

# Occurrence and Leave Extractable Essential Oil of *Lippia multiflora* M. (Verbenaceae) as Affected by Soil Acidity, Carbon, Nitrogen and Phosphorus Contents in North Cote d'Ivoire

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## Abstract

For generating a strategy of quantitative and qualitative productions of invasive *Lippia multiflora*, influence of soil pH and the contents of organic carbon (C), total nitrogen (Nt), total (Pt) and available (Pa) phosphorus were explored via dominant-abundance (DAI) and aggregation (AI) index of the species as well as the leave extractable essential oil. In three sites (Labelekaha, Taoura and Zievogo) of *L. multiflora* ecosystem in north Cote d'Ivoire (Sudan savanna), corresponding DAI and AI were recorded coupled with soil and leave sampling at different topographic sections. Soil pH and the contents of C, Nt, Pt and Pa were determined as well as the concentrations of essential oils. Occurrence of *L. multiflora* was highest in the down slope position of the landscapes with significant influence of Pa also noticed for essential oils mainly characterized by highest concentrations of Geranial, Neral,  $\alpha$ -Phellandrene, Para-Cymene, Limonene+ $\beta$ -Phellandrene and  $\alpha$ -Humulene. The effect of soil organic C, and Nt were accounting for hydromorphic condition as occurred at Ziévogo. The cultivation of *L. multiflora* may be possible in down slope position applying P for high yields of leaves and essential oils mainly composed of terpenoid derivatives. Further applications of organic C and N are required for hydromorphic soil.

**Keywords:** toposequence, chemotype, terpenoid, ecosystem, degraded soil, nutrient interactions

## 1. Introduction

*Lippia multiflora* Mondenke (savanna tea) is a woody shrub in tropical ecologies, with increasing interest because of its biomedical virtues: Tea-like infusions are traditionally used as remedy against malaria fever, stress, hypertension, gastro-intestinal trouble and caught as well as laxative (Noamesi, Adebayo, & Bamgbose, 1985; Pham & Koffi, 1998; Jim, Wudeneh, Mariana, & Dan, 2000; Abena et al., 2003). Moreover, *L. multiflora* contains some adjutants for cosmetic (Porspi, 1992; Kanko, Sawaliho, Koné, Koukoua, & N'guessan, 2003) and pesticides (Oladimeji, Orafidiya, Ogunniyi, & Adewunmi, 2000; Oussou et al., 2008; Etienne et al., 2011) produces. These qualities are attributed to the properties of the leaf extractable essential oils (Irvine, 1961) accounting for 42 volatile compounds from *L. multiflora* and more than 126 from *Lippia* spp. (Maia, Silva, Andrade, & Carreira, 2005) with specific properties that could interfere the plant attributes as individual effect (Lahlou, 2004). Specific effectiveness of terpenoids,  $\alpha$ - and  $\beta$ -pinenes were observed on scabies by Oladimeji et al. (2000) as well as the therapeutic effects of terpene, eugenol, camphor, and menthol against divers' skin problems (Lawless, 1995; Pearlstine, 2006). In the light of these utilities of the extractable chemical compounds of essential oil of *Lippia* spp., it may significantly contribute to the development of pharmaceutical and cosmetic manufacturing activities in its natural growing ecosystems, as observed in savanna zone of Sub-sahara Africa. This potentiality can substantially contribute to alleviation of poverty in this region where the population

wellbeing is more depending on informal economy (Programme des Nations Unies pour le Développement [PNUD], 2013).

For this purpose, available quantity and quality of essential oil are required meanwhile; *Lippia* spp. is yet an invasive plant characterized by a wide variability in the quality of essential oils (Oussou et al., 2008; Gouollaly, 2010) even within the same ecological zone and for a given genotype involving effects of seasons and environment including the soils (Bassolé et al., 2010). Previous studies of chemotypes in different ecological zones of West Africa were only descriptive (Folashade & Omoregie, 2012; Kanko, Koukoua, & N'guessan, 1999), missing causal and effect relationship analysis that could guide the control of both qualitative and quantitative productions of essential oil. However, such analysis was established between soil chemical characteristics and specific weed occurrence in Sub-sahara Africa ecosystems involving soil contents of C, N, P and K along toposquence and fertilizer effects (Udoh, Ogunkunle, & Ndaeyo, 2007; Koné et al., 2013a; Koné, Traoré, & Touré, 2014) somewhat depending on soil pH. Indeed, there is variability of soil fertility potential along toposquence in tropical ecosystem (Koné et al., 2009) that can affect the plant growth.

