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Effects of Nitrogen and Phosphorus Fertilizers on Bioavailable Aluminum and Uptake of Nutrients and Aluminum in *Hibiscus Sabdariffa*

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***Abstract* – The rural-urban migration has led to depopulate the African villages for the benefit of cities. Farmers become urban residents are forced to adopt an intensive agriculture. The result has been the decline in crop yields and depletion of the resource base. The aims of this study were to assess effects of N or P fertilization on bioavailable Al and their contribution on Al and nutrient uptake in Hibiscus sabdariffa. Thus, a pot experiment was led to supply a tropical soil with nitrogen and phosphorus fertilizers at different levels. The phosphorus amendment decreased significantly Al in soil solution, not nitrogen amendment. P and N fertilizers had significant effects on Al and nutrient uptake in roots and leaves of *Hibiscus sabdariffa*. The results also shown the uptake of Al and nutrients simultaneously depends on Al level on soil solution and N or P supply, the strongest effects being on the uptake of Al in roots and P in leaves.**

***Keywords* – Acid Soil, Mineral Amendment, Nutrient Uptake, Soil Solution, Urban Garden.**

I. Introduction

Food crops in Africa are cultivated by rural subsistent farmers. They practice slash and burn and/or shifting cultivation, over the years, to sustain yield even though at a low level. The rural-urban migration has led to depopulate the villages for the benefit of cities. Farmers became urban residents continue their activities in cities with new difficulties such as a limited availability of arable land. The inability to practice fallow as in rural areas led farmers to cultivate the same plot continuously [1]. The result has been the decline in crop yields and depletion of the resource base. Soil fertility depletion in smallholder farms is the biophysical root cause of declining per capita food production, and soil fertility replenishment should be considered as an investment in natural resource capital. Per capita food production in Africa will continue to decrease unless an adequately solution to the problem of soil fertility depletion is found.

When exposed to intensive cultivation in the equatorial African climate of West Gabon, soils rapidly lose organic matter and nutrients, and are subject to soil erosion and acidification. Acidification causes several limiting factors, such as: deficiencies of some essential elements, such as: phosphorus (P), nitrogen (N), calcium (Ca), magnesium (Mg), some micronutrients, and toxic levels of aluminum (Al). The combined effects generally limit plant production, especially that of crop species[1] – [2].

Nitrogen and P are essential nutrients for vegetative growth and crop yield[3], and in the urban farms of Libreville, West Gabon, N and Pdeficiencies and acidic soil conditions are widespread [4]. Deficiencies of P, N and other nutrients are made worse by limited inputs of organic matter and fertilizer. These weathered soils have extremely low soil solution P concentrations and hostile conditions for P availabilitydue to the large amounts of Al and Fe oxides which retain P by ligand exchange at their surfaces [5]- [6]- [7]. Nitrogen is the element most frequently deficient in crops and is the most limiting nutrient in acid soils[8]. It is very mobile and lost by both leaching and volatilization [9]. Nitrogen limits crop production in the tropical soils with low organic matter and the symptoms are pale green or yellowish-green color of the foliage and premature necrosis of the old leaves[10].

To improve the nutrient status in order to meet plant demands of potentially high yielding crops N and P fertilizers are used in limited amounts because mineral fertilizersare an expensive but often necessary input for agricultural systems[11].

Roselle (*Hibiscus sabdariffa*. L) is an annual or biennial vegetable cultivated in tropical and subtropical regions for its stem, fibers, edible calyces, leaves and seeds. It belongs to the Malvaceae family. Roselle is a short day plant, and thrives in areas with a high humidity and a temperature of 25-35°C. Other optimal pH of 6-7 and rainfall of 450-500 mm that is well-distributed over the 90-120 days growing season[12]. Currently, *Hibiscus sabdariffa* is an important leafy vegetable due to its high nutritional value and high antioxidant capacity[13]- [14]- [15]. At present, there are no data on the interaction of P or N fertilization, and Al in the soil solution on the growth and mineral nutrition of *Hibiscus sabdariffa*.

This study assesses the effects of N and P fertilizers on the growth and mineral nutrition of *Hibiscus sabdariffa* on acidic soils from Libreville. In addition, the effects on a chemical measure of bioavailable soil Al were assessed. The study was conducted using large pots in an open shade structure.

