation of Al and Fe by the organic acids produced as the result of mineralization of the manure may compute with the citrate ion to extract Al and Fe. This may result in the reduction of the amount of Fe and Al to be extracted by citrate dithionite bicarbonate.

Conclusion

The result of this study showed that applications of lime and wood ash to obtain a target pH of 6.5 significantly increased the pH of the soil, decreased the exchangeable acidity and exchangeable Al almost equally. However, wood ash performed better in increasing the available P, the easily soluble P, Al-P, Fe-P, P_{ox}, and DPS compared to lime. Application of manure P and mineral P plus lime and wood ash at target pH value of 5.5 performed very well in alleviating acidity related problems compared to the corresponding separate applications. Mineral P application with wood ash gave the highest available P, easily soluble P fraction, P_{ox}, and DPS. Application of manure P plus wood ash also resulted in comparable values.

Applications of manure and other organic residues to soils in the highlands of Ethiopia, where there is acidity and P fixation problems, could result in improved soil and fertilizer P use efficiency by crops partly as a consequence of increasing availability P and pH, reducing exchangeable acidity and Al and supplying N to the soil. Therefore, knowledge of the extent to which applications of wood ash and organic residues to reduce lime and fertilizer P requirement is needed so that integrated soil fertility management programmes can be planned. In line with this, use of wood ash for soil acidity management must be controlled to maximize its benefits and prevent adverse effects on the environment or future fertility/sustainability of the farms to which thay are applied.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by Center for International Mobility (CIMO), Finland, the University of Helsinki Future Funds (Tulevaisuusrahasto) and Ethiopian Ministry of Education.

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Vol. 11(2), pp. 100-104, 14 January, 2016
DOI: 10.5897/AJAR2014.10334
Article Number: 9F7D93656666
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Economic and financial analysis of the implementation of a water-tube boiler in the process of grain drying using a column dryer

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Received 22 August, 2015; Accepted 29 October, 2015

The aim of this study was to carry out an economic and financial evaluation of the use of a dryer column with steam system replacing a conventional furnace. Seeking an energetic and economic evaluation of the process of grain drying, a research was carried out on the drying of corn kernels using a dryer column (Comil CM 150 DR, double reuse) and a water-tube boiler (CW 40). In order to perform economic and financial analysis, the net present value (VPL), internal rate of return (TIR), profitability index (PI), rate of return (RR) and discounted payback were calculated. In the economic analysis, an investment of US$ 155,258.84 and a minimum acceptable rate of return (MARR) of 3.5% per year were considered. The VPL within 15 years was US$ 200,612.85. The TIR was 17.89% per year. The project presented a PI of 1.29% and RR of 29%, which were considered acceptable. The payback period, considered as the time required to recover the capital invested in the project was 7 years, which indicates economic feasibility.

Key words: Energy, firewood, storage.

INTRODUCTION

The purpose of drying agricultural products is to ensure their quality. This is done by reducing the moisture content, biological activity, chemical and physical changes that occur (Oliveira et al., 2014). The drying study provides information on the heat and mass transfer occurring between the biological material and drying element (atmospheric air normally heated or unheated), which is crucial for the design, operation and simulation drying systems dryers (Corrêa et al., 2003).

According to Pacheco (2010), grain drying is defined as the unit of operation where liquid is removed from a solid to an unsaturated phase, by means of thermal

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Figure 1. AWD 40 water-tube boiler. Source: Picture taken by the authors (2013).

evaporation. It occurs at a temperature inferior to that at which that liquid boils in the system pressure.

As stated by Afonso (2011), high moisture content in the product during storage contributes to the loss of germination power and seed vigor. It affects the quality of starch and protein and also induces acidity. It may lead to microbial contamination and affect physical structures (whole grains). High moisture content also impairs a proper preservation of the product, causes loss of nutritional content, which is required by the industrial sector, and affects the average chemical compositions and energetic values of the species during storage or industrialization.

Therefore, drying is the stage of pre-processing of agricultural products whose main purpose is to remove part of the water contained in them. According to Bremm and Chiodelli (2012), drying is the post-harvest process that has the highest energy consumption, thus, it is primordial to use dryers that consume a minimal amount of energy to remove as much water as possible from the product to reach the desired final condition.

According to Groff (2002), the energy required in the drying process is an expensive and scarce input, as the energy consumed will always be higher than the latent heat of vaporization of water, which is around 540 kcal/kg H_2O.

Nogueira (1985) presented a viable alternative to the use of biomass as a source of heat, aiming to preserve the quality of the final product by installing a system consisting of a boiler associated with one or more heat exchangers and replacing the solid fuel furnaces. In this process, the steam supplied by the boiler flows through the heat exchanger, which is heated. The dryer fan forces the passage of ambient air through the heat exchanger, injecting it into the mass of grains, which causes the drying. Some advantages of employing this system are: the possibility of installing several dryers in parallel; fuel and labor economy; lower risk of fire; reduction of environmental pollution levels and uniformity of drying air temperature. As a major disadvantage, the cost of the initial investment for implementation and system maintenance is high.

Benecke (2013) and Sobrinho (2001) presented the water-tube boiler system, which consists of boilers with water walls or water tubes. The water flows through the tubes, which are heated by the flames of a furnace with indirect heating, and circulates in the heat exchanger.

Afonso (2011) indicates that the dryer column is the most recent equipment to be introduced in the drying process. It has a series of internal vertical modules (columns) through which the product flows. In order to carry out the economic and financial analysis of an energy project, the following factors must be calculated: net present value (NPV), internal rate of return (IRR), profitability index (PI), rate of return (RR) and discounted payback (Kassai, 2005).

The aim of this study was to carry out an economic and financial evaluation of the use of a dryer column with steam system replacing a conventional furnace, by analyzing the potential for reducing fuel consumption (eucalyptus firewood), with a system proposed for the drying of corn.

MATERIALS AND METHODS

The study was conducted in the grain storage unit of an agricultural cooperative located in the West of Paraná State, Santa Helena, at a latitude of 24°51'37" south, longitude of 54°19'58" west, and altitude of 258 m.

A complete boiler system was installed (Figure 1) with water pump for high temperature; a furnace induced for burning wood and other solid fuels was properly sized to a dryerwith capacity of 150 tons. A water-tube boiler with heat exchanger was used (Figure 2); there were three gas passages inside the furnace, which was dimensioned to ensure smoke tubes at the exit of gases, steam production of 4000 kg/h, working pressure of 8 kgf/cm^2 (7.845 bar; 113.79 psi), hydraulic test pressure of 12 kgf/cm^2 (11,768 bar; 170.68 psi), heating area of 160 m^2, fuel wood and other solid fuels, maximum temperature of 446°F (230°C), corresponding to a thermal efficiency of 85%. The control panel is fully automatic with manual option.

The boiler AWD, model CW 40 Water tube type, operated with a working pressure of 8 kg/cm^2 (7,845 bar; 113.79 psi); it used wood Eucalyptus (45% moisture content and higher calorific value of 16 082 kJ/kg^{-1} and 3,842 kcal/kg firewood) as a solid fuel, and operating temperature varying from 185°F (85°C) to 221°F (105°C) (Oliveira, 2014).

Water-tube boilers

In the water tube boiler, the water passes through the tubes, which

Figure 2. Heat exchanger. Source: Picture taken by the authors (2013).

in turn are heated by the flames of a furnace with indirect heating circulating in the heat exchanger.

Column dryers

Column dryers are drying towers made up of panels and intercalated ducts. It is where the grain mass moves vertically and receives the flow of hot air from the furnace, providing the drying of grains. They have shutters for air intake, which enable the ambient air to mix with the air from the furnace, thus providing the ideal mixture for drying.

The monitoring of the drying air temperature is held at three points of the tower, using sensors located at the entrance to the first drying stage, at the second stage and at the outlet for the fan.

The volumetric discharge is a system consisting of a set of trays in a galvanized steel plate that oscillates horizontally and moves up to the grain column, loading up a specific volume and then unloading it to the lower funnel. The discharge and the lower funnel are modulated, fitted with inspection covers and devices that allow better access for maintenance and cleaning.

Economic analysis of the investment

For the economic analysis, it was considered the calculations of discounted payback, net present value (NPV) and internal rate of return (IRR) (Kassai et al., 2005). These alternatives are the most consistent for investment analysis.

In the analysis of economic and financial indicators, the first step is to determine the cost structure, selling price and the breakeven point. It was analyzed the system of financing, the projection of cash flow and the projection of the Profit and Loss Statement (P&L). Braga (1989) cites the following as the most widespread methods for evaluating investment proposals: accounting rate of return, payback period, net present value and internal rate of return. According to Oliveira (2005), in a projection of investments, the residual value of the investment should be taken into account in the last year. This value is 30% for buildings (civil works) and 10% for machinery and equipment. Thus, in the financial calculations for the last year (15th year) in this study, it was added an extra revenue to the income concerning the residual value. In the structure of expenses, it was developed a cost structure in order to observe more clearly what happens with the development of the business. Fixed costs are expenses that are independent of the quantity produced. They do not vary and are recorded at each production period, that is, they remain constant in a certain period of time and do not increase due to the volume of production and sales (Braga, 1989).

Costs of repairs and maintenance of assets and facilities are the expenses necessary to preserve the buildings, improvements, facilities, machinery and equipment in working condition (Ocepar, 2003). Maintenance of equipment should always be carried out in order to preserve proper functioning and long life cycle. Maintenance expenses must be counted.

Variable costs are expenses that happen according to the amount produced, in other words, they represent the inputs used in the production process (raw materials, sales commissions) and other expenses (Hoji, 2003).

For calculation purposes, it was considered net revenues as the price differences between the installation of the steam drying system and the conventional system of furnaces.

The total cost is the sum of fixed costs and variable costs, that is, the revenue that can be achieved with a reduction in the consumption of firewood.

According to Matarazzo (2003), cash flow is the discrimination of amounts of money being transferred into and out of a business during a specified period of time, thus leading to the assessment of the cash balance and enabling several economic analyses. The main goals of the discrimination of cash flow include: assessing alternative of investments; assessing and controlling important decisions that have monetary consequences taken by the company; assessing the current and future cash flow situation in order not to achieve a liquidity situation; certifying that the current cash excesses are being correctly applied.

Braga (1989) states that in the forecast of the profit and loss statements, the profit and loss of the fiscal year can be verified by the difference between the revenue and expenses incurred.

According to Hoji (2003), in order to achieve economic and financial results based on a budget framework, one should analyze the financial transactions of several equity accounts, as well as profit and loss accounts. The economic indicators of investments are described below.

Setting a minimum acceptable rate of return or hurdle rate is necessary for the financial analysis of a project as a source of comparison for the investment to be made. That is, in case this rate is not acceptable, the project should be rejected (Kassai, 2005).

The net present value is a sophisticated and useful tool to assess proposals of capital investment as well as to show wealth of the investment in monetary values. It is measured by the difference between the present value of cash inflows and the present value of cash outflows, discounted by a determined sum (Kassai, 2005).

The values for calculation refer to the net results of each year obtained from the P&L statement, which are considered as positive flows, and to the investment, which is the negative flow. The following equation was used for the computation of the NPV:

$$NPV = \left[\sum_{t=0}^{n} \frac{CF_t}{(1+i)^t} \right.$$

where: NPV, Net present value; t, Time (months or years); n, Project lifespan (total of months or years); i, minimum acceptable rate of return; CF, net cash flow at time t.

Table 1. Financial indicators overview.

Investment	R$ 350,000.00
Repayment	15 years
MARR	3.5% per year
NPV	US$ 200,612.85.
IRR	17.89%
PI	1.29
RT	29%
Discounted payback	7 years

Source: author (2013).

For the minimum acceptable rate of return, any investment with NPV equal or above zero is considered acceptable. The internal rate of return is used in capital budgeting to measure and compare the profitability of an investment. It is the discount rate at which the net present value of costs (negative cash flows) of the investment equals the net present value of the benefits (positive cash flows) of the investment (KASSAI, 2005).

$$\text{IRR = i when } \left[\sum_{t=0}^{n}\frac{CF_t}{(1+i)^n}\right]=0$$

where: IRR: Internal rate of return.

Any investment that presents an IRR superior to the MARR is economically viable (KASSAI, 2005).

The profitability index and rate of return refer to the ratio of payoff to investment of a proposed project. That is, a PI greater than 1.0 indicates that the profitability is positive (Neto, 2000).

So:

$$\text{PI} = \frac{\text{PV (positive cash flow)}}{\text{PV (investment)}}$$

The profitability index of the proposed project is larger than 1, what is considered acceptable. The rate of return of an investment is represented by relative values, that is, percentage. Every investment with a rate of return above zero is considered acceptable. The rate of return of the investment is given by the following equation:

RR= (PI – 1) x 100

The discounted payback period is considered the exact time period required to recover the capital invested in a project (Gitman, 2002). With analysis of financial index, it is easy to ascertain the period of time that a company will need to recover what was invested before, having a better view of the business that is proposed.

