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Aluminum Bioavailable and Nutrient and Aluminum Uptake in *Hibiscus sabdariffa* under Effect of Gypsum Fertilization

Jean Aubin Ondo[1*], Alain Bissielou Koumba[1], Bertrand Ngou Akue[1], Estimé Rolant Ngodoua[2] and Richard Menye Biyogo[1]

[1]Laboratoire Pluridisciplinaire des Sciences, Ecole Normale Supérieure, B.P. 17009 Libreville, Gabon.
[2]Laboratoire de Substances Naturelles, de Synthèse Organométallique et de Chimie des Matériaux Inorganiques, Université des Sciences et Techniques de Masuku, B.P. 901 Franceville, Gabon.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The rural exodus and immigration contribute to the development of urban agriculture in developing countries. The unsuitable practices of this intensive farming contribute reduce soil fertility. The result is the decline in crop yields and depletion of the resource base. The aim of this study was to assess the gypsum application in acid soil on available aluminum and the combined or not effect of gypsum application and aluminum in soil solution on aluminum, calcium, magnesium and phosphorus in roots and leaves of *Hibiscus sabdariffa*. A pot experiment was led by calcium as gypsum amendments in different levels. Al concentration in soil solution rapidly decreased significantly below the toxicity threshold value. The gypsum application was a good amendment for Al level decreasing, particularly in roots, and an increase of Ca, Mg and P levels in *Hibiscus sabdariffa*, exception of Mg in roots and P in leaves. The effect of both gypsum application and Al in soil solution showed a significant decrease of Mg in the roots of *Hibiscus sabdariffa*.

**Corresponding author: E-mail: laplus_ens@yahoo.fr;*

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1. INTRODUCTION

Ferralitic soils are the most abundant in the world. They derive from many parent materials and cover large areas in Gabon. Thus, Libreville soils are mostly ferralitic soils with "islands" of raw mineral and podzols soils. They are characterized by some major properties such as an organic matter with high levels but few developed mostly giving fulvic compounds specifically acid [1]. The soils speedily lose nutritive elements and organic matter under harsh environmental conditions or an intensive agriculture. Great precipitations in equatorial areas usually contribute towards important biomass production, but cause soil erosion and soil acidification [2,3]. The main problems in these soils are acidity and the toxic and available forms of aluminum (Al) in the soil solution, a metal which is very detrimental for plants. As result water and nutrient uptake are reduced. Some limiting factors occur because of soil acidification: deficiencies of some nutrients such as calcium (Ca), magnesium (Mg), phosphorus (P), nitrogen (N), and high levels of aluminum in soil solution. Nutrient deficiency and low pH commonly reduce plant yield on acidic soils, especially that of crop species [2-4].

Al ubiquity is correlated to its abundance as the third most abundant element in the Earth's crust [5]. Al appears in soils in mineral forms such as aluminosilicates, hydrous oxides, phosphates and sulphates, and in ions and complexes in soil solution. In acidic soils, the cation exchange capacity is largely made of Al ions. It is currently accepted that Al toxicity decreases in the presence of inorganic and organic complexing ions in soil solution [6]. Now, there is no evidence that Al is essential for plant growth, although it is beneficial for some plant species. However, in acid soils, Al appears as potentially toxic ionic forms into the soil solution and inhibits root growth. Therefore, Al toxicity is an important factor controlling crop production and sol-plant nutrient transfer in acid soils [7].

Another method often used to decrease Al toxicity in acid soils is the employment of Ca amendments such as lime or gypsum or phosphogypsum. The beneficial effects of Ca in ameliorating Al toxicity in crops growing in acid soils are often reported [4,8]. Thus, acid soils amendment with gypsum is able to reduce Al content in soil solution by the formation of Al sulfate complexes which are less toxic to plants. This practice also helps to keep the soil pH and make available Ca for plants [9].

