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Analysis and Application of Leaf Chemical Concentrations in *Heveabrasiliensis*nutrition: Compositional Nutrient Diagnosis Norms

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Abstract: *The main source of natural rubber is the rubber tree (Heveabrasiliensis), an important industrial crop. In order to improve yields while maintaining tree sustainability, nutrient norms must be derived for proper nutrient and sustainable soil fertility management. The aim of this study therefore, was to analysis and use leaf chemical concentrations to derive compositional nutrient diagnosis (CND) norms for Heveabrasiliensisnutrient management. A survey of 130 Heveabrasiliensis fields was carried out to collect leaf samples and yield data. The sampled leaves were under shade of canopy and at least 100 days old and the yield data was measured as kg/ha per year of dry rubber. The leaves were analysed for N, P, K, Ca, Mg, S, Fe, Mn, Mo, Na and Zn. Using the yield data, the sampled population was divided into high- and low–yielding sub-populations. The CND norms were derived from a high-yielding sub-population (fields yielding >1,667 kg/ha) following standard procedures. The CND indices were calculated and used for the assessment of the nutrient status of a low- yielding sub-population of Hevea. The preliminary CND norms obtained for the different elements were as follows:* $V^*_N = 3.61 \pm 0.14$, $V^*_P = 0.94 \pm 0.16$, $V^*_K = 2.53 \pm 0.23$, $V^*_{Ca} = 2.47 \pm 0.24$, $V^*_{Mg} = 1.43 \pm 0.18$, $V^*_S = 1.1 \pm 0.10$, $V^*_{Ca} = 2.47 \pm 0.24$, $V^*_{Na} = -2.38 \pm 0.15$, $V^*_B = -3.63 \pm 0.27$, $V^*_{Cu} = -4.04 \pm 0.18$, $V^*_{Fe} = -1.41 \pm 0.39$, $V^*_{Mn} = -0.96 \pm 0.50$, $V^*_{Mo} = -8.18 \pm 0.35$, $V^*_{Zn} = -2.73 \pm 0.15$ *and* $V^*_R = 11.25 \pm 0.10$. *Where* V^*_R *represents the norm for nutrients not analysed. The results showed that the micro nutrients except for Cu, Zn and Mo were deficient. The obtained nutrient norms are recommended for nutrient assessment in H. brasiliensisplantations.*

Keywords: *Concentrations, leaf, Heveabrasiliensis, norms, yields*

1. INTRODUCTION

Natural rubber is an important crop with many applications like in the manufacture of heavy duty vehicle tyres, items of medical and paramedical use and *Heveabrasiliensis*has remained the main commercial source of natural rubber [1] In order to improve and obtain sustainable yields, proper management ofessential nutrients is required leading to minimized waste, economic losses andenvironmental impacts [2]. Proper nutrient management begins with adequate nutrient diagnosis where the nutrient status is accurately determined. Plantchemical analysis have often been used for nutrient diagnosis based on the assumption that causal relationshipsexist between growth rates (yield) and nutrient content in the plant part tested [3]. Adequate procedures for the interpretation of analytical data must be available for the fullbenefit of using foliar analysis in assessing plant nutrient status [4].

The most common approach in diagnosing nutritional status of plants is the critical nutrient approach 5,6]. The critical nutrient approach however, has some limitations such as variation in critical value as the magnitudeof the backgroundconcentrations of other nutrients vary in crop tissue [7]. In order to overcome this limitation, Beaufils in 1973, and Walworth and Sumner (1987), proposed the Diagnosis andRecommendation Integrated [3] System (DRIS) method for nutrient evaluation. This methodconsiders nutrient ratios in order to avoid the effect of leaf age and physiological interactions [8, 9], which limits the applicability of leaf tissue analysis for nutrient diagnosis [4]. The major drawback of the DRIS method for nutrient diagnosis is that each time it is used it indicates that one or two nutrients are limiting [10].