RESULTS AND DISCUSSION

According to Silva (2004), the decisions about investments refer to the application of assets, as well as to the expected return and the risks posed by these assets.

The price quoted for the supply of the boiler and the two radiators described above, properly dimensioned for the dryer, was US$ 155,258.84. The Storage Incentive Program for National Grain Companies, known as BNDES *Cerealistas*, provides financial support with repayment periods from 12 to 15 years and annual interest of 3.5% (BNDES, 2013).

In the cost structure, the National Bank for Economic and Social Development (BNDES) will finance US$ 155,258.84 through the net of financial agents associated with the development bank. The budget will be part of the investment support program (ISP), which finances the construction and expansion of silos and auxiliary structures. The loans will last for 180 months (15years) at an annual interest of 3.5%. The investment of US$ 155,258.84 will be made by the company AWD Metalúrgica em Geral Ltda. The annual maintenance of the boiler costs US$ 6,210.35, according to the Ocepar (2003).

The variable cost for the drying system (annual value of electric energy) will be US$ 3,619.39. The total annual value, represented by the sum of fixed and variable costs, will be US$ 9,829.75. For calculation purposes, the revenue will be the difference of price between the installation of a steam drying system and the installation of the conventional firewood boiler system, according to the projections for reception and drying of 18,000 tons of soybean, 48,000 tons of maize and 3,000 tons of wheat (Oliveira, 2014). Considering an average price of US$ 19.96 for the cubic meter of firewood, it is possible to achieve an annual revenue increment of US$ 39,923.70, with an average reduction of 40% in the consumption of firewood (= 5,000 m^3/year) for the parameters obtained for corn kernel drying, using a tube dryer in a water-tube boiler system. The financial indicators are summarized and depicted in Table 1.

The cash flow forecast presented a net present value of US$ 200,612.85 considering an investment of US$ 155,258.84 and annual gross revenue of US$ 39,923.70, with capital return and revenue at the 15th year of US$ 55,449.58. The minimum acceptable rate of return was considered 3.5% per year; the same value for the line of credit (according to the BNDES) for financial investments supported by the Harvest Plan (Plano Safra) 2013/2014 (Mapa, 2013). All economic index shows that the proposed project is feasible (NPV larger than zero, IRR larger than MARR, PI larger than 1 and RT larger than zero).

The discounted payback period is considered the exact period of time necessary to recover the costs of the investment, considering the annual cash inflow (Gitman, 2002). Based on the cash flow forecast, the discounted payback period was reached in the 7th year, as the annual positive cash flow was able to return the invested capital.

Conclusion

According to the analysis of the economic indicators, based on the cash flow and revenue growth forecasts, the project requires an investment of US$ 155,258.84 (approximately R$ 350,000.00). Considering a minimum acceptable rate of return of 3.5% per year, the net present value for a period of 15 years will be US$ 200,612.85. The internal rate of return is 17.89 per year. The project presented a profitability index of 1.29% and rate of return of 29%, which is considered acceptable. The payback period is 7 years, which indicates economic feasibility.

Conflict of Interests

The authors have not declared any conflict of interests

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academicJournals

Vol. 11(2), pp. 105-116, 14 January, 2016
DOI: 10.5897/AJAR2014.10363
Article Number: 0504B1B56674
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Extraction and characterization of the essential oils from *Spondias mombin* L. (Cajá), *Spondias purpurea* L. (Ciriguela) and *Spondia* ssp (Cajarana do sertão)

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Received 30 August, 2015; Accepted 20 November, 2015

This research aimed at extracting and characterizing the phytochemistry of the essencial oils from *Spondias mombin* L. (Cajá), *Spondias purpurea* L. (Ciriguela) e *Spondias* sp (Cajarana do sertão). We selected five vigorous and healthy trees located in the city of Patos, PB. The leaves were collected and taken to Natural Products Research Lab (NPRL) in the Regional University of Cariri - URCA - in the city of Crato, CE. The extraction method was the steam-dragging distillation through a Clevenger extractor. The chemical analysis of the oils were done by means of a Shimadzu spectrometer. The greatest components found in the Cajá essential oil were: octadecane (43.51%), heptacosane (21.98%) e hexatriacontane (15.37%); the predominant substances in the Cajarana do sertão oil were: Octadecane (31.5%), Indene (22.53%) and tetraacantane (10.51%), and in the Ciriguela, there were: heptacosane (28,80%), nonadecane (19,47%) e tetracosane (17.02%). Among the terpenes, we emphasize the β-caryophyllene (Cajá e Cajarana do sertão) e o α-humuleno (Ciriguela), that present important antimicrobial action. In the Cajarana do sertão, we identified the phytol, known for enhancing the tenacity and balancing the flow of skin natural oil, causing great anti-aging benefits.

Key words: *Spondias*, essential oils, gas chromatography.

INTRODUCTION

The Caatinga is the only exclusively Brazilian biome, with an area of 844 453 km^2, with a native vegetation area of 518 635 km^2 order, which is equivalent to 62.77% of the mapped area of the biome. The biome is approximately 54% of the Northeastern region and 11% of the Brazilian territory. It is between the parallels of the 2°54'S to 17°21' and involves areas of the states of Ceará, Rio Grande do Norte, Paraiba, Pernambuco, Alagoas, Sergipe, southwest of Piauí, interior parts of Bahia and part of Minas Gerais (IBGE, 2010), being considered the richest semi-arid biome in the world in biodiversity (MMA, 2009).

According to Araújo et al. (2007), the northeastern semi-arid region of the polygon of droughts, its characterized by low rainfall (average 700 mm).

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Precipitation almost always focuses in the months from February to May. The potential evaporation is very high (can reach to 3,000 mm / year), this region has average temperatures ranging from 20 to 28°C.

Araújo et al. (2007) report that the forest cover in the semiarid region has declined dramatically, a fact caused by lack of proper management and the type of operation adopted. Emphasize that the exploitation of Caatinga vegetation is used for various purposes, highlighting the use of wood for energy, rural buildings, commercialization of fruits such as medicinal plants, perfume and cosmetics. The essential oils have been shown to be an alternative to add value to forest species of semiarid region. The International Standard Organization (ISO) defines essential oil as the product obtained from parts of plants by distillation with water steam and the product obtained by expression from pericarp of citrus fruit.

For Bakkali et al. (2008), many consider essential oil equivalent to the soul of the plant - it is exactly the produced substance that lends flavor and taste by which it is known. Unlike olive oils and lipid (fatty) oils, essential oils are produced in minimum quantity and are quite volatile (hence its odor fills the entire environment quickly). In general, several hundred of kilograms of fresh plants is needed to produce only one essential oil kilogram. This is to say that the use of a single drop (or less) of the product aromatically equivalent to several hundred grams of the herb, which opens up numerous culinary and therapeutic possibilities.

According to Angnes (2005) and Almeida et al. (2010) the popular medical practice, essential oils have a long tradition of use as medicinal agents. There are pictorial records of six thousand years ago, among the Egyptians, of religious practices associated with curing ills, the royal anointing, and physical well-being search through the aromas, obtained from specific parts of certain plants, such as resins, leaves, flowers and seeds. Flavour were already popular in ancient China and India, hundreds of years before the Christian era, when they were incorporated into incense, potions and various types of accessories, used directly on the body.

The term itself "aromatherapy" would have been created by a French chemist in 1928 and whose name was Maurice Rene Gattefosse. Gattefoss came to be fascinated by the therapeutic possibilities of essential oils and from a personal experience with lavender oil. Until then, Gattefosse used essential oils in their products and creations for the purpose of perfuming, but without any therapeutic basis. Making a distillation in his laboratory, there was an accident where the product was flammable, fell into his arms, causing a serious burn. In an act without thinking, he plunged his arms in a lavender tub, he thought was water and realized immediately that the sensation of pain soon passed. Within days, the wound had healed and in the burn place was not even a scar. This led him to become interested in researching the therapeutic possibilities of essential oils (Mccaffrey et al., 2009).

The antimicrobial properties of extracts and essential oils obtained from medicinal plants have been empirically recognized for centuries, but only recently have been confirmed scientifically. Several researchers study the biological activity of medicinal plants from different regions of the world, guided by the popular use of native species, showing that its extracts and essential oils are effective in controlling the growth of a wide variety of microorganisms, including fungus, yeasts and bacteria (Almeida et al., 2010).

Besides antimicrobial activity, essential oils present analgesic properties, anti-inflammatory, antimalarial, anti-carcinogenic, anticonvulsant, antioxidant, gastro-protective and acetylcholinesterasic. This latter property is of great interest in controlling Alzheimer's disease, progressive neurodegenerative disease that primarily affects the elderly population accounts for 50 to 60% of cases of dementia in people over 65 years (Barbosa-filho et al., 2008; Asuquo et al., 2013).

One of the most promising treatments for this disease is the increased levels of the neurotransmitter acetylcholine by inhibiting the enzyme acetylcholinesterase. Essential oils of *Salvia lavandulaefolia* inhibited the enzyme in question both in vitro and in vivo test. Some monoterpenes isolated as alpha- and beta-pinene, and fencholfenchone also were effective in inhibiting acetylcholinesterase (Barbosa-filho et al., 2008; Almeida et al., 2010).

In Brazil, one of the sources initially exploited for the extraction of essential oils, was the rosewood. Its exploitation was such that until today, the InstitutoBrasileiro do MeioAmbiente (IBAMA) included in the Species List in Danger of Extinction (Decree 37/92 of 04/03/1992) (Ferraz et al., 2009). Other vegetables were also explored, such as eucalyptus, lemon grass, mint, orange and cinnamon. Due to the difficulty to import essences, and increased global demand for Brazilian production caused by the Second World War, Brazil with it had most of its sales aimed at exports, which helped to significantly increase production. In the 50's, another factor contributed to the increased extraction of essences within the country; International companies producing perfumes, cosmetics and pharmaceuticals and foodstuffs have settled in the country (Bizzo et al., 2009).

According to Bizzo et al. (2009) flowers, leaves, bark, rhizomes and fruits are raw materials for their production, like the essential oils of rose, eucalyptus, cinnamon, ginger and orange, respectively. They have wide application in perfumery, cosmetics, food and as adjuncts in drugs. They are mainly used as flavorings, fragrances, fragrance fasteners in pharmaceutical compositions and oral and commercialized in raw form or benefited by providing purified substances such as limonene, citral, citronellal, eugenol, menthol and safrole. In general, the species have specific times in which they contain higher amount of active ingredient in its tissue, which change may occur both within one day as in times of the year (Almeida et al., 2010).

The food plants, feed and fiber culture are not the only of importance on the market today. Other species, whose secondary metabolism are valued for its aromatic and therapeutic characteristics, or because they are raw materials for industry, either as active ingredients and flavor and perfumery industry fragrances, or as constituents in product formulations for hygiene and health, and are also widely used in alternative medicine, also have economic importance (Almeida et al., 2010). Because of this, demand has increased by essential oils. Some specific components of these oils are used as aids in synthetic organic chemistry and common structures of transformations, in order to obtain highly functional substances of recognized economic value.

According to Bakkali et al. (2008), there is a lack of chemical composition of essential oils not only from the Myrtáceas, as well as all Brazilian flora rich in relation to secondary metabolism. According to Bakkali et al. (2008), there are about 3000 essential oils known, of which 300 have commercial importance for the pharmaceutical, food, cosmetics and perfumes and agronomy. Among the species that produce essential oils that contribute significantly in global sales volume, the study can highlight the mint (*Mentha pipperita*), citronella (*Cymbopogon winterianus*), capim-limão (*Cymbopogon flexuosos*), eucalyptus (*Eucalyptus*), the rosa (*Rosa damacena*), geranium (*Pelargonium graveolens*), lavender (*Lavanda officinalis*) chamomile (*Chamomilla recutita*), sândalo (*Santalum álbum*), manjerona (*Origanum majorana*) and sálvia (*Salvia officinalis*).

Among the species of the genus *Spondias* belonging to the family *Anacardiaceae* attracting interest from agribusiness are Cajarana do sertão, Umbu, Cajazeira, Umbu-cajá and ciriguela. The demand for the fruits of this genre is mainly due to the interest for industrialization and consumption "in nature". The fruits of *Spondias* are well accepted in the industry for having good quantity of nutrients and good looks. For this reason, its pulp has been quite popular and utilized by the food industry. They are consumed raw, sold in local markets or on some Brazilian highways margins (Santos and Oliveira, 2008).