II. Experimental

This study was conducted from soils sampled in an urban garden of Libreville. The city is situated in West Gabon (9°25′ east longitude and 0°27′ north latitude). The climate type is equatorial. The annual rainfall varies from 1,600 to 1,800 mm. The average temperatures oscillate between 25 and 28 °C with minima (18 °C) in July and maxima (35 °C) in April, with a humidity of 80 to 100 %. Three cultivated soil samples were randomly collected with a stainless steel shovel. The samples were put in plastic bags immediately and stored at −4 °C. They were air-dried, crushed in a mortar, sieved through a 100-mesh sieve (2 mm), then crushed with a tungsten-carbide blade grinder and subsequently sieved through a 0.2 mm titanium mesh.

The experiment was carried out with 63 pots containing each 24 kg dry soil. Sevennutrient additions were made by adding appropriate amounts of N as potassium nitrate or P as potassium dihydrogen phosphate as solid powdered : 0 mg/kg; 12,5 mg/kg; 25 mg/kg; 50 mg/kg; 100 mg/kg; 200 mg/kg; 400 mg/kg. The acidification can results from using different fertilizers. Some nitrogenous fertilizers, for example urea, Calcium nitrate, potassium nitrate and sodium nitrate have few effects on pH if all the nitrogen is utilized by the plant or if all nitrate is leached. Elemental sulfur is acidifying and requires 3.125 kg of lime for each kg of sulfur to neutralize its effect. This effect can be avoided by using products that contain sulfur in the sulfate form such as gypsum, potassium sulfate and superphosphate[16]. A basal application of 24 g of NPK fertilizer per pot was also made at the same time. Deionised water was added to bring the samples to field capacity.

Roselle (*Hibiscus sabdariffa*) was chosen as the potted vegetable because, it is one of staple leafy crops in Africa diet and available all year. It belongs to the family of Malvaceace. This vegetable has been found to flourish on a wide range of soil conditions but for economic purposes, a soil well supplied with organic materials and essential nutrients is important in the productions. Roots orally used as a stomachic and externally as an emollient and leaves are eaten as vegetable after cooking[17]. Roots and leaves of *Hibiscus sabdariffa* are rich in β-carotene, vitamin C, riboflavin, organic acids and mineral elements[18]- [19].

It was sown in a rate of 12 seeds per pot. These were allowed to germinate and establish for 10 days in an ambient environment, in an open shade structure. After this time, young plants were removed and there were six plants per pot. There were three replicates of each treatment and the pots were placed in three blocks under an open shade structure. Each block contained one pot of each treatment arranged randomly within the block. The pots were watered regularly with deionised water to keep them close to field capacity. The experiment was finished 30 days after sowing and the plants were uprooted.

Soil sampling was done in each pot. Soil samples were air-dried and then dried at 105°C in a stove until their weight was constant.In order to determine the mobile or “potentially available” fraction of Al in the studied soils, 1 mol/LKCl solution were mixed with 3 g of soil sample into 30 mL plastic centrifuge tubes. The tubes were shaken for 24 hours. The solutions were separated from the soil by centrifugation, filtered through a 0.43 µm filter paper and conserved in flasks.

The sample vegetables were firstly washed with distilled water, and secondly with deionized water. Roots, stems and leaves were separated. They were air-dried and then dried at 70°C in a stove until their weight was constant. Dried leaves samples were ground into a fine powder using a mill of IKA A10 type, thereafter stored in polyethylene bags kept at room temperature. Plant samples were digested for 1 h at 150°C in a microwave mineralizer, using a mixture of nitric acid, hydrogen peroxide and ultra-pure water with a volume ratio of 2:1:1[19]. The resulting solution was then filtered at 0.45 µm and stored at 4°C before the quantifying of element concentrations.

The soil properties were assessed according to Association Française de Normalisation (AFNOR) protocols. They included: particle size, pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), available phosphorus (P), Ca and Mg exchangeable cations and cation exchange capacity (CEC). Considering, the average content of carbon in soil organic matter of 58 %, a conversion factor of 1.724 was used to calculate the percentage of organic matter (OM) from the content of organic carbon[4]. Contents of exchangeable Al in soil and Al, Ca, Mg, in roots and leaves of *Hibiscus sabdariffa* were determined using a GBC 932 AA atomic absorption spectrometer (Australia). Calibrations were performed using mixed standard calibration solutions of element studied, which were prepared daily by dilution of commercial standard solutions, for atomic spectroscopy, in ultrapure water. N and P were analyzed by spectrophotometry with Genesys 10UV spectrophotometer (USA).

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. The reagents were of analytical grade. Table I presents data on standard plant reference materials (DC 73349) from China National Analysis Center for Ironand Steel (NSC) and soil reference materials (CRM-SS1, EPA -3050A) that were analyzed as a part of the control protocol(accuracies within 100 ± 10%). Blank and drift standards were run after ten determinations to maintain instrument calibration. The coefficient of variation of replicate analyses was determined for the measurements to calculate analytical precision.