For best diagnosis results to be obtained, the Compositional Nutrient Diagnosis (CND) method was proposed and developed by Parent &Dafir (1992) [11] and is based on the principles of Compositional Data Analysis (CDA). The CND approach was thus developed to improve tissue diagnosis as compared to univariate (CVA) or bivariate (DRIS) approaches. The CND takes into consideration the interdependence of nutrient concentrations in plants and that the sum of all dry matter concentrations always totals up to 100 %, or sums up to 1. The multivariate nutrient diagnosis and principal component analysis use "row centered log ratios” of nutrient fractions. Nutrient indices are used for the interpretation of compositional nutrient data. It represents the difference between a particular nutrient and its geometric mean relative to the difference of the same nutrient to the geometric mean of the high-yielding sub population [11]. The nutrient imbalance index is an inherent component of CND. Despite the usefulness of the CND method of nutrient diagnosis, CND norms for *Heveabrasiliensis* are not readily available. Thus the objective of this study was to analyse and use leaf chemical concentrations to derive CND norms for *Heveabrasiliensis*nutrient assessment.

2. MATERIALS AND METHODS

2.1. The Study Area

A total of 130 leaf samples were collected from rubber estates belonging to the Cameroon Development Corporation of Cameroon and SocièteForestiereAgricole du Cameroon (SAFACAM) (Fig. 1). The climate of the study site is characterized by high temperatures and seasonal rainfall. The mean temperature ranges from 25 - 28ºC and rainfall ranging from 700 - 1250 mm. The soils depth ranged from shallow to deep and the texture from sandy loam through clay loam to sandy clay. Following the FAO classification the soils ranged from Ferric/HumicAcrisols to orthic/xanthicFerrasols.

2.2. Leaf Sampling and Analysis

2.2.1. Leaf Sampling

The *Hevea*trees sampled were all matured trees in tapping (exploitation) and the leaves sampled were at least 100 days old. Leaves from the shaded canopy were sampled from 15 trees/site and all samples were mixed together to make a composite sample. Upon arrival in the laboratory, the samples were washed in running water to remove dirt, followed by the removal of the leaves stalk. The leaves were cut into smaller pieces and placed in paper bags for oven drying at 70 to 75ºC for 48 hours. After drying, the leaves were crushed into a powder using a home use blender (Mulinex) and sealed in polythene bags for latter analysis.

2.2.2. Leaf Analysis

Leaf N was analysed using the Elemental analyser, vario MAX CNS and the macro nutrients (P, K, Ca, S and Mg) by inductively coupled plasma optical emission-spectrometry (ICPOES) after dry combustion followed by dilution in nitric acid + Hydrogen peroxide. The leaf micro nutrients (Fe, Mn, B, Na, Mo and Zn) were microwave digested in nitric acid and hydrogen peroxide. The extract was read using the Inductively Coupled Plasma Optical Emission-Spectrometry (ICP-OES) method.