The *Anacardiaceae* family is represented by about 80 genera and 600 species, which are known to produce tasty fruits, excellent wood, compounds used in industry and medicine. The *Spondias* genus has 18 species, some are grown in the Northeast and are tropical fruit trees in domestication and exploited for their commercial value. Among the species of the genus *Spondias* that stand out in the semi-arid northeast are Cajarana do sertão (*Spondia* ssp), Umbu (*Spondias tuberosa* Arruda Câmara), Ciriguela (*Spondias purpurea* L.) and Cajá (*Spondias mombin* L.).Being the umbuzeiro, the only endemic in the Brazilian semi-arid. Other species also grown in Northeast Brazil are the Umbu-cajá *Spondias* sp.), Cajá-manga (*S. cytherea* Sonn) and (umbuguela (*Spondia* ssp) (Agra et al., 2007; Santos and Oliveira, 2008; Almeida et al., 2010).

The Cajá (*Spondias mombin* L.) also called ambaló, cajá-Mirim, cajazeira, cajazinha, taparebá or tapiriba originating in the tropical region of the Americas. It is tall, reaching up to 15 feet tall, deciduous leaves and trunk covered with thick, rough skin that esgalha and branches at the back, giving a high size plant. The canopy is large, showy and imposing when flowering and fruiting phase. The fruits are fragrant nuculânios with fleshy mesocarp, yellow sweet and sour flavor, containing carotenoids, sugars, vitamins A and C (Barbosa-filho et al., 2008), are well appreciated in kind or in the form of pulp, jams and ice cream.

The Cirigueleira (*Spondias purpurea* L.) is also called siriguela, cajarana-of-spain, ciroela, ciriguela and Mexican ciruela. It is a deciduous tree 3 to 6 m in height. Pinnate leaves measuring 18 to 24 cm and 9 to 11 pairs of membranous leaflets about 2.5 cm long. Discrete flowers, unisexual (male and female) and androgynous on the same plant are formed during spring along with the sprouting of new foliage. Fruits of the drupe type, with sweet-acidic pulp are very tasty. The fruit of 15 to 20 g and ellipsoidal form of 3 to 5 cm long, smooth and shiny, purple or wine with firm epicarp, presents a yield of 50% pulp and is used to make juices, ice cream, liquor, wine, jam, compotes and soft drinks in natura. It has a fleshy mesocarp, yellow, 5 to 7 mm thick, is sweet, sour, very pleasant taste. The endocarp (seed) occupies most of the fruit (Asuquo et al., 2013). It is one of the most cultivated species of the genre. It is a species native to Central America but is distributed in Mexico, the Caribbean and several countries in the northern region of South America, probably dispersed by man. According to Almeida et al. (2010), produces, in well-drained soil in tropical and subtropical climates and edible fruit with peculiar and pleasant taste. The adult plant rarely exceeds 7 m high, hardly propagates by seed and its multiplication by human action takes place by stakes. Begin fruiting in the third year after planting in the field. A mature plant can produce between 80 and 120 kg per year. Under the alimentary point of view, this is an extremely rich fruit in carbohydrates, calcium, phosphorus, iron and vitamins A, B and C. The fruits and leaves can be seen on Figure 1.

Cajarana do sertão (*Spondia s*sp) is a plant of the Society Islands in Oceania. It is fast-growing tree, with thick and brittle branches, compound leaves of 11 to 13 leaflets. Flowers arranged in large terminal panicles. The fruits come in bunches. They are Ellipsoids drupes or slightly obovóides of thin yellow skin, measuring approximately 3.0 cm. The pulp is compact, pale yellow, juicy, sweet or acidified, covering a bristly seed long timber beams deeply ingrained in the mass of pulp. Edible natural, tasty, fruit give good soft drinks, popsicles and ice cream (Asuquo et al., 2013).

Barbosa-Filho et al. (2008) studying the umbu-cajazeira

Figure 1. *Spondias purpurea* L. fruits and leaves.

(*Spondias* spp), which is a similar hybrid to Cajarana do sertão (*Spondia* ssp), reports that the fruits have excellent taste and aroma, good appearance and nutritional quality, very consumed in the form "in natura", with average yield of 55 to 65% in pulp, with potential for use as processed as frozen pulp, juice, nectars and ice cream. For descriptions of Almeida et al. (2010), the species of *Spondias* are better adapted to lowland tropical hot. The trees grow best in well-drained fertile soils, but, if properly nurtured, can also develop satisfactorily in several poor soils. It can be seen on Figure 2.

Due to the diverse use of Caatinga species, and the lack of information on the vegetation of the same, it is clear the importance of knowledge of the phytochemical characteristics of the occurrence of species in the Brazilian semi-arid region, in order to create a species database present in the Caatinga and hence indicate the species with the potential to produce essential oils, adding economic value to these species (Agra et a., 2007)

MATERIALS AND METHODS

Characterization and location of study area

The present study was conducted in the city of Patos-PB in the semi-arid backlands depression, distant 301 km from the capital João Pessoa and its headquarters is located in the heart of the state. The state of Paraiba is located in the eastern portion of the Brazilian Northeast, between the meridians 34°45'45" to 38°45'45" West longitude and 06°02'12" and 08°19'18" latitude South, occupying an area of 56,732 km 2. Following the East to the West, in other words from the Coast to the Hinterland, we have: Coastal vegetation, Fields and Forests of Restinga, Mangroves, Humid Forest, Cerrado, Wasteland, Caatinga and MatasSerranas. The climate in Paraíba is characterized by high temperatures and scarce and irregular rainfall. In the inner part of the state the semi-arid climate dominates, registering this area high average monthly temperature of 25 to 30°C, and low rainfall 300 to 1000 mm bad rain distributed throughout the year, which conditions the strongly xerophytic vegetation (Tolke et al., 2011).

The Patos County is located in the state of Paraíba (Figure 3), in the semi-arid backlands depression, distant 301 km from the capital João Pessoa and its headquarters is located in the heart of the state, with road vectors connecting all the Paraíba and enabling access the states of Rio Grande do Norte, Pernambuco and Ceará. Its land area is approximately 512.79 square kilometers with a population of 100,732 inhabitants (IBGE, 2010). Its geographical coordinates are: 7,02°S and 37,27°W, with an altitude of 242 m. The climate according to the Köppen classification is the hot-Bsh semi-arid and dry, average annual temperature of 28°C and relative humidity of 55%. The driest period covers the months from July to February and the wettest in the period from March to June. The average annual rainfall is 675 mm with irregular rainfall distribution, although in recent years this index has been exceeding 1000 mm. Its soils are mostly shallow and rocky, typical of lithic soils, basically represented by the non-calcium-based Bruno soils.

Collect of samples

Five trees were selected from *Spondiasmombin* L. (Cajá), *Spondiaspurpurea* L. (Ciriguela) and *Spondiassp* (Cajarana do sertão) who had good health, located in the city of Patos-PB, with the following coordinates:. Cajá: 07°03'593' 'S and 37°16'498' 'W and altitude of 253 m; Ciriguela: 07°03'550" S and 37°16'539"W

Figure 2. *Spondia ssp* tree.

Figure 3. Map of Paraíba showing the location of the city of Patos. Source – IBGE (2010).

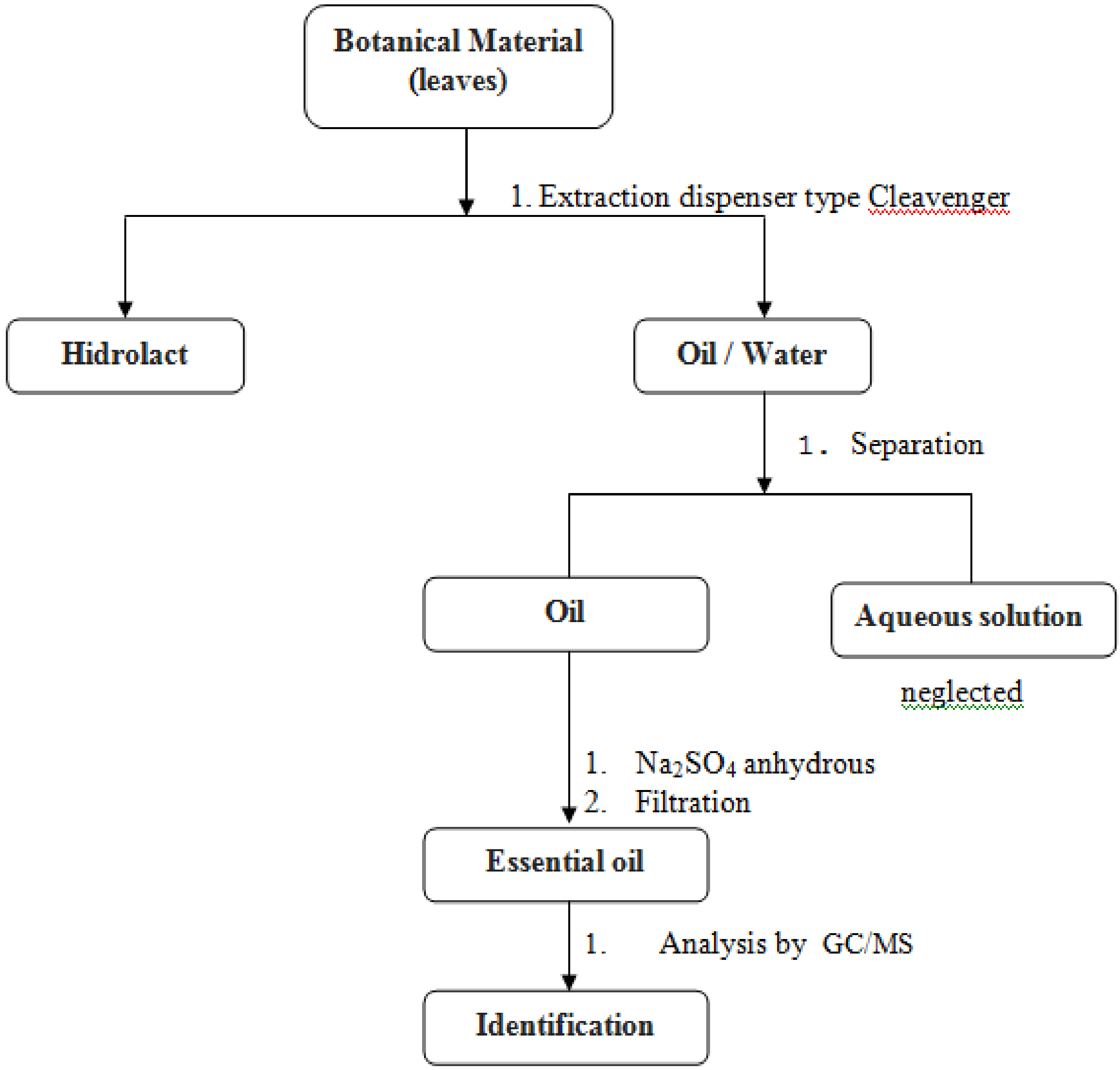

Figure 4. Flow chart depicts the method for the extraction of essential oils from the hog plum leaves.

and altitude of 256 m and Cajarana do sertão: 07°03'608"S and 37°16'488" W and altitude of 256 m, of which samples were collected while fruit, branches with leaves, flowers and fruits, at 7, 10, 14 and 17 h, with the help of pruning shears in ten different points of the canopy of each tree and mixed to form uniform samples, packed in plastic bags black in color, identified and put in the refrigerator until the travel time (23 h of the same day) to the Research Laboratory of Natural Products (LPPN) Regional University of Cariri - URCA - in Crato-CE. Part of excicatas were made of the samples that were deposited in the Herbarium CaririenseDárdano de Andrade- Lima Regional University of Cariri - URCA and the rest were for the extraction of essential oils.

Essential oil extraction

The extracted essential oils (1000 g leaves of each plant) (Figures 4 and 5) were obtained by hydrodistillation using a Clevenger type apparatus for a period of 3 h. Then the mixtures oil / water were collected, dried over anhydrous sodium sulfate (Na_2SO_4), filtered and stored under refrigeration until analysis.

Analysis by gas chromatography connected to a mass spectrometry (GC/MS)

The analyzes of the chemical compositions of the obtained oil were performed using a Shimadzu GC-17A spectrometer / QP5050A MS (GC / MS): DB-5HT capillary column (30 mx 0.251 mm); Carrier gas: helium 1.0 ml / min; 72.3 kPa pressure column; line speed = 37.2 cm / sec; the total flow 85 ml / min; carrier flow 85 mL / min; injector temperature 280°C; detector temperature 280°C; column temperature 60 (2 min) - 180°C (1min) at 4°C / min, then 180 to 260°C at 10°C / min (10 min). Operating under ionization energy of 70 eV. Hydrocarbon standards were injected with Kovats indices corrected by the straight line equation. The identification of the chemical compositions were carried out after analysis of the chromatograms of the respective oils (Figures 6 and 7), based on the spectral fragmentation using computer library (Wiley 229), the retention rates and comparison with the literature data (Adams,

Figure 5. Essential oil extractor, model Clevenger do LPPN-– URCA - Crato-CE.