Therefore, a survey was conducted in the natural prevailing occurrence zone of *L. multiflora* in Cote d'Ivoire for chemical analysis of essential oils extracted from the leaves sampled at different topographic sections of landscapes considering soil pH as well as soil contents of organic carbon (C), total nitrogen (N), total (Pt) and available (Pa) phosphorus. The aim was to identify how and which of these soil chemical characters could affect the occurrence of *L. multiflora* and the composition of its leaves essential oils in order to recommend a strategy for soil fertility management when developing agricultural systems for quantitative and qualitative productions.

## 2. Methodology

### 2.1 Physiographical Description of the Studied Zone

The study was conducted in the north of Cote d'Ivoire (West Africa) around the region of Korhogo concerning three sites (Labélékaha: N 8°58' - W 5°30' - 325 m elevation; Taouara: N 9°43' - W 5°41' - 330 m elevation; Ziévogo: N 9°28' - W 5°54' - 335 m elevation) naturally colonized by *L. multiflora*. The region belongs to the sudanean climatic sector as a tropical sub-humid zone with plateau landscapes of 300 – 400 m in altitude. Rainfall pattern is monomodal characterized by a long dry season period (November – May) and shorter rain season (June – October). Annual average rainfall amount and temperature are 1217 mm and 26.95 °C, respectively, and coupled with 1609 mm as evapotranspiration. The bed-rock is characterized by granite and schist (Beaudou & Sayol, 1980).

### 2.2 Vegetation

The region is characterized by dispersed blocks of secondary forests in a dominant derived savanna where, forest bands are also associated to hydrographic network (Beaudou & Sayol, 1980). Deciduous and hardwood trees are encountered in the summit and the upper slope positions of landscape across the studied zone somewhat contrasting with the dominance of savanna vegetation species in the middle and foot slope positions. Arborescent species (*Acacia sieberiana*; *Bridelia ferruginea*; *Vitellaria paradoxa*; *Daniella oliveri*; *Hymenocardia acida*; *Lophira lanceolata*; *Mitragyna Inermis*; *Nauclea latifolia*; *Pterocarpus erinaceus*; *Terminalia glaucescens* and *Piliostigma thonningii*) are occurring with DAI and AI values of 1 respectively. This biotope of savanna includes *L. multiflora* associated with different herbaceous species as presented in Table 1.

Table 1. Average dominant abundance and aggregation index of herbaceous species in *L. multiflora* community as encountered in the studied region (Labélékaha, Taouara & Ziévogo)

Identification	DAI	AI
<i>Cochlospermum planchonii</i>	1	1
<i>Aframomum latifolium</i>	3	3
<i>Melanthera scandens</i>	2	3
<i>Euclasta condylotrica</i>	4	4
<i>Hyparrhenia dissoluta</i>	4	4
<i>Hyparrhenia diplandra</i>	4	3
<i>Pennisetum polystachion</i>	4	3
<i>Sorghastrum bipennatum</i>	4	3

DAI: Dominant-abundance index; AI: Aggregation index.

### 2.3 Landscape and Soil

Dominant morpho-pedological landscapes in the region are characterized by plateaus with or not degraded hardpan layer in summit position accounting for 33.3% and 28.5% of total surface of the studied region respectively while; convexo-concave side interfluves are recovering 12.3% according to Beaudou and Sayol (1980). At Labélékaha, Taoura and Zievogo, *L. multiflora* community was encountered along the middle slope, the foot slope and peneplanated lowland with alluvial deposit respectively. Soils of hill slope were Plinthosols with Leptic character somewhere while, Acrisols and Arenosols were prevailing in the down slope. Fluvisol was exclusively occurring in lowland.

### 2.4 Sampling of Plant and Soil

In randomly selected area of about 1 ha, equally stratified sampling method (Webster & Olivier, 1990) according to topographic sections defined as summit, upper slope, middle slope and foot slope (Rhuhe & Walker, 1968) was applied along the hillside in each of the studied site (Labélékaha, Taoura & Zievogo). Toposequence with azimuths of 119, 121 and 135 grades were laid in Labélékaha, Taoura and Zievogo respectively. Each of topographic section was about 100 – 150 m in length along 500 – 700 m of hillside characterized by a gentle slope (2 – 5%) and high invasion of *L. multiflora* in places. At flowering stage of *L. multiflora* (November) corresponding to the physiological maturity, leaves were sampled around 11h – 12h from randomly selected 32 plants dispersed within 1 ha. For a given topographic position, samples of leaves were kept together before taking 1kg as composite sample which was saved in carboglass before air drying in a room condition during 7 – 10 days. This sampling was coupled with soil profile study for characterization and 32 elemental soil samples were taken using hand augur within 0 – 20 cm, 20 – 40 cm, 40 – 60 cm and 60 – 80 cm depth neighboring (5 - 10 cm apart) the plants respectively. A composite sample of 2 kg was done for each of the soil depths in a given topographic position for all the three sites.