Table and Fig.s present the results as means ± standard deviation of three replicates. The significance of differences between the means of metals in edible parts of plants was evaluated by Tukey’s test ($P<0.05$). Regression analyses were carried out to find out how the Al, Ca, Mg and P uptake in plant varied with P and available Al or N and available Al in soil solution. Statistical analyses were performed with the software XLSTAT, Version 2010 (Addinsoft, Paris, France).

III. Results and Discussion

The soils were loamy sand in texture, poor in organic matter, Ca, Mg and P available, and hence infertile and strongly acid. The pH_{KCl} of the soils was $>pH_{water}$ (Table 1). This result might indicate the presence of gibbsite in these soils. The cation exchange capacity (CEC) values were low in the soils indicating that the potential for positively charged cations adsorption had low. The Al levels in soil solution were ranged between 93 and 150 mg/kg(Table I). Many works revealed a toxicity threshold of 50 mg/kg of exchangeable Al[20] - [21]. The exchangeable aluminum exists only in acid soils. Al

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toxicity is one of the most important single factors limiting crop production in acidic tropical soils. Recent studies in this city showed that the urban cultivated soils were acidified because an intensified cultivation and the increasing human population[4] - [19]. At low pH (4–6) the concentration of Al in soil solution is influenced by several processes among whose decreasing of amount of exchange sites of complex and dissolution of gibbsite whose releases soluble Al[22].

Table 1. Soil physicochemical characteristics before amendments.

	pH_{H2O}	pH_{KCl}	TKN	OM	Sand	Silt	Clay	CEC	$P_{avail.}$	$Ca_{exch.}$	$Mg_{exch.}$	$Al_{exch.}$
			g/kg					méq/100g	mg/kg			
Soil	5,6	4,3	1,3	192	753	155	9,3	5,7	0,2	138	15,6	123

TKN: Total Kjeldahl Nitrogen; OM: Organic Matter; CEC: Cationic Exchange Capacity; $P_{avail.}$:Phosphore available; $Ca_{exch.}$, $Mg_{exch.}$, $Al_{exch.}$: Calcium, Magnesium and Aluminium exchangeable.

The exchangeable Al as function of both mineral amendments is shown in Fig.1. KCl-exchangeable Al content decreased significantly for P amendments. All the concentrations of P supplemented in soil contributed to decrease exchangeable Al^{3+} concentration. The concentration of KCl-extractable Al decreased of 115 mg/kg for control before to stabilize around of 9 mg/kg from amendment of 100 mg/kg (Fig. 1). Besides, the levels of Al in soil solution were remained high (between 128 and 69 mg/kg) for all treatments with potassium nitrate. No significant difference between Al levels of these treatments was not appeared (Fig. 1).

The acidification causes the dissolution of minerals and the release of Al^{3+} ions which attach themselves to vacant sites of the complex. It is recognized that increasing of soil solution P can reduce soil solution Al. Application of P fertilizers in soils can be an actual possibility of depressing soil extractable Al to non-toxic levels. Of course, the relevant literature show that P fertilization increases soil pH and means to formation of insoluble Al and P co-precipitates such as $Al(OH)_2H_2PO_4$[14]- [23].

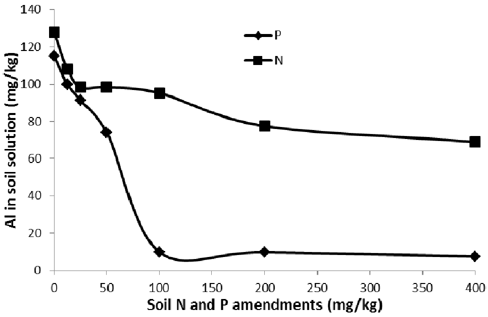
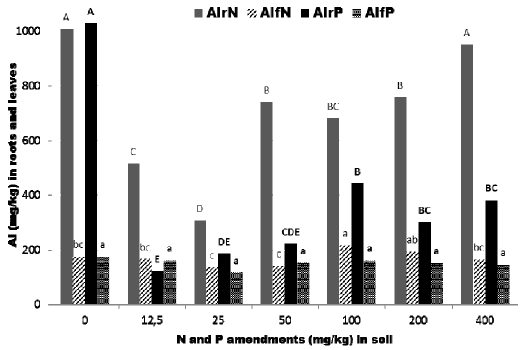


Fig. 1. Effect of N and P fertilizer content on Al content in soil solution.