2001; Alencar et al., 1990).

RESULTS AND DISCUSSION

In this experiment the first collection, the study found that the distillation did not contain essential oils and the second collection the study found the presence of essential oils, which shows the influence of time on the production of essential oil, which is in agreement with studies by Gonçalves et al. (2009) and Miranda et al. (2013). The sampling time for the extraction of essential oils has influence in some species. Thus, the material harvest time can be an important aspect in the production of oils.

According to Rocha et al. (2011) who studied the effect of lemon grass cutting time [(*Cymbopogom citratus* (DC) Stapf] as to campim citronella (*Cymbopogon winterianus* Jowitt) found that the actual harvest between 9 and 11 h provided better essential oil content and higher percentages of major constituents.

Nascimento et al. (2006) studying basil capim-santo

Table 1. Sample and oil mass and its percentage product.

Espécie	Leaves mass (g)	Oil mass (g)	Yield (%)
Spondiaspurpurea	2000	0,0518	0.0026
Spondiasmombin	1550	0,0564	0.0036
Spondiassp	1850	0,0746	0.0040

Table 2. Chemical compounds of the Cajá leaves essential oils (*Spondiasmombin* L.).

Constituent	Retention Time (min)	Percentage (%)
Ethyl acetate	3.56	0.70
4-hydroxy-4-methyl-2-pentanone	4.08	6.41
β-caryophyllene	30.83	1.24
2,4,10,15 tetrametilheptadecano	52.11	1.41
Octadecane	53.10	43.51
Tetracosane	55.33	8.62
Heptacosane	61.90	21.98
Hexa-triacontane	65.37	15.37
Total identified	-	99.24

(*Andropogum* sp) noted that the most essential oil content was obtained reaping the 8 h and distilling fresh leaves. Miranda et al. (2013) (*Cymbopogon citratus* (D.C) found that, when tested six harvest times, the best content occurred at 9 am and the worst at 7:17 h. In this study experiment was made a first crop (fresh leaves) on March 16 at the times of 7, 14 and 17 h and found that the distillation did not contain essential oils. Studying some articles, the study found that some species had best essential oils of income when the samples were collected in the time between 9 and 11 h. So the study made a second crop and selected 10 h time. Made distillations the study found the presence of essential oils.

Table 1 presented relationship between the data from the collection and the extraction of essential oils from species *Spondias mombin* L. (Cajá), *Spondias purpurea* L. (Ciriguela) and *Spondias sp*. (Cajarana do sertão). It is observed in Table 1, that the *Spondia ssp* had the highest production of essential oils followed by the species *Spondias mombin* and *Spondia* ssp although they are very close values. The values found for the *Spondias* study were lower than those found by Dhar and Dhar (1997), studying the *C. jwarancusa* species, which in stage I (green plants), yield was 0.5% and in stage IX (when the plants showed a third of leaves in brown color) yield of 1.64%. Santos et al. (2007) studied the *Lantana camara* L., obtained an oil yield of 0.12%.

The constitution of the essential oils of the studied Cajarana do sertão although it is mono- and sesquiterpenes, showed differences in their chemical composition, with a differentiation in the relative level of these components. The major compounds found in Cajá essential oil (*Spondias mombin* L) is octadecane (43.51%), heptacosano (21.98%) and hexatriacontano (15.37%) (Table 2); the oil Cajarana do sertão (*Spondia ssp*) predominate substances: octadecane (31.5%), indene (22.53%) and tetraacantano (10.51%) (Table 3). While the essential oil of Ciriguela (*Spondiaspurpurea* L.) (Table 4) are: heptacosano (28.80%), nonadecane (19.47%) and Tetracosane (17.02%).

Essential oils extracted from plants, from the chemical point of view, mainly consist of a mixture of lipids which are called terpenes. These terpenes are hydrocarbons and some may have oxygen in their structure, which are called terpenoids. The formation of these terpenes are based units isoprene (2-methyl-1,3-butadiene) according to the number of units may be called hemiterpenes (1), monoterpenes (2), sesquiterpenes (3), diterpenes (4), sesterpenos (5), tetraterpenes (8) and polyterpenes with many units. The analysis permits us to identify some components to be common to all three essential oils studied. In Table 5 comparison can be clearly seen.

In the analysis of the chromatograms of the species studied Cajá, Cajarana do sertão (Figure 6) and Ciriguela (Figure 7), where the x axis is in minutes and temperature TIC means: total ion chromatogram and the y-axis is the intensity measured in the presence of compounds IK (Kovats index). Tables 2, 3 and 4 showed the presence of terpenoids, among them mono- and sesquiterpenes on which 30,000 terpenes are known, classified according to the number of isoprene units: hemiterpenóides, C5; monoterpenoids C10; sesquiterpenoids. C15; diterpenoids, C20; triterpenoids andtetraterpenóides C30, C40 (Rocha et al., 2011). They have different functions in plants. The monoterpenes are the main constituents of the essential oils, acting in

Table 3. Chemical compounds of the Cajarana do sertão leaves essential oils (*Spondias* sp).

Constituent	Retention time (min)	Percentage
Indene	31.06	22.53
Decyl-octahydro-indene	37.62	6.19
Palmitic acid	50.78	8.22
Phytol	52.20	6.33
Acid eicosatrieno	52.48	3.07
Octadecane	54.21	31.5
Di-ethyl Hexanodionato	55.36	9.12
Tetra-acantane	61.91	10.51
Total identified	-	97.47

Table 4. Chemical compounds of the Ciriguela leaves essential oils (*Spondias purpurea* L.).

Constituent	Retention time (min)	Percentage
2-hydroxy-2-methyl-4-pentanone	4.06	2.70
β-caryophyllene	30.83	2.35
Indene	31.03	1.43
α-humulene	32.66	0.85
Germacremo-D	34.00	2.34
γ-cadineno	35.95	1.94
Caryophyllene oxide	38.94	1.20
Nonadecane	53.35	19.47
Adinol	55.35	1.07
Tetracosane	55.52	17.02
Lupenol	59.11	4.93
Heptacosano	59.23	28.80
Octadecane	63.36	9.81
Total identified	-	93.91

Table 5. Common components of the Cajá, Cajarana do sertão and Ciriguelas leaves' essencial oils.

Constituent	Leaves
Octadecane	Cajarana do sertão, Ciriguela e Cajá
Heptacosane	Cajá e Ciriguela
β-caryophyllene	Cajá e Ciriguela
Tetracosane	Cajá e Ciriguela
Indene	Cajarana do sertão, Ciriguela

attracting pollinators. The sesquiterpenes, generally have protective functions against fungi and bacteria, while many diterpenoids lead to plant growth hormones. Triterpenoids and derivatives steroidal feature a range of functions such as protection against herbivores, some are antimitotic, others act on seed germination and inhibition of root growth (Joy et al., 2007).

Among the terpenes found in the chromatograms in the species studied, the study highlights the β-caryophyllene (Cajarana do sertão, Ciriguela) and the α-humulene (Ciriguela), which has important antimicrobial action. Studies made by Rocha et al. (2011), with essential oil of Abiesbalsamea, found that 96% of the essential oil was composed of monoterpenes α and β-pinene, α and β-caryophyllene, humulene, which have significant antimicrobial activity against distinct pathogenic microorganisms.

The anticancer activity of some sesquiterpenes have also been reported, such as α-humuleno whose action was observed in solid tumor cells MCF-7, PC-3, A-549,

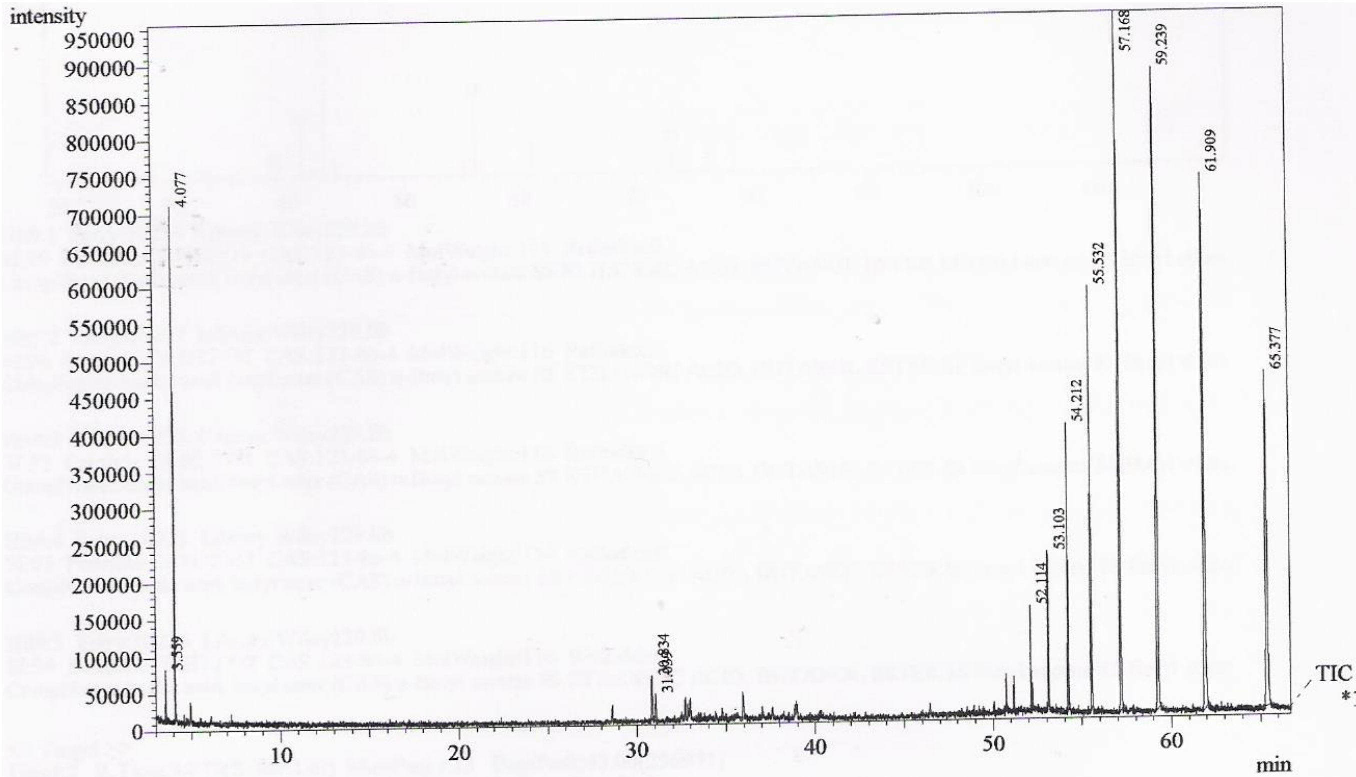

Figure 6. Chromatographic analysis of the Cajá essential oil.

DLD-1, M4BEU and CT-26. This compound was able to induce a dose and time-dependent decrease of cellular glutathione content and increased production of reactive oxygen species (ROS). These results suggested that both glutathione depletion and the production of ROS such as may be implied in its cytotoxic action (Martins et al., 2010).

Morais et al. (2006) reports that recent research with *Croton. zenhtneri*, *Croton. Nepetaefolius* and *Croton. argyrophylloides*, which is scientifically recognized by the anti-inflammatory properties, revealed that the anti-inflammatory action plan is a result of the essential oil action and that the active ingredient responsible for the action was identified as the α-humulenesesquiterpene.

Constantin et al. (2001) studying essential oils of *Piper reginelli* leaves, whose major components were myrcene and linalool and *Piper cernuum* with bicyclogermacrene and β-caryophyllene as major, showed activity against *Candida albicans* and *Staphylococcus aureus*, the authors attributed this activity to the major compounds. Costa et al. (2007) studying essential oils of *Lantana camara* L. and *Lantana sp*., found the predominance of sesquiterpenes, bicyclogermacrene (19.42%), isocariofileno (16.70%), valencene (12.94%) and sesquiterpene (12.34%) in the oil *L. camara*, while bicyclogermacrene (13.93%), germancreno D (27.54%) and β-caryophyllene (31.50%), stood out as major in *Lantana* sp oil. The *in vitro* antibacterial activity tests showed *L. camara* oil as inhibitor of growth of almost all tested bacteria (mainly *Proteus vulgris* (ACTCC 13315) and *Escherichia coli* (ATCC 25922) *S aureus* appear as resistant to the action of components present in the oil. The oil of *Lantana sp.* was more significant against *S. aureus* (ATCC 10390).