### 2.5 Estimation of DAI and AI for *L. multiflora*

The dominant-abundance indice (DAI) of *L. multiflora* was estimated according to Braun-Blaquet, Roussine and Negre (1952) method of species score (1 – 5) of soil recovering rates: 1 = < 5% of recovering; 2 = 5 – 25% of recovering; 3 = 25 – 50% of recovering; 4 = 50 – 75% of recovering and 5 = 75 – 100%. Guinochet (1973) score of species dispersion (1 – 5) were also used for the estimation of species aggregation indice (AI): 1: individual occurrence; 2 = dispersed small groups; 3 = dispersed larger groups; 4 = almost continuous population and 5 = continuous population.

### 2.6 Analysis of Essential Oil

The essential oil from the leaves of *L. multiflora* was extracted by hydrodistillation (water and stem distillation) in a Clevenger-type apparatus (Clevenger, 2006) during 3 hours, yielding 1.0% (v/w). Gas chromatography analyses were performed coupled with spectrometry (Hewlett-Packard, CG 5890 serial II) for chemical volatile compounds (constituents of mono- and sesquiterpene) identification as described by Etienne et al. (2011) including comparison between the experimental gas chromatographic retention indices (RI) and fragmentation pattern with corresponding reference data (NBS5K/NIST98) as done by Adams (2007). A standard solution of n-alkanes (C7 – C26) was used to obtain the retention indices. Identified elements were grouped according to the molecular radicals as monoterpene and sesquiterpene characterized by two isoprene units (C<sub>10</sub>H<sub>16</sub>) and three (C<sub>15</sub>H<sub>24</sub>) respectively. Further differentiation was done referring to oxygenated derivatives among both.

### 2.7 Soil Analysis

Soil composite samples were air dried in a room condition, grounded, and sieved (2 mm) before analytical process including pH measurement with electrode glass in soil/water (1/2.5) solution. The content of organic carbon (C) was determined by colorimetric measurement of unreduced amount of Cr<sub>2</sub>O<sub>7</sub><sup>-</sup> by C according to Walkley and Black (Pansu & Gauteryrou, 2003). Kjeldhal method including mineralization of organic matter at 300°C was used to determine soil content of total-N using sulphuric acid (K<sub>2</sub>SO<sub>4</sub> + CuSO<sub>4</sub> + Se) during three hours. Mineralization process and selective extraction were applied to determine the amount of total and available phosphorus using EDTA (Pansu & Gautheryrou, 2003). Results of soil analysis were interpreted according to Baillie (2010) referring to the critical levels respectively.

### 2.8 Statistical Analysis

Mean value of soil pH and the contents of C, N, Pt and Pa were determined by descriptive statistic for every site according to soil depths along the toposequence. Pearson correlation analysis was done between abundance index (DAI) of *L. Multiflora* and soil contents of C, N, Pt and Pa in 0 – 20 cm, 20 – 40 cm, 40 – 60 cm and 60 – 80 cm for each topographic positions respectively. Similar analyse was also done for aggregation index of

sociability (AI) and repeated for studied sites respectively. Average value of studied chemical contents of soil in 0 – 40 cm and 40 – 80 cm was similarly used, especially, with DAI. Pearson correlation was also processed between soil characters and essential oil concentrations in leave. SAS (version 8) was used for statistical analysis considering  $\alpha = 0.05$ , meanwhile extension up to 0.10 was accepted for correlation data interpretation.

### 3. Results

#### 3.1 Soil Characteristics

Table 2. Mean values and standard deviation (SD) of C, Nt, Pt and Pa as well as pH in 0 – 20 cm, 20 – 40 cm, 40 – 60 cm and 60 – 80 cm soil depths according to topographic sections for the three sites