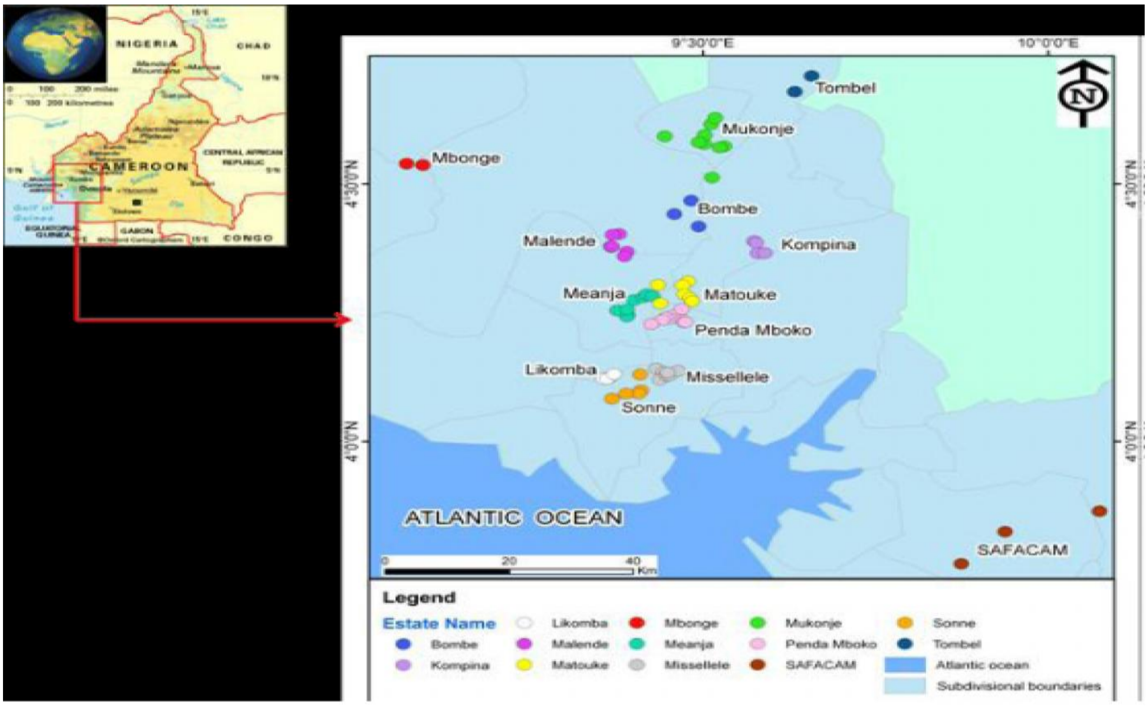

Figure 1. *The map of Cameroon showing the sampling sites*

2.3. Yield Recording

Harvested latex from the various blocks/sites was subjected to three days coagulation in the field to obtain field coagula (cuplumps). The dry rubber from each block was collectedby their respective managements, weighed and the annual yields recorded in kilogram perhectare (kg/ha).

2.4. CND Methodology and Data Analysis

Using *Hevea*yield and leaf chemical concentration data, CND norms and standard deviationsvariation (SDs) were derived according to the procedure by Parent and Dafir (1992) [11].The yield cut off point was determined following the Cate and Nelson statisticalprocedure [12].Using the yield cut-off, the CND norms were established ($V_N{}^* + V_P{}^* + V_K{}^* +\ldots+ V_{Rd}{}^*$) as means of row-centred log ratios of *d* nutrients (CND-norms) and their standard deviations, $SD_N{}^*$, $SD_P{}^*$, $SD_K{}^*$, …,$SD_{Rd}{}^*$. Independent plant samples were assigned to individual row-centred log ratios to form CND-indices [11]: as shown in the (1).

$$I_N = V_N - V_N{}^*/SD_N{}^*,\ I_P = V_P - V_P{}^*/SD_P{}^*,\ I_K = V_K - V_K{}^*/SD_K{}^*,\ \text{---------------},\ I_{RD} + V_{RD} - V_{RD}{}^*/SD_{RD}{}^* \quad (1)$$

Where: I_X is the index for nutrient X, and $V_X{}^*$ and $SD_X{}^*$ are the CND norms

The CND nutrient imbalance index ($CNDr^2$) of each specimen was calculated following (2).

$$CNDr^2 = I_N{}^2 + I_P{}^2 + I_K{}^2 + \ldots + I_{Rd}{}^2 \quad (2)$$

3. Results and Discussion

3.1. Leaf Chemical Concentration Data

The descriptive statistics for *Hevea* natural rubber yields and leaf chemical concentrations arepresented in Table 1. The yield data ranged between 369 kg/ha and 2,550 kg/ha with a mean of 1,407 kg/ha. Leave N values range between low and deficient. Plants absorb nitrogen from the soil as both NH_4+ and NO_3^- ions. Once inside the plant NO_3^- is reduced to an NH_2 form and is assimilated to produce more complex compounds Leaf P, K, Mg and Ca values ranged from low to high values [13] or deficient to adequate levels according to Reuter and Robinson (1997) [14]. Once inside the plant, P may be stored in the root or transported to the upper portions of the plant. Through various chemical reactions, it is incorporated into organic compounds, including nucleic acids (DNA and RNA), phosphoproteins, phospholipids, sugar phosphates, enzymes, and energy-rich phosphate compounds like adenosine triphosphate. This high-energy phosphate is the source of energy that drives the multitude of chemical reactions within the plant [15]. Although K does not become a part of the chemical structure of plants, it plays many important regulatory roles in development. Potassium activates at least 60 different enzymes involved in plant growth [15]. Mg^{2+} is the coordinating metal ion in the chlorophyll molecule and in plants where the ion is in high supply, about 6 % of the total Mg^{2+} is bound to chlorophyll [15].