In the chromatogram of the Cajarana do sertão, terpenephytol was identified, it is known to improve the toughness and balance of the natural flow of oil from the skin, causing large anti-aging benefits and according to Dewick (2002) and Silva-Junior and Almeida (2013), the phytol is the reduced form of geranylgeraniol, that is one of the simplest diterpenes and important nature, since forming the lipophilic side chain of vitamin K and clorofilas. Also it was found in the hog plum fatty acid, palmitic, which according Melos et al. (2007) there are many studies in the literature reporting allelopathic activities relevant to many of the substances in green fronds *A. tetraphyllum*. For example, seeds of twenty-five different species of economic significance were treated with vegetable oils with the objective of evaluating the effect of such oils on the germination of such seeds. It was observed that among the species tested, there was complete inhibition of germination of *Allium cepa*, *Tritium aestivum* and *Zea mays*. The oil contained in its composition, long chain saturated fatty acids, palmitic and oleic acids. There are also reports in the literature identifying oleic, stearic and myristic, palmitic, linoleic and

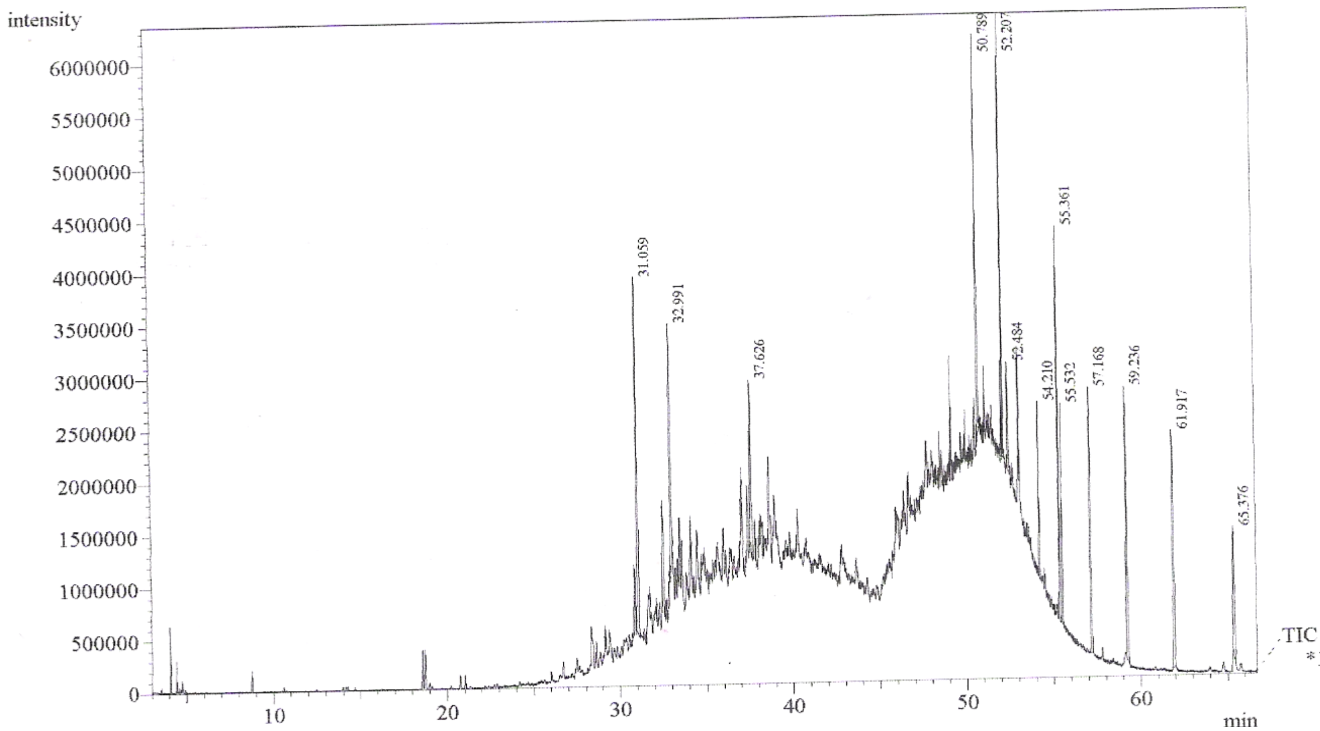

Figure 7. Chromatographic analysis of the Cajarana dosertão essential oil.

linolenic and other long-chain fatty acids as allelopathic agents, and the myristic acid and palmitic are the most potent sorghum germination inhibitors and the inhibitory capacity is inversely proportional to the size of the carbonchain acids. Saturated and unsaturated fatty acids in vegetable substances are common and evolutionarily are important to ferns, as a barrier against water loss.

Conclusions

Based on the results obtained, it can be concluded that sample collection time (leaves) interfere with the production of essential oils, since the analysis of the extracts from leaf samples collected 7 h did not contain essential oil. The presence of linear saturated hydrocarbons, of long chain (18 to 40 carbon atoms) were the main components in all species studied according to the chromatograms and these components mostly have even number of atoms in the literature where it quantifies the odd hydrocarbons in plant species as major. Therefore, there is little literature about his, because essential oils are rare. Some work is needed so that one can understand in detail the behavior of essential oils studied. In this sense it becomes important to submit some suggestions that may contribute to increasing the utility of these oils.

The *Spondia smombin* L species (Cajá) and *Spondias purpurea* L (Ciriguela), because they have in their essential oils terpene β-caryophyllene, even in small proportions, deserves attention and can be done *in vitro* test to try to find some possible inhibitions with antibacterial action; antiphlogistic; inhibiting activity of the enzyme acetylcholinesterase (AChE) in combination with 1,8-cineole and anti-parasitic (schistosomiasis).

The *Spondias* sp species (Cajarana do sertão) due to its essential oil in the terpenePhytol, which is a component of chlorophyll and known to improve the toughness and balance to the natural flow of oil from the skin, which becomes acid phytanic in contact with skin enzymes, resulting in broad anti-aging benefits and defense against the ravages of time and the fatty acid Palmitic Acid, trials to confirm the use of this essential oil in rejuvenating cream formulations.

The extraction of oils from these species can bring to this northeastern region of Brazil ways to sustainable development, in addition to the sale of its fruits that have an exotic flavor. As a consequence, the study has a possible way to keep people in their place of origin, working in their natural habitat, thus preserving it and reforesting it.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(2), pp. 117-129, 14 January, 2016
DOI: 10.5897/AJAR2015.10399
Article Number: 6FA9AF656676
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Distribution of gill Monogenean parasites from *Oreochromis niloticus* (Linné, 1758) in man-made Lake Ayamé I, Côte d'Ivoire

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Received 17 September, 2015; Accepted 16 October, 2015

A parasite study was conducted from August 2004 to July 2005 to highlight the distribution of gill Monogenean parasites from *Oreochromis niloticus*. A total of 370 fish collected from Ayamé man-made Lake, Côte d'Ivoire were examined for Monogenean species using routine parasitological techniques. The study revealed the presence of six species of Monogeneans (*Cichlidogyrus thurstonae*, *Cichlidogyrus halli*, *Cichlidogyrus tilapiae, Cichlidogyrus rognoni, Cichlidogyrus cirratus* and *Scutogyrus longicornis*). These species showed an aggregate pattern within the host population. Four species of parasites showed positive and significant correlation with parasitic abundance and the relative condition factor. The prevalence and intensity of the infection of these parasites were highest in the long rainy season and lowest in the dry season. The large sized fishes were more subjected to parasitic infection than smaller ones. In contrast, no sex effect was observed on the infestation of *O. niloticus* by the Monogeneans. No preference for the right or left side of the fish host was observed and the parasite species mostly colonized the middle arches (II and III). This could lead to more informed aquacultural practises, and maximization of the production of *O. niloticus.*

Key words: Disease, season, sex, infestation, gill.

INTRODUCTION

Monogenean trematodes belong to one of the most species-rich classes of fish parasites. They are commonly found on fish gills and skin. Nevertheless, some Monogenean species invade the rectal cavity, ureter, body cavity, nostrils, intestine, stomach and even the vascular system (Rohde et al., 1992; Pariselle and Euzet, 1998; Whittington et al., 2000). Their life cycle involves only one host and they mostly spread by way of egg releasing and free-swimming infective larvae (Öztürk and Özer, 2014). In the natural environment, Monogeneans are often in equilibrium with their hosts, and can cause serious morbidity and mortality during fish breeding (Buchmann and Lindenstrom, 2002; Kayis et al., 2009; Strona et al., 2010) resulting in significant economical losses (Ghittino et al., 2003; Johnson et al., 2004; Boungou et al., 2008; Velloso and Joaber, 2010). The Nile tilapia, *Oreochromis niloticus*, indigenous to the Nile basin, plays an important role in aquaculture globally

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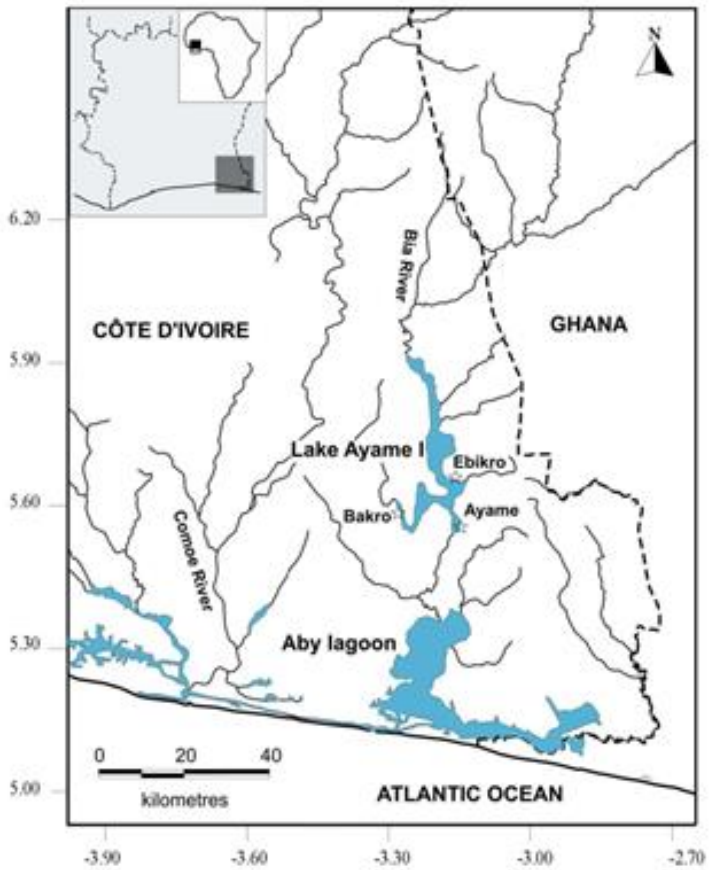

Figure 1. Geographical situation of lake Ayamé I (Côte d'Ivoire) and sampling sites (☆)

(Akoll et al., 2011) could face such problem. This fish has a great economic interest in Côte d'Ivoire (Gole-Bi et al., 2005). Indeed, the total annual yield of inland fisheries estimated at 18 000 tons are essentially dominated by Cichlidae, notably *O. niloticus* which represents between 50 to 70% of the total catches (Gole-Bi et al., 2005).

The Food and Agricultural Organization of the United Nations (FAO, 2009) reported that, to satisfy an increasing demand in freshwater fish, extensive research must include studies of their parasites for optimal production levels. The knowledge of fish parasites is of particular interest in relation not only to fish health but also to understanding ecological problems (Sures, 2001; Dudgeon et al., 2006). Baseline data, collected from infections within natural water systems, serve as reference point for management strategies.

In tropical Africa, Monogeneans parasites are mostly known in their taxonomical aspect (Pariselle and Euzet, 2009) and specificity and biological cycle (Aloo, 2002; Tombi and Bilong Bilong, 2004). Studies concerning eco-biological aspects of these parasites are scarce (Nwani et al., 2008; Keremah and Inko-Tariah, 2013, Tombi et al., 2014). To the best of our knowledge, there has only been two ichthyoparasitological studies (Blahoua et al., 2009, 2015) on infection dynamics of gill Monogenean parasites of Ivoirian fish.

Here we present some eco-biological aspects of Monogeneans' gill parasites of *Oreochromis niloticus* in Lake Ayamé I, Côte d'Ivoire.