		0-20 cm		20-40 cm		40-60 cm		60-80 cm	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Summit</b>	pH <sub>water</sub>	6.00	0.34	6.00	0.38	6.00	0.32	6.00	0.25
	C (gkg <sup>-1</sup> )	1.10	0.11	0.86	0.37	0.82	0.14	0.60	0.25
	Nt (gkg <sup>-1</sup> )	0.11	0.01	0.10	0.03	0.09	0.03	0.07	0.03
	Pt (mgkg <sup>-1</sup> )	421.00	181.31	365.00	163.36	323.00	116.09	124.00	111.12
	Pa(mgkg <sup>-1</sup> )	55.00	3.03	44.00	19.85	26.00	13.83	26.00	17.61
<b>US</b>	pH <sub>water</sub>	6.00	0.346	6.00	0.388	6.00	0.327	6.00	0.264
	C (gkg <sup>-1</sup> )	1.170	0.122	0.832	0.385	0.524	0.155	0.556	0.208
	Nt (gkg <sup>-1</sup> )	0.106	0.015	0.086	0.028	0.081	0.032	0.095	0.036
	Pt (mgkg <sup>-1</sup> )	355.33	180.31	284.33	164.36	240.66	117.09	268.00	110.12
	Pa (mgkg <sup>-1</sup> )	50.877	3.0386	33.491	19.952	21.605	13.808	27.105	17.513
<b>MS</b>	pH <sub>water</sub>	6.00	0.24	6.00	0.42	5.50	0.48	5.50	0.48
	C (gkg <sup>-1</sup> )	0.85	0.206	0.68	0.240	0.48	0.15	0.46	0.17
	Nt (gkg <sup>-1</sup> )	0.086	0.017	0.102	0.04	0.085	0.05	0.05	0.02
	Pt (mgkg <sup>-1</sup> )	315.25	145.19	375.00	136.56	390.50	130.83	379.87	156.81
	Pa(mgkg <sup>-1</sup> )	42.10	23.93	41.45	19.17	37.46	24.58	37.92	31.399
<b>FS</b>	pH <sub>water</sub>	6.00	0.482	6.00	0.470	6.00	0.47	6.00	0.50
	C (gkg <sup>-1</sup> )	1.45	0.719	1.089	0.674	0.582	0.334	0.70	0.327
	Nt (gkg <sup>-1</sup> )	0.140	0.047	0.112	0.054	0.069	0.024	0.07	0.027
	Pt (mgkg <sup>-1</sup> )	234.20	136.32	223.05	121.47	220.50	149.87	251.21	171.44
	Pa(mgkg <sup>-1</sup> )	34.77	10.26	27.90	9.79	30.13	9.89	30.92	11.93

US: Upper slope; MS: Middle slope; FS: Foot slope.

Table 1 reveals moderate acidity ( $5.5 < \text{pH} < 6$ ) of studied soils indifferently to soil depths and topographic positions. In topsoil (0 – 20 cm), soil content of C ( $> 1 \text{ gkg}^{-1}$ ) is high except for the soil in middle slope position contrasting with soil content of Nt, exclusively low ( $< 1 \text{ gkg}^{-1}$ ) whatever the topographic position when compared with the critical level respectively. Soil contents of Pt is ranging moderately with wide variability while, that of Pa is high ( $> 10 \text{ mg kg}^{-1}$ ).

#### 3.2 Occurrence of *L. multiflora* Referring to Soil

There is wide irregularity in the occurrence of *L. multiflora* along the toposequences: no occurrence is observed at the summit and the upper slope positions contrasting with the data recorded for the middle and foot slopes, especially at Taoura and Ziévo with dominant-abundances of 2 and 3 respectively, but, with the same estimated value of aggregation sociability index of 1. Dominant-abundance index of 2 is also recorded at the middle slope position in Labélékaha. Across the three sites surveyed, down slope position (middle and foot slopes) is likely more favorable to *L. multiflora* occurrence than the hill slope position (summit and upper slope).

Table 3. Soil contents of C, Nt, Pt, and Pa as well as the pH under *L. multiflora* occurrence according to soil depths in studied sites

Site	Topographic Sections	Depth (cm)	pH <sub>water</sub>	C (gkg <sup>-1</sup> )	Nt (gkg <sup>-1</sup> )	Pt (mgkg <sup>-1</sup> )	Pa (mgkg <sup>-1</sup> )
Taouara	Foot slope	0-20	6.0	0.66	0.09	534	42
		20-40	6.5	0.48	0.08	483	34
		40-60	6.5	0.30	0.06	405	30
		60-80	6.0	0.30	0.06	378	34
Labélékaha	Middle slope	0-20	6.0	0.98	0.11	475	68
		20-40	6.0	0.79	0.12	500	63
		40-60	5.0	0.49	0.04	488	63
		60-80	5.0	0.49	0.04	488	63
Ziévoogo	Foot slope	0-20	5.5	1.99	0.16	438	21
		20-40	5.5	1.81	0.17	387	16
		40-60	6.0	0.86	0.11	456	11
		60-80	6.0	0.86	0.11	456	11

The occurrence of *L. multiflora* is observed associated to wide range of soil pH (5.0 – 6.5), as well as for the contents of C (0.30 – 1.99 g kg<sup>-1</sup>), Nt (0.04 – 0.17 g kg<sup>-1</sup>), Pt (378 – 534 mg kg<sup>-1</sup>) and Pa (11 – 68 mg kg<sup>-1</sup>). However, the sites of Taoura and Labélékaha are characterized by pH range of about (6.0) and lower content than the critical level of C (< 1 g kg<sup>-1</sup>) in the topsoil (0 – 20 cm) while, lower pH (<5.5) is observed for Ziévoogo. In turn, soil contents of Nt (< 1 g kg<sup>-1</sup>) and Pt (378 – 534 mg kg<sup>-1</sup>) are likely in the same ranges across the three sites under *L. multiflora* occurrence respectively, but, soil Pa contents (21 – 11 mg kg<sup>-1</sup>) at Ziévoogo are not much as observed for the other sites (30 – 68 mg kg<sup>-1</sup>) according to Table 3.