Considering the B level of less than 20 ppm as the deficient level, most estates were deficient in boron. On the other hand, the leaves were highly supplied with zinc indicating although the levels in the soil were low; they were not yet a problem but could be a problem in the future. The range of values for Zn obtained in this study was similar to a range of 0.02-0.04 mg g-1 dry weight obtained for plants [16]. The metabolic functions of Zn are based on its strong tendency to form tetrahedral complexes with N- , O- and S-donor ligands [15]. In plants, its predominant forms are low molecular weight complexes, storage metalloproteins, free ions, and insoluble structures associated with the cell walls. At an intracellular level, Zn is inactivated throughout complexes with organic ligands or by complexation with phosphorus [17].

Table 1. *Descriptive statistics for Heveabrasiliensisyields and nutrients*

Parameter	MEAN	Min	Max	Std dev.
Yield (kg/ha)	1407.4	369.0	2550.0	465.4
N %	3.3	2.6	4.6	0.3
P	2549.9	1503.5	4521.1	612.2
K	11451.7	6194.0	21187.1	2861.8
Ca	9386.2	2151.9	16385.6	3194.0
Mg	3295.8	1340.8	6049.9	774.6
S	2656.9	1269.5	3674.8	306.6
Al	189.5	50.8	958.4	145.6
Na	102.2	57.3	302.2	47.3
B	21.0	6.4	39.3	6.4
Cu	17.6	11.3	41.5	6.3
Fe	197.3	78.3	891.9	123.1
Mn	317.6	35.6	832.2	179.2
Mo	0.3	0.1	1.6	0.2
Zn	55.2	19.7	96.9	11.4

Min = minimum, Max = Maximum, Std dev. Standard deviation

3.2. Compositional Nutrient Diagnosis (CND)

Following the procedure of Khiari*et al*. (2001a) [18], the yield cut off associated with the various nutrients were 1,333 kg/ha for N, P, B, Ca, Cu, Mg, Mn, S and Zn, 1,111 kg/ha for Mo, 1,429 for Fe and 1,667 kg/ha for R (the filling value) and K. As proposed in this method the yield cut off should be the maximum obtained no matter the nutrient to which it is associated. Thus, for this study, the yield cut off of 1,667 kg/ha was chosen. Nutrient concentrations that were transformed into row-centred log ratios were used for the derivation of CND norms. Using the row-centered log ratios for the high- yielding sub population, the preliminary CND norms and standard deviations presented in Table 2 (R being the filling value for elements not analysed) were obtained. There was however a significant difference in the mean row centred log ratios for the high and low-yielding sub populations, suggesting that the yield difference is due to nutritional disorder and thus the norms derived in this study are reliable.

So far, literature values for CND norms for *Heveabrasiliensis* are not available which makes comparison difficult. The CND norms derived in this study have both positive and negative values with all of them summing up to zero. This indicates that the method for the derivation of these norms was correctly followed. Using the norms developed in this study, the CND nutrient indices were calculated. The CND indices reveal the order of limiting nutrients and the magnitude of nutrient deficiency in each sample relative to the optimum value. Negative CND indices represent nutrient deficiencies and the most negative value indicates the most limiting nutrient. Positive CND indices are an indication of nutrient excesses and the largest value indicates the nutrient present in greatest amount [19].

Table 2. *CND Norms as means of row-centred log ratios and standard deviation of high-yielding sub population (n= 46).*

Parameter	**V^*_N**	**V^*_B**	**V^*_{Ca}**	**V^*_{Cu}**	**V^*_{Fe}**	**V^*_K**	**V^*_{Mg}**
Mean	3.61	-3.63	2.47	-4.04	-1.41	2.53	1.43
Std. Dev.	0.14	0.27	0.24	0.18	0.39	0.23	0.18
Parameter	V^*_{Mn}	V*Mo	V*Na	V*P	V*S	V*Zn	V*R
Mean	-0.96	-8.18	-2.38	0.94	1.1	-2.73	11.25
Std. Dev.	0.50	0.35	0.15	0.16	0.10	0.15	0.10

Where: Std. Dev. = Standard deviation, * = Compositional Nutrient Diagnosis (CND) norm for each nutrient.