MATERIALS AND METHODS

Study area

Lake Ayamé I, the oldest hydroelectric dam of Côte d'Ivoire being commissioned for hydroelectric generation in 1959 (Reizer, 1967) has an average surface of 90 km^2 and its maximum depth is 20 m (Gourene et al., 1999) (Figure 1). The reservoir is situated at 3°-3°5' W, 5°30'-6°N in the Southeast of the country. The climate of lake area in the Bia watershed is an equatorial transition zone with two rainy seasons separated by a short dry period in August and September and a more pronounced dry season from December to March. The lake is characterized by two high water level periods, April to June and September to November (with maximum discharge of about 500 $m^3 s^{-1}$). Conversely, low water level periods

Table 1. Prevalence, mean intensity, variance and dispersion index of parasite infra communities of *O. niloticus.*

Parasite species	Prevalence (%)	Mean intensity ±SE	Variance	Dispersion index (S^2/x)
Cichlidogyrus thurstonae	86.22	30.16±16.11	927.93	30.77
Cichlidogyrus halli	72.7	18.04±8.19	458.58	25.42
Cichlidogyrus tilapiae	49.19	17.17±4.27	346	20.15
Scutogyrus longicornis	39.46	9.3±3.48	150.8	16.26
Cichlidogyrus rognoni	29.54	5.6±2.51	57.6	10.28
Cichlidogyrus cirratus	14.86	2.44±2.03	15.7	6.42

SE = Standard Error.

are December to March and July to August (with a minimal discharge of about 0.13 $m^3 s^{-1}$). Mean annual water surface temperature in the reservoir is 28°C (Ouattara et al., 2007). Water surface temperature varied from 25.9 to 26.2°C during the small rainy season and from 29.8 to 32.7°C in the long rainy season (Blahoua et al., 2009). The average dissolved oxygen and water transparency ranged between 2.8 to 15.4 mgl^{-1} and 550 to 1371 mm respectively (Nobah et al., 2008).

Fish sampling and parasitological analyses

From August 2004 to July 2005, a total of 370 *O. niloticus* individuals were collected from the described three main landing sites every month, during 3 to 4 days per site. Fishing was done with multi-meshed nylon gillnets (8 to 90 mm mesh sizes). Each gill net measures 30 m long by 1.5 m deep. Nets were set overnight (17 to 07h) and during the following day (07 to 12h).

Once out of the water, the fish were immediately identified following Teugels and Thys van den Audernaerde (2003) keys. The weight of the fish were taken using digital weighing balance and recorded. The standard length (SL) of each specimen was measured to the nearest 1 mm on a measuring board. The effect of the parasites on the health status of the fish host was investigated from Fulton's condition factor (K-factor) with the following Formula: $Kc= W \times 10^5/L^3$, where W is the weight (grams) and L the standard length of fish (millimeters) (Klemm et al., 1992). The fish sample were divided into four different length classes of 50 mm amplitude, which are: Class I ($50 \leq SL < 100$), class II ($100 \leq SL < 150$), class III ($150 \leq SL < 200$) and class IV ($200 \leq SL \leq 250$). Fishes were killed by a single cut through the spinal cord and dissected to determine their sex. Subsequently, gill arches on each side were removed fresh from bucco-pharyngeal cavity by dorsal and ventral sections and then stored in ice (0°C).

At the laboratory, each gill arch was placed in a separate Petri dish containing water, and examined for Monogenean parasites under a binocular microscope (Olympus SZ 60). Gill arches from each side of the fish were numbered I-IV from the anterior portion of the gill arch below the operculum to the posterior. Individual worms were collected and mounted on a slide in a drop of ammonium picrate-glycerine mixture (Malmberg, 1957). The identification of the parasite species observed were done with a microscope magnification of 400 and 1000X, on the basis of available taxonomic characters as described by Pariselle and Euzet (2003, 2009).

The total number of parasites counted from the entire gill section was expressed in terms of prevalence, abundance and mean intensity following the formula proposed by Bush et al. (1997). Aggregation was calculated using dispersion index (S^2/x) to determine distribution pattern of parasites (Poulin, 1993; Shaw and Dobson, 1995).

Statistical analysis

The Sperman's correlation coefficient "rs" was used to investigate correlations of abundances of parasites with the relative condition factor of the hosts (Zar, 1996). The Chi-square (χ^2) test was used to compare two or more proportions (prevalence) of samples. Analysis of variance and Student U test were used to determine the existence of any meaningful difference in mean intensity of the parasites species. Statistical significance level was evaluated at of 5% using STATISTICA 6.0.

RESULTS

Monogenean parasite species composition

Six species belonging to two different genera: *Cichlidogyrus* (*C. thurstonae* Ergens, 1981; *C. halli* (Price and Kirk, 1967); *C. tilapiae* Paperna, 1960; *C. rognoni* Paperna, 1964; *C. cirratus* Paperna, 1964) and *Scutogyrus* (*S. longicornis* (Paperna and Thurston, 1969)), were collected on the gills of *Oreochromis. niloticus*. Prevalence, mean intensity and the aggregation index are reported (Table 1).These data revealed that all the Monogenean parasites adopted an aggregation distribution (variance is greater than mean intensity).

Four of the six parasites species (*C. thurstonae*, *C. halli*, *C. tilapiae* and *S. longicornis*) studied have an abundance significantly and positively correlated with the condition factor of the fish (rs = 0.73, 0.69, 0.59 and 0.38, respectively, $p< 0.05$) (Table 2).

Temporal variation of the occurrence of the parasites

C. thurstonae occurred on the host population during all the study period. A bimodal curve showed its maximum prevalence (100%) in October and November 2004 (small rainy season) and from April to July 2005 (long rainy season). The lowest infestation rate (60.9%) was recorded in February 2005 (long dry season) (Figure 2). The prevalence variations during the four seasons were significant (χ^2 = 70.3, df = 3, $p < 0.05$). Mean intensity values varied from 11.2 ± 1 in February 2005 (long dry season) with a peak of 61 ± 2.1 in June 2005 (long rainy season) (Figure 3). This variation curve was similar to the

Table 2. Values of the Sperman's rank correlation "rs" correlating the relative condition factor and the abundance of parasitism of *O. niloticus* in the Ayamé lake.

Parasite species	rs	*p*
Cichlidogyrus thurstonae	0.73	0.00*
Cichlidogyrus halli	0.69	0.00*
Cichlidogyrus tilapiae	0.59	0.00*
Scutogyrus longicornis	0.38	0.00*
Cichlidogyrus rognoni	0.17	0.07
Cichlidogyrus cirratus	0.16	0.09

*= Significant level atp< 0.05.

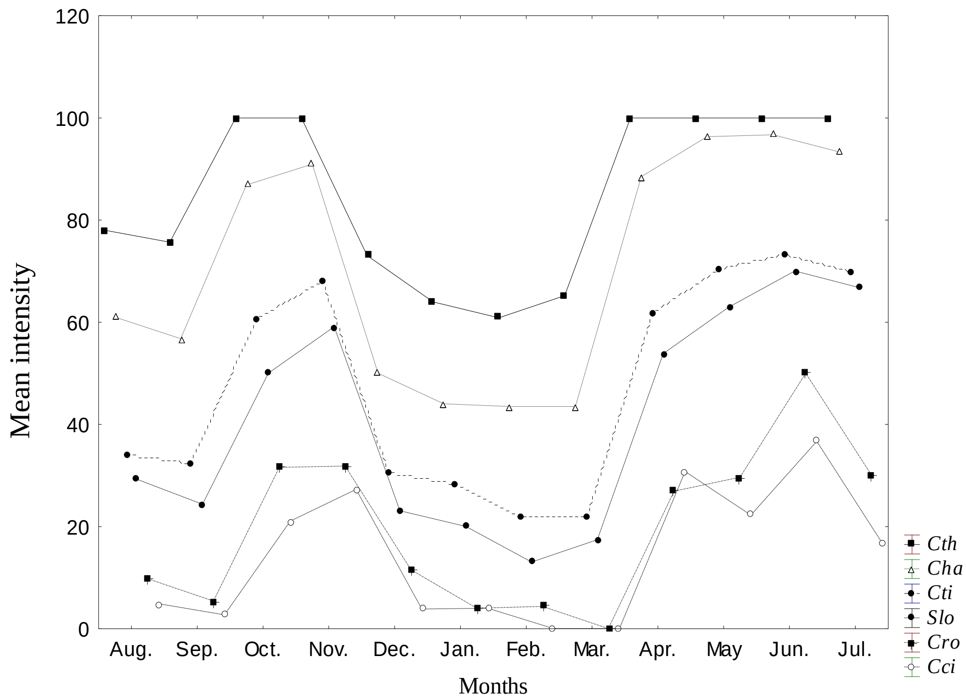

Figure 2. Month fluctuation in the prevalence of *Cichlidogyrus cirratus* (*Cci*), *Cichlidogyrus rognoni* (*Cro*), *Scutogyrus longicornis* (*Slo*), *Cichlidogyrus tilapiae* (*Cti*), *Cichlidogyrus halli* (*Cha*) and *Cichlidogyrus thurstonae* (*Cth*) infesting the gill of *Oreochromis niloticus* in the Ayamé I lake.

prevalence one. Significant difference was found in intensity of infection between seasons (Analysis of variance, F = 53.4). Parasitism changed from one season to another (Student, $p < 0.05$) (Table 3).

Scutogyrus longicornis, Cichlidogyrus tilapiae and *C. halli* were also present during the year of study. The prevalence variations of these parasites were similar to that of *C. thurstonae* but the values remained a little lower compared to the first one. For *S. longicornis* and *C. tilapiae*, the lowest prevalences (13 and 21.7%) were found in February 2005 (long dry season) and the highest (70 and 73.3%) in June (long rainy season), respectively. The number of fish infested by *C. halli* ranged from 43.5% in February and March 2005 (long dry season) to 96.7% in June (long rainy season) (Figure 2). The infestation showed a clear seasonal pattern (χ^2 = 56.6 and 54.7 and 80.3, df = 3, respectively, $p < 0.05$). The mean intensity reached the highest values 10.5 ± 0.7, 11.9 ± 0.8 and 26.4 ± 1 in November 2004 (small rainy season) and 13.1 ± 0.5, 16.8 ± 1.1 and 27 ± 1.6 in June (long rainy season), respectively (Figure 3). The statistical analyses revealed that the infection of this host with these three parasites differed significantly between seasons (Analysis of variance, F = 16.1 and 27.7 and 76.9, respectively, df = 3, $p< 0.05$). There was also significantly difference from one season to another (Student test, $p < 0.05$) (Table 3).

For *Cichlidogyrus cirratus* and *C. rognoni*, the

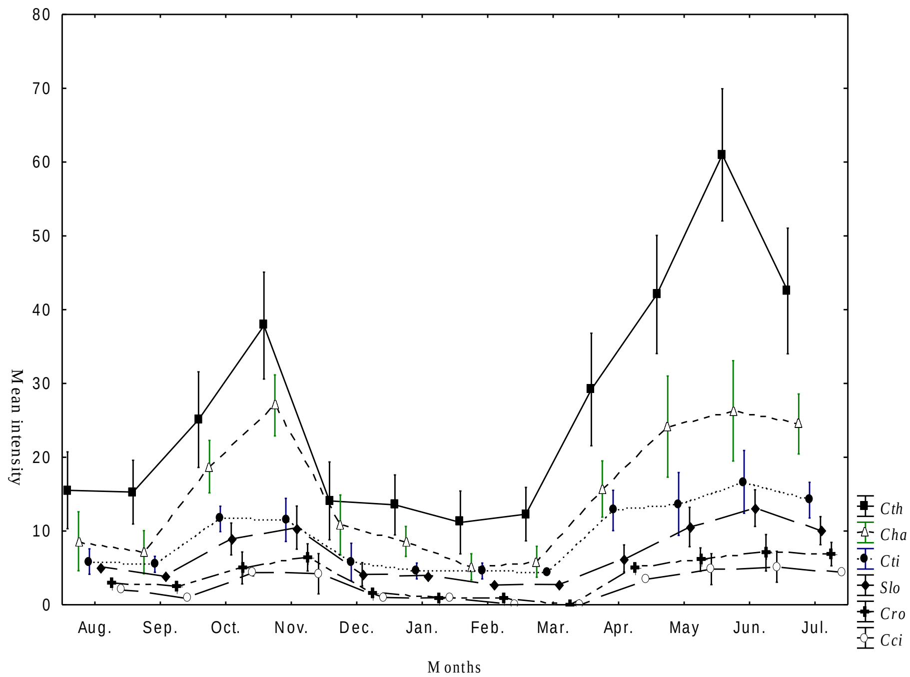

Figure 3. Month fluctuation in the mean intensity of *Cichlidogyrus cirratus* (*Cci*), *Cichlidogyrus rognoni* (*Cro*), *Scutogyrus longicornis* (*Slo*), *Cichlidogyrus tilapiae* (*Cti*), *Cichlidogyrus halli* (*Cha*) and *Cichlidogyrus thurstonae* (*Cth*) infesting the gill of *Oreochromis niloticus* in the Ayamé I lake.

prevalence of infestation (varied from 3.8% in December (long dry season) to 36.7% in June (long rainy season) and from 4% in January (long dry season) to 50% in June (long rainy season), respectively. There was a lack of parasites in February and March 2005 (Figure 2). The Chi-square (χ^2) applied to the temporal variations of the occurrence of these parasites showed that these values were statistically significant at 5% (χ^2 = 38.1 and 41.7, df = 3, respectively). In both species, the highest mean intensities were recorded in November 2004 (small rainy season) and June 2005 (long rainy season). The values of mean intensity of *C. cirratus* were 4.4 ± 0.8 and 5.2 ± 0.5 then 6.4 ± 0.4 and 7.1 ± 0.6 for *C. rognoni*, respectively (Figure 3). Seasonal changes of mean intensity of infection followed the seasonal pattern of prevalence (Analysis of variance, F = 18.7 and 16.2, respectively, df = 3, $p < 0.05$). Intensity of infection changed from one season to another except from the small to the long dry season ($p < 0.05$) (Table 3).