Roughly, there is high affinity between low occurrences of *L. multiflora* with soils characterized by high pH associated with low content of C in 0 – 40 cm depth while, the increase of Nt in 40 – 80 cm depth is more related to the high density of this species. These contrasts are not as much for Pt and Pa referring to the indicators (DAI and AI) of *L. multiflora* occurrence.

No significant correlation is noticed between DAI and soil parameters in 0 – 20 cm and 20 – 40 cm depths contrasting with the negative values (-0.76 and -0.86) significantly observed for Pa in 40 – 60 cm ( $p = 0.04$ ) and 60 – 80 cm ( $p = 0.09$ ). Although the correlation values (-0.62 and -0.66) of AI are also negative and high for Pa under 40 cm soil depth, they are not significant and low values are observed for Pt. Significant ( $p = 0.04$ ) and high correlation value of 0.78 is noticed only for soil pH with positive magnitude in 40 – 60 cm depth.

Beside the high correlation value recorded between DAI and AI ( $r = 0.99$ ;  $p < 0.001$ ), it is significantly recorded positive correlation values for DAI and soil content of Pa in 0 – 40 cm (0.96) as well as in 40 – 80 cm (0.89) at Labélékaha. No significant correlation accounts for the site of Taoura while, soil pH and the content of Nt are concerned at Ziévoogo ( $\alpha = 0.10$ ) coupled with negative magnitude of correlation for soil pH in 0 – 40 cm.

Table 4. Coefficients (r) of Pearson correlation between DAI and soil pH, C, Nt, Pt, and Pa according to topographic sections as well as for AI

Soil Parameters	Correlation Coefficient (r)							
	Summit		Upper Slope		Middle Slope		Foot Slope	
	DAI	AI	DAI	AI	DAI	AI	DAI	AI
pH	..	..	..	..	-0.13ns	0.13ns	0.20ns	0.28ns
C	..	..	..	..	0.19ns	0.19ns	0.06ns	0.07ns
Nt	..	..	..	..	0.09ns	0.09ns	0.19ns	0.05ns
Pt	..	..	..	..	0.58*	0.004ns	0.39*	0.20ns
Pa	..	..	..	..	0.64*	0.64*	-0.59*	0.44*

.. : Not available; \* : significant for  $\alpha = 0.05$ ; ns: Non significant for  $\alpha = 0.05$ .

Positive and significant ( $p = 0.011$ ) similar correlation values are observed for Pa when referring to DAI and AI respectively in the middle slope position. In opposite, negative and significant ( $p = 0.001$ ) correlation accounts for Pa and DAI as recorded in the foot slope position also contrasting with that observed significantly ( $p = 0.023$ ) between Pa and AI. In turn, correlation values for Pt are only significant for DAI in the middle slope ( $p = 0.004$ ) and foot slope ( $p = 0.040$ ) positions with positive magnitude. No significant correlation is observed for the other soil parameters whatever the coefficient of *L. multiflora* occurrence (Table 4).

### 3.3 Essential Oil Composition and Relation With Soil

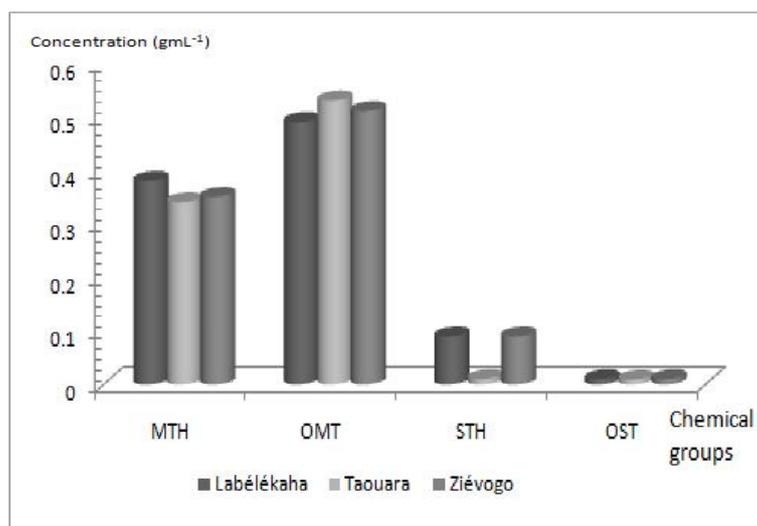


Figure 1. Average concentrations of different groups of chemical compounds (MTH, OMT, STH and OST) as determined in essential oil of *L. multiflora* from the topographic sections of Labélékaha, Taoura and Ziévogo (MTH: Monoterpene hydrocarbon; OMT: Oxygenated Monoterpene; STH: Sesquiterpene hydrocarbon; OST: Oxygenated Sesquiterpene)