3.3. Diagnosis of the Nutrient Status of a Low-Yielding Sub-Population

In order to know the nutrient(s) limiting yield, the derived CND norms were used to diagnose the nutrient status of the low-yielding sub population in this study. With the yield cut off of 1,486 kg/ha a total of 76 samples were in this low-yielding sub population. The average of the CND indices is presented below for each nutrient (Fig. 2). The theoretical approach to the interpretation of CND indices as negative being deficient and positive meaning sufficiency was explained by Parent and Dafir (1992) [11]. The macronutrients (NPK) had positive indices showing that they were sufficient and no fertilization was needed. The micronutrients (Fe, B and Mn) were deficient. As a whole, the most deficient nutrients were B and Mg (with most negative indices). This suggests that the low yields could be due to the low levels of micronutrients. There was an apparent excessive consumption of P (Ip = 0.7) and Na (I_{Na}= 0.54). The disadvantage of having high values of nutrients like Na is that it may accumulate in the leaf at luxury or excessive levels at the expense of other nutrients or the filling value (R) [20]. In DRIS (as is also the case for CND) it was assumed that luxury consumption of a single nutrient by the crop results in a lack of balance or low yields [20]. Apparent luxury consumption may also occur when nutrient norms developed for a given biogeographic region are misapplied to another region, since an interaction between nutrient norms and regional conditions may occur. Excess P may inhibit the uptake and translocation of Fe [21]. This could account for the negative (deficient) Fe values obtained in this study.

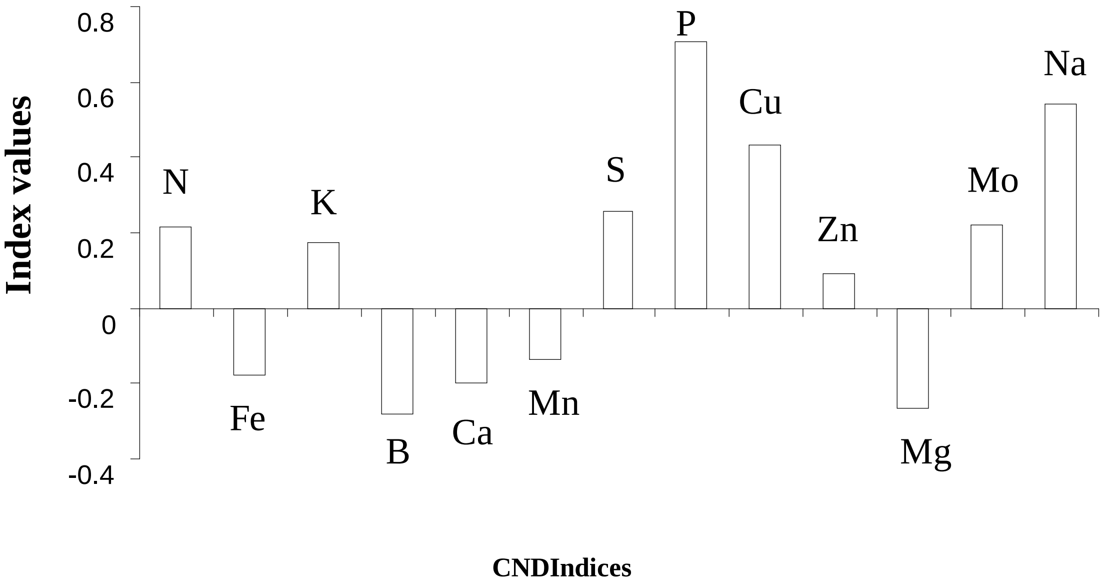

Figure 2. *Mean CND indices for a low-yielding sub population of Heveabrasiliensis plantations in Cameroon*

4. Conclusion

The chemical concentrations of *Heveabrasiliensis* leaves were analysed and found to range between low and high values. Using leaf chemical concentrations, preliminary CND norms for *Heveabrasiliensis* have been derived. Literature CND norms for *Heveabrasiliensis* are not readily available thus it was not possible to compare the obtained norms. This work is thus a foundation work on CND norms derivation for *Heveabrasiliensis*. The elements; N,P and K had positive indices showing that they were sufficient and no fertilization was needed while Fe, B and Mn were deficient. The obtained norms will be used for the assessment of *Heveabrasiliensis* nutrition.

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