Hibiscus sabdariffa (Roselle) is a multi-product and multipurpose annual plant belonging to the family Malvaceae cultivated in the tropical and sub-tropical regions for its shoot parts. The dry calyces give local and commercial beverages while the leaves are eaten as green vegetables and cooked in the same manner as spinach [10]. Medical treatments based *Hibiscus sabdariffa* are many. In Africa, calice or leave infusions are traditionally administered for their effects on alleviates diarrhea, diuresis, cholera, hypotension, heart and nervous diseases, improved blood viscosity. A calices infusion is also used to help lower body temperature to treat intoxication, sore throat, cough and genital problems. In Nigeria, the decoction of the seeds is used to improve lactation in cases of low milk production [11,12]. It is an important source of natural coloring and flavoring agent in food and fruit processing industries. The seeds are a valuable source of edible oil, and protein [10,13]. The leaves of *Hibiscus sabdariffa* were found to contain useful amounts of micronutrients such as iron, manganese, copper and zinc, but also toxic Al levels when it is cultured in acidic soils [14].

The possibility to employ amendments such as gypsum ($CaSO_4$) which reduce Al phytotoxicity is an important agricultural practice for crops which tolerate acidic soils for their development and growth, but are sensitive to toxicity of Al as in the case of *Hibiscus sabdariffa*. However, little is known about the impact of gypsum fertilization and Al in soil solution on the chemical elements in this plant species. Therefore, the aim of this work was to assess the effects of $CaSO_4$ fertilizer on Al in soil solution and nutrient and Al uptake in root and leaves of *Hibiscus sabdariffa*.

2. MATERIALS AND METHODS

2.1 Study Area and Soil Properties

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℃. 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.

2.2 Pot Experiment on *Hibiscus sabdariffa*

The soil used was a sandy loam from the A horizon (0-15 cm depth) from soil near an urban garden area of Libreville city. Its pH (water, 1: 2.5 soil: water ratio) was 5.4, and it had a carbon content of 39.6 mg/kg and cation exchange capacity of 13.6 meq/100 g.

The experiment was carried out with 21 pots containing each 24 kg dry soil. Seven nutrient additions were made by appropriate amendment amounts of Ca as calcium sulfate or gypsum as solid powdered: 0 mg/kg; 1.5 mg/kg; 3 mg/kg; 6 mg/kg; 12.5 mg/kg; 25 mg/kg; 50 mg/kg.

A basal application of 24 g of NPK 15-15-15 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 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 deionized 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℃ in a stove until constant weight. In order to determine the mobile or “potentially available” fraction of Al in the studied soils, 1 M KCl solution were mixed with 3 g of soil sample into 30 mL plastic centrifuge tubes. The tubes were shaken for 24 h. 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℃ 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℃ in a microwave mineralizer, using a mixture of nitric acid, hydrogen peroxide and ultra-pure water with a volume ratio of 2:1:1 [15]. The resulting solution was then filtered at 0.45 μm and stored at 4℃ before the quantifying of element concentrations.

2.3 Analysis of Element Concentrations in Roots and Leaves of *Hibiscus sabdariffa*

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 spectrophotometer as Molins-Legua et al. [16] and Okalebo et al. [17] methods, respectively.

2.4 Quality Control and Statistical Analysis

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. The reagents were of analytical grade. Table 1 presents data on standard plant reference materials (DC 73349) from China National Analysis Center for Iron and 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 figures 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 Ca amendment and Al in soil solution. Statistical analyses were performed with the software XLSTAT, Version 2010 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

3.1 Availability of Al as Function of Gypsum Amendment

The available Al as function of different gypsum amendments is shown in Fig. 1. Soil Available Al content decreased significantly for gypsum amendments.