Spatial distribution of Monogeneans on gills

The prevalence and mean intensity values of *Cichlidogyrus thurstonae* were 96.7% and 27.1 ± 10.9 on the left side and 93.3% and 26.7 ± 11.3 on the right side of fish, respectively (Table 4). These values were not statistically significant at 5% (χ^2 = 0.3, df = 1; analysis of variance, F = 0.06, $p > 0.05$). This species was more frequent and concentrated on arches II and III (χ^2 = 38.9; df = 3, analysis of variance, F = 5.5, $p < 0.05$) (Table 5).

Scutogyrus longicornis, *Cichlidogyrus tilapiae* and *C. halli* were present in 66.7, 83.3 and 90% on the left side and 83.3, 80 and 86.7% on the right one, respectively. There was no significant difference between the Monogenean prevalence of left and right pairs of fish (χ^2 = 2.2, 0.1 and 0.2 df = 1, $p > 0.05$). Mean intensity values were 19.6 ± 7.3, 21.6 ± 10 and 23.1 ± 7.6 on the left set of gill arch of the fish while they were 16.4 ± 5.9, 21.7 ± 9.8 and 23.3 ± 7.8 on the right one, respectively (Table 4). In these three cases the differences were not statistically significant (Analysis of variance, F = 0.23, 0.03 and 0.02, df = 1, respectively, $p > 0.05$). The data analysis showed that *S. longicornis*, *C. tilapiae* and *C. halli* were more frequently found on the median arches while the arch IV was the least infected (respectively χ^2 = 13, 25.9 and 37.3, df = 3, $p < 0.05$). These species were more concentrated on the median arches (Analysis of variance, F = 7.1, 5.7 and 5.7 respectively, df = 3, $p < 0.05$) (Table 5).

The prevalence values of *Cichlidogyrus cirratus* and *C. rognoni* were 33.3 and 46.7% on the left side and 43.3 and 46.7% on the right one, respectively. These Monogeneans did not show preference for the left or right side of the gills (respectively (χ^2 = 0.6 and 0.1, df = 1, $p > 0.05$). Intensity of infection was 9.6 ± 3.8 on the left side

Table 3. Prevalence (%) and mean intensity in relation season.

Parasites species	Seasons	Number of Examined fish	Prevalence (%)	Mean intensity ± SE	F	*p*-value	Comparison two by two	t	*p*-value
	SDS	78	76.9	15.4±0.1			SDS-SRS	-6.4	0.02*
							SDS-LDS	4.6	0.01*
Cichlidogyrus thurstonae	SRS	82	100	34.8±4.3	53.4	0.00*	SDS-LRS	-3.4	0.02*
	LDS	97	65.8	12.8±1.3			SRS-LDS	9.8	0.00*
							SRS-LRS	0.6	0.6
	LRS	113	100	43.7±13.1			LDS-LRS	-8.3	0.00*
	SDS	78	42.9	7.9±1.1			SDS-SRS	-4.5	0.04*
							SDS-LDS	2.3	0.09
Cichlidogyrus halli	SRS	82	87.4	17±5.9	76.9	0.00*	SDS-LRS	-4.3	0.01*
	LDS	97	37.7	7.6±2.6			SRS-LDS	6.1	0.00*
							SRS-LRS	1.1	0.34
	LRS	113	96	19.2±4.7			LDS-LRS	-7.1	0.00*
	SDS	78	31.5	5.6±0.2			SDS-SRS	-9.3	0.01*
							SDS-LDS	3.5	0.02*
Cichlidogyrus tilapiae	SRS	82	66	11.7±0.3	27.7	0.00*	SDS-LRS	-6.8	0.00*
	LDS	97	28.3	5.3±0.5			SRS-LDS	9.5	0.00*
							SRS-LRS	0.5	0.6
	LRS	113	70.1	14.4±1.7			LDS-LRS	-11	0.00*
	SDS	78	23.9	4.6±0.8			SDS-SRS	-4.4	0.04*
							SDS-LDS	2.8	0.05
Scutogyrus longicornis	SRS	82	52.2	9.9±0.8	16.1	0.00*	SDS-LRS	-3.2	0.03*
	LDS	97	18.7	3.4±0.8			SRS-LDS	6.5	0.00*
							SRS-LRS	0.6	0.59
	LRS	113	62.5	10±2.8			LDS-LRS	-6.9	0.00*
	SDS	78	23.9	2.7±0.3			SDS-SRS	-4.7	0.04*
							SDS-LDS	2.0	0.14
Cichlidogyrus rognoni	SRS	82	52.2	5.7±1.0	16.2	0.00*	SDS-LRS	-4.6	0.01*
	LDS	97	18.7	0.9±0.7			SRS-LDS	5.3	0.01*
							SRS-LRS	0.6	0.55
	LRS	113	62.5	6.3±0.9			LDS-LRS	-6.7	0.00*
	SDS	78	23.9	1.5±0.7			SDS-SRS	-4.2	0.05
							SDS-LDS	1	0.42
Cichlidogyrus cirratus	SRS	82	52.2	4.3±0.1	18.7	0.00*	SDS-LRS	-5.4	0.01*
	LDS	97	18.7	0.2±0.5			SRS-LDS	19.9	0.00*
							SRS-LRS	0.9	0.43
	LRS	113	62.5	4.5±0.7			LDS-LRS	-11.3	0.00*

*= Significant level at $p < 0.05$; SE = Standard Error; SDS = small dry season; SRS = small rainy season; LDS = long dry season; LRS = long rainy season.

and 7.8 ± 2.8 on the right side for *C. cirratus* while was 14.3 ± 7.3 on the left and 14.3 ± 5.7 on the right side for *C. rognoni* (Table 4). There was no significant difference between the intensity of infection (Analysis of variance, F = 0.04 and 0.69, df = 1, respectively, p > 0.05). In these two cases the significance difference was observed between the fourth and the rest of the gill arches (respectively, χ^2 = 8 and 9.5, df = 3, analysis of variance

Table 4. Prevalence (%) and mean intensity in relation to host side.

Parasite species	Number of fish examined	Prevalence (%)		Mean intensity ± SE	
		Left side	Right side	Left side	Right side
Cichlidogyrus thurstonae	30	96.7	93.3	27.1±10.9	26.7±11.3
Cichlidogyrus halli	30	90	86.7	23.1±7.6	23.3±7.8
Cichlidogyrus tilapiae	30	83.3	80	21.6±10	21.7±9.8
Scutogyrus longicornis	30	66.7	83.3	19.6±7.3	16.4±5.9
Cichlidogyrus rognoni	30	46.7	46.7	14.3±7.3	14.3±5.7
Cichlidogyrus cirratus	30	33.3	43.3	9.6±3.8	7.8±2.8

Table 5. Prevalence (%) and mean intensity as a function to the gill arch.

Parasite species	Prevalence (%)				Mean intensity ± SE			
	Arch I	Arch II	Arch III	Arch IV	Arch I	Arch II	Arch III	Arch IV
Cichlidogyrus thurstonae	93.3	96.7	96.7	46.7	7.7±2	8.7±2.7	7.9±2.5	3.5±1.8
Cichlidogyrus halli	90	93.3	93.3	40	6.5±1.7	7.3±1.2	6.7±1.6	3±0.2
Cichlidogyrus tilapiae	80	93.3	83.3	40	6.2±2.7	6.5±2.9	6.2±2	3±1.6
Scutogyrus longicornis	66.7	66.7	56.7	26	6.9±1.8	6.7±1.4	6.8±0.8	2.9±0.4
Cichlidogyrus rognoni	30	40	43.3	10	4.4±1.1	5.8±1.8	4.6±0.5	1.7±0.2
Cichlidogyrus cirratus	26.7	36.7	40	10	2.7±0.1	3.5±0.6	2.9±0.4	1.7±0.1

$F = 1.7$ and 9.4, $p < 0.05$) (Table 5).

Relationship between body length and degree of infection

All the Monogenean species infested individuals of all any length class, except for *C. cirratus* and *C. rognoni*. For each parasite, the prevalence was lowest in the smallest length class (standard length ranges 50 to 100 mm) (Table 6).

The highest prevalence 100% of *C. thurstonae* was observed in the fish of the classes with standard length ranges greater than 150 mm. Statistical tests showed significant difference in prevalence according to the size classes ($\chi^2 = 124.96$, df = 3, $p < 0.05$). The values of mean intensity were 9.5 ± 0.8 in the first class and 12.0 ± 15.1, 31.7 ± 28.1 and 39.9 ± 35.4 in the other ones respectively (Analysis of variance, $F = 33.1$, dl = 3, $p < 0.05$). Globally, there was also a significant difference from one size class to another (Student test, $p < 0.05$) (Table 6).

The prevalence of *Scutogyrus longicornis*, *Cichlidogyrus tilapiae* and *C. halli* increased progressively with host size and reached the highest values 54.5, 64.7 and 86% in the largest individuals, respectively ($\chi^2 = 43.7$ and 58.6 and 59.5, df = 3 ; $p < 0.05$).

The values of mean intensity of *S. longicornis* were 3.0 ± 2.6, 3.5 ± 3.2 and 9.8 ± 2.6 in the first three classes respectively and 10.7 ± 16.4 in the latter class, while they were 5.4 ± 3.5 and 5.8 ± 3.3 in the z first two classes then 12.0 ± 14.1 and 12.5 ± 19.4 in the two latter classes for *C. tilapiae.* The lowest rates infestation (prevalence and mean intensity) of *C. halli* were observed in the smallest length class (36.7%, 7.6 ± 3.4) respectively. The highest values were observed in the largest individuals (standard length ranges 200 to 250 mm) (86%, 23.2 ± 22.7). Intensity of infection was varied significantly by host length class (Analysis of variance, $F = 22.6$ and 25.2 and 32.1, respectively, df = 3, $p < 0.05$). Student test revealed that there was significant difference from one size class to another ($p < 0.05$) (Table 6).

For *C. cirratus* and *C. rognoni*, the lowest prevalence 20 and 12% were recorded in the fish of the classes with standard length range 150 to 200 mm and 100 to 150 mm, respectively and the highest values 25.3 and 30.7% in the latter class. The Chi-square (χ^2) indicated that the prevalence of these two species depends on the size of the host ($\chi^2 = 38.3$ and 79.6, respectively, df = 3; $p < 0.05$). The values of mean intensity varied from 2.0 ± 1.6 to 5.1 ± 4.6 for *C. cirratus* whereas varied from 1.5 ± 0.7 to 7.1 ± 10.7 for *C. rognoni.* The difference was statistically significant (Analysis of variance, $F = 4.4$ and 11.6, respectively, df = 3, Student, $p < 0.05$) (Table 6).

Relationship between host sex and degree of infection

The prevalence of *C. thurstonae* was 85% for male fish and 87.7% for female one. On female fish the mean intensity was slightly higher (31 ± 0.6) than on male fish

Table 6. Prevalence (%) and mean intensity in relation to host size.

Parasites species	Host length classes	Number of examined fish	Prevalence (%)	Mean Intensity ± SE	F	*p*-value	Comparison two by two	t	*p*-value
	Class I	60	46.7	9.5±0.8			Classes I- II	0.7	0.46
							Classes I-III	-2.8	0.01*
Cichlidogyrus thurstonae	Class II	75	74.7	12.0±15.1	33.1	0.00*	Classes I- IV	-7.6	0.00*
	Class III	85	100	31.7±28.1			Classes II-III	-4.1	0.00*
							Classes II- IV	-10.2	0.00*
	Class IV	150	100	39.9±35.4			Classes III- IV	-3.7	0.00*
	Class I	60	36.7	7.6±3.4			Classes I- II	-0.7	0.50
							Classes I-III	-2.5	0.01*
Cichlidogyrus halli	Class II	75	64.0	7.7±3.0	32.1	0.00*	Classes I- IV	-7.2	0.00*
	Class III	85	82.3	18.8±31.6			Classes II-III	-2.9	0.00*
							Classes II- IV	-9.5	0.00*
	Class IV	150	86.0	23.2±22.7			Classes III- IV	-5.1	0.00*
	class I	60	15.0	5.4±3.5			Classes I- II	-0.2	0.83
							Classes I-III	-5.3	0.00*
Cichlidogyrus tilapiae	class II	75	30.7	5.8±3.3	25.2	0.00*	Classes I- IV	-4.9	0.00*
	class III	85	62.4	12.0±14.1			Classes II-III	-6.4	0.00*
							Classes II- IV	-6.9	0.00*
	Class IV	150	64.7	12.5±19.4			Classes III- IV	-2.1	0.04*
	Class I	60	11.7	3.0±2.6			Classes I- II	-0.2	0.88
							Classes I-III	-3.1	0.00*
Scutogyrus longicornis	Class II	75	22.7	3.5±3.2	22.6	0.00*	Classes I- IV	-4.3	0.00*
	Class III	85	47.1	9.8±2.6			Classes II-III	-3.8	0.00*
							Classes II- IV	-6.2	0.00*
	Class IV	150	54.5	10.7±16.4			Classes III- IV	-4.9	0.00*
	Class I	60	0	0					

Table 6. Continued.