Four groups of chemical compounds are extracted from essential oil of Lippia: Monoterpene and Sesquiterpene varying as hydrocarbon and oxygenated derivatives. Relatively, the concentrations within a chemical group are in the same ranges for all the localities. Roughly, hydrocarbon (MTH) and oxygenated (MTO) monoterpenes have almost the same concentrations meanwhile; the concentration of sesquiterpene hydrocarbon (STH) is likely 2 – 3 times greater than that of oxygenated sesquiterpene (OST). Moreover, the concentrations of monoterpenes appeared to be 2 – 4 times greater than that of sesquiterpene derivatives respectively. The concentrations are ranging from 0.05 g ml<sup>-1</sup> (OST) to 0.5 g ml<sup>-1</sup> (MTH) as 10 times increasing ratio (Figure 1).

Table 5. Volatile substance concentrations in essential oil of *L. multiflora* according to studied localities

Volatile Substance Concentrations (g ml <sup>-1</sup> )			
	Labélékaha	Taouara	Ziévo
<b>Monoterpene Hydrocarbons</b>			
$\alpha$ -Pinene	0.10	0.08	0.09
Sabinene	0.05	0.07	0.06
Myrcene	0.10	0.10	0.10
$\alpha$ -Phellandrene	1.39	1.08	1.15
Para-Cymene	0.97	1.05	1.01
Limonène+ $\beta$ -Phellandrene	0.87	0.84	0.85
(E)- $\beta$ -Ocimene	0.19	0.14	0.15
Linalol	0.15	0.12	0.13
<b>Total (g ml<sup>-1</sup>)</b>	<b>3.82</b>	<b>3.48</b>	<b>3.54</b>
<b>Oxygenated monoterpenes</b>			
6-methyl-5-hepten-2-one	0.16	0.16	0.16
1,8 Cineol	0.04	0.07	0.05
Cis-para-menth-2-en-1-ol	Trace	0.03	Trace
Citronellal	0.04	0.04	0.04
(Z)-isocitral	0.09	0.07	0.08
(E)-isocitral	0.15	0.12	0.13
Nerol	0.06	Trace	Trace
Neral	1.95	2.09	1.99
Geraniol	0.05	0.06	0.05
Geranial	2.32	2.66	2.48
Thymol	0.04	Trace	Trace
<b>Total (g ml<sup>-1</sup>)</b>	<b>4.90</b>	<b>5.30</b>	<b>5.15</b>
<b>Sesquiterpene hydrocarbons</b>			
(E)- $\beta$ Caryophyllene	0.20	0.25	0.23
$\alpha$ -Humulene	0.55	0.55	0.55
(E)- $\beta$ -Farnesene	0.06	0.08	0.07
Germacrene D	0.10	0.06	0.08
$\beta$ -Bisabolene	0.05	0.06	0.06
<b>Total (g ml<sup>-1</sup>)</b>	<b>0.96</b>	<b>1.00</b>	<b>0.99</b>
<b>Oxygenated sesquiterpenes</b>			
(E)-Nerolidol	0.08	0.05	0.07
Caryophyllene Oxide	0.05	0.04	0.05
<b>Total (g ml<sup>-1</sup>)</b>	<b>0.13</b>	<b>0.09</b>	<b>0.12</b>
<b>Overall concentration (g ml<sup>-1</sup>)</b>	<b>9.87</b>	<b>9.81</b>	<b>9.63</b>

Monoterpene hydrocarbon is composed of eight volatile substances against 11 as oxygenated derivatives which are minor (2) among sesquioxides containing 5 hydrocarbon derivatives. The concentrations of volatile substances are relatively similar in a given site for monoterpenes hydrocarbon as well as for sesquioxides compounds. However, essential oil extracted from the Lippia of Labélékaha is characterized by Nerol and

Thymol concentrations which are missing monoterpenoids in the essential oil from Taoura and Ziévogo. Exceptionally, Cis-para-menth-en-1-ol is determined in the essential oil of *L. multiflora* from Taoura. In turn, major substances (high concentrations) are recorded in the same range of concentrations in essential oil extracted from the leaves of *L. multiflora* across the studied sites, including, 3 ( $\alpha$ -Phellandrene, Para-Cymene, Limonene+ $\beta$ -Phellandrene) of monoterpene hydrocarbons and 2 (Neral and Geramial) of oxygenated monoterpenes. Only  $\alpha$ -Humulène out stands among the sesquiterpene strongly contrasting with oxygenated derivatives which have lowest concentrations (Table 5).