It is known that N fertilization sustains or increases exchangeable Al and Al saturation but decreases exchangeable base cations Ca^{2+} and Mg^{2+}[24]. The nitrate amendments as N source increase the pH in the rhizosphere due to nitrate nutrition. In most soils, nitrate as an anion is not adsorbed to the soil particles, thus it is mobile in the soil. The increase of the pH in the rhizosphere often alleviates Al ions toxicity and allows root elongation[25]. Even if it was no significant, the decreasing of Al in soil solution (Fig. 1) could effectively indicate that pH increased.

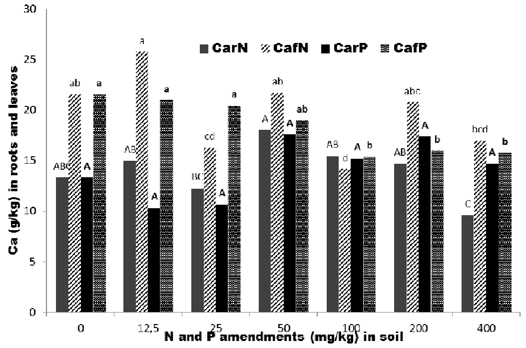
Al levels ranged from 103 to 1129 mg/kg and from 76 to 295 mg kg^{-1}in roots and leaves, respectively (Fig. 2). Contents of Al in plants varied weakly in leaves and widely in roots. Al ions translocate very weakly to the upper parts of plants. Most plants contain no more than 200 mg/kg of Al in dry weight. The concentrations of Al were generally near this value. Ondo *et al.*[4] indicated that similar concentrations of Al in leaves of vegetables could entail to a high target hazard quotient above 0.14 mg/kgday, the provisional tolerable daily intake value for Al. Although N and P nutrition plays a major role in tolerance of Al, the mechanisms are still poorly unspoken. Likewise, interactions between Al and macronutrients in plant are few documented.



AlrN: Al uptake in roots under N amendment; **AlfN**: Al uptake in leaves under N amendment; **AlrP**: Al uptake in roots under P amendment; **AlfP**: Al uptake in leaves under P amendment. The columns of the same type accompanied by different letters are significantly different ($p < 0.05$).

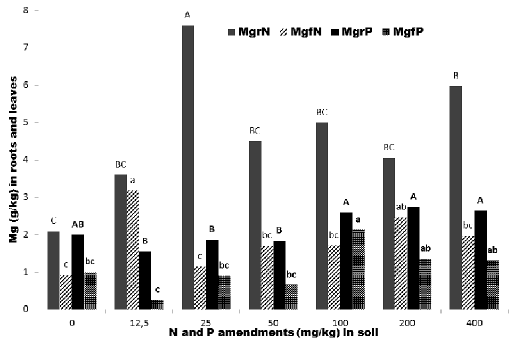
Fig. 2. Al uptake in roots and leaves as a function of N and P fertilizer content.

Ca, Mg and P levels ranged from 9.6 g/kg to 25.8 g/kg, from 0.26 g/kg to 7.6 g/kg, and from 2.0 g/kg to 12.4 g/kg, respectively (Fig.3, 4 and 5). Ca, Mg and P levels differed among treatments and were sufficient for plants grown at all N and P supplies (P sufficiency range : 2 to 5 g/kg; Mg sufficiency range : 1.5 to 4.0 g/kg; Ca sufficiency range : 5 to 15 g kg^{-1}) except Mg levels in leaves of *Hibiscus sabdariffa* growing under 0 to 50 mg/kg P supplies. N and P supplies significantly affected Al, Ca, Mg and P accumulation in roots and leaves of *Hibiscus sabdariffa*. Levels of Mg and P in leaves, and P in roots increased from different levels of N in soil. Levels of Al in roots and leaves, and Ca in leaves decreased and then increased when N increased in soil. Levels of Al and Mg in roots, and Ca and Mg in leaves decreased and then increased when P increased in soil. Ca concentration in roots decreased significantly when P increased in soil. P concentration in roots and leaves increased significantly from high P levels in soil. It was expected to have an accumulation of P proportional to the amendment of this element in the soil. However, the increase of P in the leaves and roots of *Hibiscus sabdariffa* was significant from 100 or 200 mg/kg of P amendment. These results are in accordance with others works showing that from 90-120 mg/kg of P fertilization, P uptake is important in plants[26]- [27].