Cichlidogyrus rognoni	Class II	75	12.0	1.5±0.7	11.6	0.00*	Classes II-III	-3.1	0.00*
	Class III	85	24.7	4.0±2.8					
							Classes II- IV	-4.1	0.00*
	Class IV	150	30.7	7.1±10.7			Classes III- IV	-2.7	0.01*
	Class I	60	0	0					
Cichlidogyrus cirratus	Class II	75	0	0	4.4	0.00*			
	Class III	85	20	2.0±1.6			Classes III- IV	-2.1	0.01*
	Class IV	150	25.3	5.1±4.6					

* = Significant level at p < 0.05; SE = Standard Error.

(29.4 ± 0.3). Host sex no significantly affects the infection ($\chi^2 = 0.5$, df = 1; Analysis of variance, F = 3.7, p > 0.05).

The species *S. longicornis*, *C. tilapiae* and *C. halli* were present in 38.5, 52.8 and 69% of male host and 46.5, 50.6 and 77.1% of the female host, respectively. The intensities of infection were 7.8 ± 0.6, 11.2 ± 1.03 and 18.7 ± 0.01 in the male and 9.5 ± 0.3, 11.2 ± 1.5 and 17.4 ± 0.3 in the female. These values were not statistically significant at 5% (χ^2 = 2.8, 0.1 and 3.01 respectively, df = 1; Analysis of variance, F = 0.12, 0.01 and 0.02, respectively, p > 0.05).

Prevalence and mean intensity of *C. cirratus* were 15% and 3.8 ± 0.9 for male fish and 14.7% and 4.4 ± 0.7 for female fish. The prevalence of *C. rognoni* was 20% in the male and 21.2% in the female. Intensity of infection was 5.7 ± 0.3 for male fish and 21.2% and 5.6 ± 0.7 for female fish. These parasites were also partitioned equally between males and females (χ^2 = 0.08 and 0.01, respectively, df = 1, p > 0.05; Analysis of variance, F = 0.1 and 0.16, respectively, dl = 1, p > 0.05).

DISCUSSION

The present study recorded six species of Monogenea community on *Oreochromis niloticus*: *Cichlidogyrus thurstonae*, *C. tilapiae*, *C. halli*, *C. rognoni*, *C. cirratus* and *Scutogyrus longicornis.* This species richness is different of those reported by the previous studies. Boungou et al. (2008) found out five on the six Monogenean species in the same fish from the dam of Loumbila (Burkina Faso) whereas Tombi et al. (2014) reported four species in Melen station in Yaoundé (Cameroon). Usually, the number of Monogenean species per fish host varied (Pariselle, 1996) and this variability of parasite richness has been associated to various factors related to experimentation (Walter et al., 1995), the phylogeny of hosts and parasites (Bush et al., 1997; Sasal et al., 1997), host (Morand et al., 1999), ecology (Zharikova, 2000) and water quality (Galli et al., 2001; El-Seify et al., 2011). Our result suggests that the water of Lake Ayamé I may provide better eco-climatic conditions for the development of these parasites and can facilitate the contact between the infesting stages of these Monogenean and the host fish.

The exploitation of a host fish by several genera of Monogeneans has been already reported. Blahoua et al. (2009) mentioned the presence in *Sarotherodon melanotheron*, the genus *Scutogyrus* and several species of *Cichlidogyrus*. Ibrahim (2012) showed that eight Monogenean species colonized the gills of *Tilapia zillii*. Moreover, the colonization of hosts by several congeneric species was also reported by Bittencourt et al. (2014) and Tombi et al. (2014). This simultaneous infection could be explained by the fact that in natural environment, the parasitic densities are generally weak and therefore, the niches are always available on the gill biotope (Buchmann and Lindenstrom, 2002; Simkova et al., 2006).

Regarding parasite dispersion, in most cases, the parasites are almost universally aggregated between their hosts (Krasnov and Poulin, 2010).

Indeed, most of the hosts have few if any parasites, while a small number of hosts are infected with many parasites (Poulin, 1993).

This pattern is expected in most animals in nature, as observed within Monogeneans (*C. thurstonae*, *C. tilapiae*, *C. halli*, *C. rognoni*, *C. cirratus* and *S. longicornis*) parasitizing the gills of *O. niloticus* in the present study. This trend has been also evidenced for *C. thurstonae*, *C. tilapiae*, *C. halli* and *S. longicornis* Monogenean gill parasites of the same host by Tombi et al. (2014). According to Combes (1995), an aggregative distribution may indicate heterogeneity in the relationship between the host and the parasite populations. The probability for the parasite to meet its host and its chances of surviving in the latter, it may vary from one host to another. In addition, Kennedy (1977) stated that aggregative distribution increase the opportunities for parasites to meet a partner in order to reproduce.

In several studies, there has been a growing interest on the total number of parasites per host fish with the condition factor of the fish. Yamada et al. (2008) found significant and positive correlation of the condition factor of cichlids with the abundance of a species of Monogeneans. Lizama et al. (2007) found negative and significant correlation when relating the *Piaractus mesopotamicus* (Holmberg, 1887) condition factor of fish farming with the levels of parasitism of two species of Monogeneans. Tozato (2011) found no differences in condition factor of *Corydoras aeneus* (Gill, 1858) parasitized and not parasitized by Monogeneans, and concludes that these parasites did not affect the welfare of the host. The author also stated that the parasite community of fish studied has low pathogenicity to the host in the different environments of the basin, indicating the proper response of fish against parasitism. In this study, it was observed that the condition factor of *O. niloticus* had significant and positive correlation with the abundance of parasitic species *C. thurstonae*, *C. tilapiae*, *C. halli*and *S. longicornis.* Cone (1995) stated that larger fish and a better condition factor can withstand higher intensities of infection by Monogeneans parasites despite being pathogenic. This is possibly the reason for the significant and positive correlation between condition factor studied fish and abundance of parasitism by Monogeneans.

Concerning the temporal variation of the occurrence of parasites, except for *C. rognoni* and *C. cirratus* absent during in February and March, all other parasite species were present throughout the year in the host fish *O. niloticus*. Thus, this host is vulnerable at any period. This is consistent with the results of Blahoua et al. (2015) who observed the omnipresence of *Tilapia zillii* gill parasites in Lake AyaméI. The occurrence of Monogenean presented its maximal prevalence and mean intensity during the rainy seasons (LRS and SRS) with peaks in June and November, respectively. Seasonal variations in Monogenean abundances observed in this study have also been reported by various authors. For example, Bilong and Tombi (2005) showed that two Monogeneans namely *Dactylogyrus in solitus* and *D. bopeleti* gills parasites from *Barbus martorelli* reached their highest prevalence and their maximum intensity during the rainy seasons. Blahoua et al. (2009) also observed that *C. acerbus*, *C. halli* and *Scutogyrus minus* appeared to be more prevalent and more abundant in long rainy season. Our results can be explained by water temperature which is assumed to be an important factor in controlling the occurrence of Monogeneans (Koskivaara et al., 1991; Simkova et al., 2001). In fact, higher water temperature must have been harmful on the adult worms (Bilong and Tombi, 2005). It also appears from the work of Bilong (1995) that temperature is not the only factor, and that other abiotic factors (concentration of suspended solids, conductivity, water transparency) might influence the seasonal abundance of parasites.

Several authors have investigated the relationship between the level of Monogenean infection and the size of the host fish. Mierzejwska et al. (2006) found that the infection intensities of white bream and roach with *Dactylogyrus sphyrna* and *D. crucifer*, respectively, increased with fish body length. Tombi et al. (2014) also agreed with them reporting positive relationship between the prevalence and mean intensity of *C. thurstonae*, *C. halli*, *C. tilapiae* and *S. longicornis* and the host, *O. niloticus*. In contrast to these results, Boungou et al. (2008) found that the size of *O. niloticus* in the dam of Loumbila (Ouagadougou) had no influence on the prevalence of its Monogenean gill parasites. The increase of parasitism with the size of *O. niloticus* from Ayamé Lake can be attributed to the fact that, larger fish offer large colonized surfaces area to parasites (Cable et al., 2002, Bilong Bilong and Tombi, 2004). In fact, an increase in the branchial surface can provide a larger area of infestation, and thus more potential sites for attachment of these parasites (Aydoğdu et al., 2003; Tekin-Ozan et al., 2008). This result can also dueto the strongest water current passing through the gill of larger fish, thus creating convenient conditions for parasites settlements (Guttiérrez and Martorelli, 1999; Turgut et al., 2006).

We found no significant variation on the prevalence and the mean intensity by host sex in Monogenean species. Boungou et al. (2008) had made the same observation with all Monogeneans of *O. niloticus*, except for *C. halli*. Tombi et al. (2014) also observed no sex influence on the infestation of the same host gill filaments by *C. thurstonae*, *C. halli* and *C. tilapiae*. This result suggests that very few parasites species have a preference in relation to the sex of the host (Rohde, 1993).

In this study, no significant preferences were found in the distribution of Monogenean species on the gill arches between the left and right sides of its host. These results are similar to those of Le Roux et al. (2011), who found no significant difference between the number of *Cichlidogyrus philander* on the left and right gill arches of the *Pseudocrenilabrus philanderphilander*. Similarly, Tombi et al. (2014) reported that *C. thurstonae*, *C. halli*, *C. tilapiae* and *Scutogyrus longicornis* colonized the two parts of the gill system of the Nile tilapia, *O. niloticus* in the same way. This could be due to the fact that similar volumes of water flowing through the left and right side of the gill might have brought equal amount of infective larval stages to the gill (Raymond et al., 2006). On the contrary, Hendrix (1990) found an asymmetrical distribution of *Bothithrema bothi* (Monogenea) on *Scophtalmus aquosus* (Bothidae). Bilong (1995) interpreted this phenomenon to be linked to Bothidae morphology. For Dessoutter (1992), Bothidae has a flat and dissymmetrical body. According to Rohde (1993) a preference for one side of the host body is associated to body asymmetry of some parasites. This suggests that equitable distribution of parasites on both sides obtained in this study is due to the bilateral symmetry of Nile tilapia associated with that of its gill Monogeneans.

In *O. niloticus*, the Monogeneans were most preferentially attached to the middle arches II and III. In this same host, Tombi et al. (2014) found that *C. halli* and *S. longicornis* were more concentrated on arches II and III, respectively. Le Roux et al. (2011) had reported that *Cichlidogyrus philander* mostly colonized the median arches of *Pseudocrenilabrus philander philander*. A number of factors have been recognized as determinants of microhabitat choice among monogeneans. Some authors include intrinsic factors such as the need to increase chances of locating a mate by having a narrow microhabitat niche and environmental conditions. Among the environmental factors, water current has been identified as among the most important factor determining site specificity (Madanire-Moyo et al., 2010; Le Roux et al., 2011) with the suggestion that middle arches offer a hydrodynamically protected site (Lo et al., 2001).

It is an established fact that many parasites have free living stages (eggs or larvae) which have complimentary behavioral reactions to the ambient environment (Echi and Ezenwaji, 2009), and therefore, the settling of oncomiracidia is likely to be influenced by water currents. Indeed, the strongest water current flowing through the middle part of the gill arches and the high respiratory water can provide a high number of parasites (Gutiérrez and Martorelli, 1994; Lo and Morand, 2001). For Buchmann (1989) and Koskivaara and Valtonen (1991), the median preference arches may also be related to the large colonized surfaces they offer to parasites. Other factors such as parasite load (Lo and Morand, 2000) and the host immunity can also influence microhabitat selection (Koskivaara and Valtonen, 1992; Gutiérrez and Martorelli, 1994).

Conclusion

This study adds to a further understanding of Monogeneangill parasitizing *O. niloticus* in a natural water system.

This could lead to more informed aquacultural practises, and maximization of the production of *O. niloticus.*

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Researchers are grateful to the staff of Hydrobiology Laboratory of the Félix Houphouët Boigny University (Côte d'Ivoire) for fieldwork assistance and to the Ministry of Animal Resources and Fisheries Productions of Côte d'Ivoire for providing financial support. Also thanks to Dr Gonedele Bi Séry Ernest for his valuable comments on the manuscript.

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