Similarly, all the major volatile substances are significantly ( $p < 0.01$ ) correlated to soil contents of Pt (0.61) and Pa (0.72) with positive magnitude. Significant ( $p = 0.01$ ) correlations with similar value (-0.49) are observed only for soil content of Pt indifferently to volatile substances of the essential oil with negative magnitude at Taoura.

As observed for Labélékaha, soil contents of Pt ( $\alpha = 0.05$ ) and Pa ( $\alpha = 0.10$ ) at Ziévogo are significantly correlated with all the major volatile substances of essential oil as well as for soil content of Nt ( $\alpha = 0.10$ ) with positive correlation values (0.39) referring only to  $\alpha$ -Phellandrene, Para-Cymene and Limonene+ $\beta$ -Phellandrene respectively. Positive and significant correlation values (0.49 and 0.41) are also observed for soil content of C with no restriction among the volatile substances.

#### 4. Discussion

##### 4.1 Occurrence of *Lippia* as Affected by Soil Characteristics

Soil pH values were ranging between 5.5 and 6 as moderate acidity that might be favorable for the growth of many vegetal species in the studied area (Koné, Saïdou, Camara, & Diatta, 2010a; Koné, Ettien, Amadji, Diatta, & Camara, 2010b) including *L. multiflora* as asserted by Alui et al. (2011). However, preferential occurrence of this species was noticed along the toposequence during the survey: Lowest occurrence accounted for the hill slope (summit and upper slope) position of landscape contrasting with the highest occurrence observed along the down slope (middle and foot slope) position. Soil morphological variability along the landscapes of the studied region could be involved in this clustering. In fact, there is highest content of gravels in the soil of hill slope contrasting with that of juxtaposed soils of down slope position which are dominated by colluviums from hill slope (Wambeke, 1974; Koné et al., 2009). This mechanical constraint could have impaired the root development of *L. multiflora* as described for similar species in such conditions (Boa, 1989) meanwhile, woody trees with more robust roots are frequently observed in the biotope of hill slope (Beaudou & Sayol, 1980) further constraining the occurrence of *L. multiflora* as C4 plant requiring sunlight (Yao-Kouamé & Fako, 2008).

Soil acidity and the content of phosphorus in subsoils (40 – 60 cm and 60 – 80 cm) significantly influenced DAI and AI respectively: Increase of soil pH in 40 – 80 cm depth was associated with highest DAI of *L. multiflora* in contradiction with the result relative to 0 – 40 cm depth. Indeed, the increase of pH in subsoil could be a consequence of cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$ ) accumulation resulting from leaching of topsoil (Dabin, 1985) hence, becoming more acidic. Thereby, the opposite correlations (positive and negative) established between DAI and soil pH in 0 – 40 cm and 40 – 80 cm respectively. Moreover, the increase of soil content of Nt in 40 – 80 cm depth was likely associated to similar trend of DAI at Ziévogo. Referring to soil pH, the increase of Nt could be more related to nitrate ( $\text{N-NO}_3^-$ ) nitrogen (Kleiner, 1981; Dyhr-Jensen & Brix, 1996) that could be leached as described above before the conversion into ammoniac ( $\text{N-NH}_4^+$ ) nitrogen (Wayne, 2001). In the light of these analyses, depletion of topsoil contents of cations can be favorable to *L. multiflora* occurrence in soil with poor content of gravels. In turn, anionic compounds as P ( $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ) should be high throughout the rooting profile (0 – 80 cm) for increasing of DAI of *L. multiflora*. Therefore, P is likely the most important nutrient for the cultivation of *L. multiflora* when developing specific agricultural systems (Yao-Kouamé & Fako, 2008). However, there was no trend of soil contents of P across the studied sites in relation with DAI. Thus, we assume indirect effect of soil P content on *L. multiflora* occurrence as result of some interactions with other nutrients such as specific cations (Marschner & Cakmak, 1986; Koné et al., 2011), which need to be confirmed by further study.

However, hydromorphic character of the soil at Ziévogo was most pronounced compared with that of the other studied sites illustrating highest moisture availability that could have supported the growth of *L. multiflora* (Rumasz-Rudnicka, Koszanski, & Woroniecki, 2008) as attested by the highest density of 3 recorded during the survey.