The available Al was higher in control soil. All the concentrations of Ca supply in soil contributed to decrease available Al^{3+} concentration. The concentration of KCl-extractable Al decreased of 135 mg/kg for control until of 17 mg/kg from amendment of 6 mg/kg (Fig. 1). The level of available-Al rapidly diminished with the calcium sulfate amendments in comparison to the control (Fig. 1). Available Al in soil was replaced by available Ca from dissolution of gypsum. pH decreased as a result of gypsum application, but this result was not significant (data not presented). The use of gypsum as fertilizer to reduce soil acidity effects has been applied by several authors [18-20]. The base supply particularly of Ca in soil solution decreases Al which is able to transfer in plants by roots. Tropical soils which are strongly weathered such as many soils in Central Africa have very small concentrations of Ca and crops developed on these soils reveal Ca deficiency because Ca concentration is below 25 mg/kg [19]. Yields are significantly improved for many crops when Ca is added in soils. This consequence is frequently due to augmentation of available Ca and decreasing of available Al in the soil. It is probable that available Al decreasing could not be precipitated as $Al(OH)_3$, but as insoluble Al-hydroxysulfate minerals as suggested by Shamshuddin et al. [21]. However, very little is known about the effect of $CaSO_4$ on other forms of Al also present in the soil [20].

3.2 Effects of Gypsum Amendment on Al and Nutrient Uptake in Roots and Leaves of *Hibiscus sabdariffa*

In roots, the level ranges of Al, Ca, Mg and P were 223-1071 mg/kg, 11752-15384 mg/kg, 1754-4356 mg/kg and 2963-6820 mg/kg, respectively. In leaves, these level ranges were 127-212 mg/kg, 17025-21898 mg/kg, 1759-4126 mg/kg and 4009-6357 mg/kg, respectively (Fig. 2). Al contents varied weakly in leaves and strongly in roots. Most plants did not contain more than 200 mg/kg of Al in leaves. The concentrations of Al were generally near this value. Ondo et al. [15] indicated that analogous levels of Al in leaves of vegetables could involve to a high target hazard quotient above 0.14 mg/kg/day, the temporary tolerable daily intake value for Al.