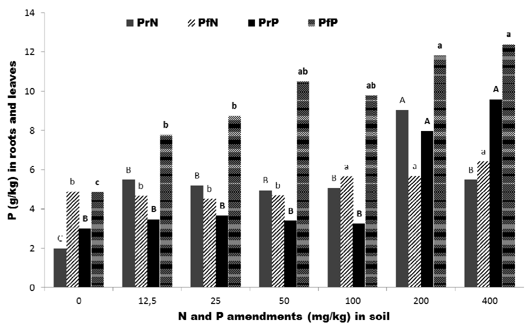
CarN: Ca uptake in roots under N amendment; **CafN**: Ca uptake in leaves under N amendment; **CarP**: Ca uptake in roots under P amendment; **CafP**: Ca uptake in leaves under P amendment. The columns of the same type accompanied by different letters are significantly different ($p < 0.05$).

Fig. 3. Ca uptake in roots and leaves as a function of N and P fertilizer content.



MgrN: Mg uptake in roots under N amendment; **MgfN**: Mg uptake in leaves under N amendment; **MgrP**: Mg uptake in roots under P amendment; **MgfP**: Mg uptake in leaves under P amendment. The columns of the same type accompanied by different letters are significantly different ($p < 0.05$).

Fig. 4.Mg uptake in roots and leaves as a function of N and P fertilizer content.



PrN: P uptake in roots under N amendment; **PfN**: P uptake in leaves under N amendment; **PrP**: P uptake in roots under P amendment; **PfP**: P uptake in leaves under P amendment. The columns of the same type accompanied by different letters are significantly different ($p < 0.05$).

Fig. 5. P uptake in roots and leaves as a function of N and P fertilizer content.

Irrespective of N and P fertilization, Al exchangeable concentration in soil had effects on Al and nutrients in plant. This concentration was highest in control. Thus, Al concentration in roots decreased and increased with Al decreasing in soil solution. Aluminum content was higher in roots than in leaves (Fig. 2). In fact, Al accumulation occurs preferentially in the roots of plants. The retaining of Al in the roots is an important factor for limiting Al tolerance in plants, by preventing the damaging effects of this toxic metal in shoot tissues[28]. Mg and P concentration in roots and leaves increased when Al decreased in soil solution. In this study Al in soil solution had no clear impact on Ca uptake. High Al concentrations in soil solution often influenced the uptake of cations such as Mg and P. Al interaction with P uptake might result in increasing of P uptake in plants grown on acid soils amended with P or N fertilizers[29]. As a result, the Al decreasing in solution increased the concentration of P in the leaves (particularly with P fertilization). Soil acidity limits nutrient use efficiency and crop production through reducing root growth. It significantly restricts the ability of the plant to develop its roots in soil to search for nutrients and water. This also leads to restricted uptake of P, Ca, and Mg by plant roots because antagonistic effects of Al in soil solution and nutrients in plant roots[30]- [31]. The lowest P content measured in the leaves of *Hibiscus sabdariffa* (Fig. 5) was due to the fact that the precipitates of Al with P in root apo plast when Al in soil solution when high, were solubilized and allowed P translocation to leaves[28]. Studies suggest that the formation of precipitates occurs in the wall of cell and outside the plasma membrane of cell of the root, or in the vacuole of the root cells.

A multiple regression analysis (N or P supply and exchangeable Al as qualitative variables) revealed that exchangeable Al and P supply could be explained largely Al, Mg and P uptake in roots and P in the leaves ($R^2 > 0.346$, $p < 0.022$; $R^2 > 0.553$, $p < 0.001$; $R^2 > 0.876$, $p < 0.0001$; $R^2 > 0.870$, $p < 0.001$, respectively), and exchangeable Al and N supply could be explained largely P uptake in roots and leaves ($R^2 > 0.299$, $p < 0.041$; $R^2 > 0.460$, $p < 0.004$).

IV. Conclusion

The results conclude that application of P fertilizer decreased significantly Al levels in soil solution, but not application of N fertilizer. P and N fertilizations had significant effects on Al and nutrient uptake in roots and leaves of *Hibiscus sabdariffa*. This uptake was also explained simultaneously by exchangeable Al and N or P supply, particularly for Al in roots and P in leaves. It could be difficult to ask the African market gardeners to remove mineral nitrogen fertilizers. Also, studies that aim to combine these with other fertilizers able to reduce the Al toxicity in soil solution and ameliorate cultivation yield are necessary. The soil infertility must to be combatted as well by use of organic fertilizers as composts who are few known of these farmers.

V. Acknowledgments

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VI. References

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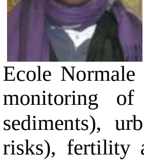

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