Beside of the characterization of *L. multiflora* occurrence, our study confirms that soil chemical degradation is more related to invasive vegetation occurrence (Koné et al., 2013b) emphasizing the importance of soil cations in this process while contrasting with that of anions. However, further investigations are required in other

ecological zones for the generalizing such assumption.

#### 4.2 Essential oil and Soil Relationship

The monoterpenes compounds (8.52 – 8.78 mg L<sup>-1</sup>) were the most important chemical components of the essential oils as extracted from the leave of *L. multiflora* harvested across the studied sites. This result is concordant with that of Loza-Tavera (1999) likewise for Bruneton (1993). The highest concentrations of oxygenated derivatives as monoterpenoids further confirm the work done by Oussou et al. (2008). However, the major volatile substances were identified as Geranial, Neral,  $\alpha$ -Phellandrene, Para-Cymene, Limonene+ $\beta$ -Phellandrene and  $\alpha$ -Humulene somewhat differing with that of lowest latitude (< 7° N) localities even in Cote d'Ivoire (Kanko et al., 2003; Kanko, 2010; Etienne et al., 2011) and Burkina Faso (Bassolé et al., 2010). The difference observed can account for genetic diversity of *Lippia multiflora* (Adou et al., 2011) at certain level in addition to the effect of the latitude variation (Purseglove, Brown, Green, & Robbins, 1981). However, environment effect as described by Besombes (2008) can stand out among variability factors of essential oil quality in a restricted area as much as our studied region. Soil contents of P as total and available P has shown significant influences on the concentrations of major volatile substances determined in extractable essential oils across the studied sites. This result could be related to temporal character of bounded P (e.g. Al-P, Fe-P and Ca-P) which may be stable in low soil pH (< 5.5) condition (Sanchez, 1976) while, higher soil pH values were often determined for the studied sites. Hence, releasing process of bounded -P can occur as consequence of isomorphic substitution against other anions (Colomb, Debaekey, Jouan, & Nolot, 2006). Therefore, a positive correlation between Pt and volatile substances of essential oil are coupled with that observed with Pa at Labélékaha while, it was recorded negative correlations at Taoura as consequence of limited release of -P contributing to induce no significant relationship between Pa and the concentrations of volatile substances. This assumption is also related to the results observed at Ziévogo differing to the other studied sites with hydromorphic soil and the influence of soil contents of C and Nt in addition to the opposites influences of Pt vs. Pa. Moreover, Neral, Geranial and  $\alpha$ -Humulene concentrations were not affected by soil content of Nt at all ( $\alpha = 0.05$  and  $\alpha = 0.10$ ).

In the light of these analyses, phosphorus is unarguably the most important nutrient for qualitative production of extractable essential oil of *Lippia multiflora*. This potential effect of P-nutrition in essential oil production was tested by R. Tunctürk and M. Tunctürk (2006) and optimum rate was about 40 kg P ha<sup>-1</sup> but no similar investigation was known for the production of essential oil of *Lippia multiflora*. Therefore, further studies should explore the effect of P-rates in quantitative and qualitative productions of volatile substances contained in the essential oil of this aromatic plant. In fact, P is a component of active isoprene (Isopentenyl pyrophosphate), the radical of terpenoid compounds (Nes & Mckean, 1977) known to be widely involved in the physiology of aromatic plants with significant influence of phosphorus in biosynthesis of essential oils (Olle & Bender, 2010).

However, nutrient interaction with phosphorus and moisture availability in soil can be of interest in essential oils synthesis: soil moisture can affect Linalol synthesis (Arganosa, Sosulski, & Slikard, 1998) with increasing effect on organic matter contribution as well as for nitrogen (Hussien, 1995). The actual study mainly contributed to identify the components of mineral nutrition of *Lippia multiflora* emphasizing their influence on chemotype variability of the extractable essential oil. Therefore, knowledge is improve for development of sustainable production strategy of this species with high economical potential but still growing as invasive vegetal.

#### 5. Conclusion

In addition to soil acidity already identified as criteria of the occurrence of *Lippia multiflora* in West Africa ecosystems, our study revealed the importance of mechanical constraints as induced by soil morphology and that of bioavailability of phosphorus. The major volatile substances were Geranial, Neral,  $\alpha$ -Phellandrene, Para-Cymene, Limonene+ $\beta$ -Phellandrene and  $\alpha$ -Humulene. Phosphorus has influenced their concentrations respectively and soil moisture was likely favorable to similar effect of nitrogen somewhat contrasting with that of organic matter. Study of response to the rates of P and N was suggested considering different ecologies in hydromorphic and aerobic conditions in order to deepen knowledge for quantitative and qualitative productions of *L. multiflora*.

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