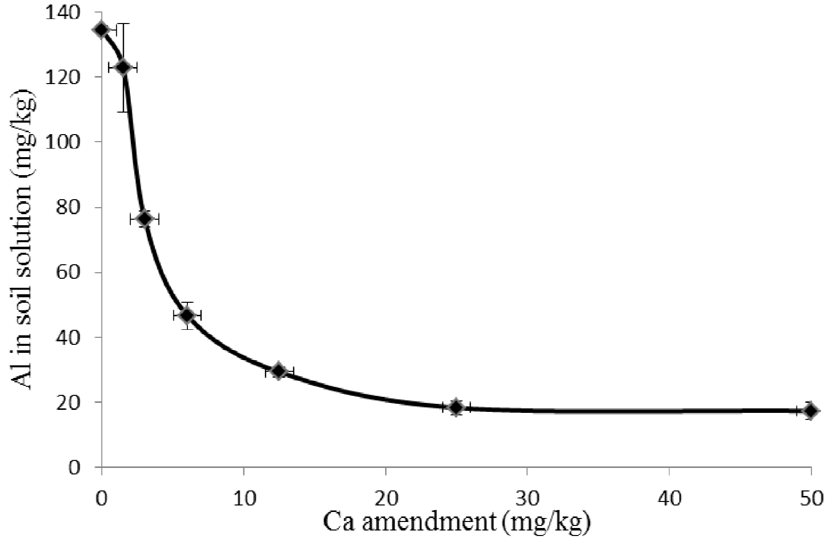

Fig. 1. Impact of Ca fertilization on Al bioavailable in soil solution

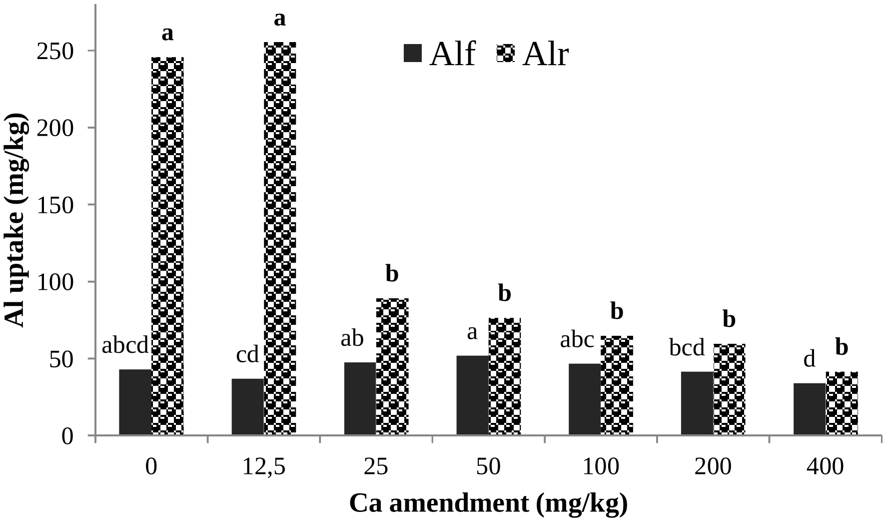

Fig. 2. Al uptake in roots and leaves as a function of Ca as gypsum amendments

Alr: Al level in roots; Alf: Al level in leaves

Table 1. Matrix of correlation coefficients between amended Ca, Al in soil solution and selected elements contained in leaves and roots of *Hibiscus sabdariffa*

	Ca	Alss	All	Alr	Cal	Mgl	Pl	Car	Mgr	Pr
Alss	**-0,700**	**1**								
All	**-0,518**	-0,108	**1**							
Alr	**-0,618**	**0,960**	-0,173	**1**						
Cal	**0,913**	**-0,825**	-0,390	**-0,731**	**1**					
Mgl	0,181	**-0,479**	0,415	**-0,565**	0,104	**1**				
Pl	0,233	-0,163	-0,007	-0,193	0,096	0,371	**1**			
Car	**0,703**	**-0,823**	-0,204	**-0,759**	**0,872**	0,188	0,054	**1**		
Mgr	-0,113	0,428	**-0,445**	**0,489**	-0,135	**-0,456**	-0,169	-0,091	**1**	
Pr	-0,197	0,028	0,257	-0,034	-0,146	0,417	0,280	0,025	-0,150	**1**

Alss: Al in soil solution; El: Element (Al, Ca, Mg or P) in leaves; Er: Element (Al, Ca, Mg or P) in roots; P= 0.05 is in bold

Agricultural practices about use of calcareous amendment to ameliorate the toxicity effect of Al on plants growing in acid soils have been reported [4,22]. However, gypsum addition on *Hibiscus sabdariffa* growing in acid ferralitic soils has been little studied. The results of this study showed that gypsum amendment was able to reduce the Al concentration in both roots and leaves of *Hibiscus sabdariffa* (Fig. 1). Similar results were found in cultivars of highbush blueberry (*Vaccinium corymbosum* L.) by Reyes-Diaz et al. [4]. These authors explained that Ca supply to soil solution reduced the accumulation of Al in tissues. Complexes of soluble aluminum sulfate which are harmless to plants train in soil solution and reduce plant Al uptake.

Ca, Mg and P levels ranged from 1.92 g/kg to 4.59 g/kg, from 1.89 g/kg to 4.21 g/kg, and from 2.89 g/kg to 7.00 g/kg, respectively (Figs. 3, 4 and 5). Ca, Mg and P levels differed among treatments. Ca levels were below the range 5-15 g/kg proposed by Burnett et al. [23] for cultivated plants. Mg and P were sufficient for plants cultivated (P sufficiency range: 2 to 5 g/kg; Mg sufficiency range: 1.5 to 4.0 g/kg) [23]. This study showed that Ca and Mg in leaves increased significantly with gypsum supply. Gypsum supplies significantly affected Ca, Mg and P accumulation in roots and leaves of *Hibiscus sabdariffa*. Moreover, Ca in roots increased slightly significantly from gypsum supply of 100 mg of Ca / kg of soil. Lopez-Lefebre et al. [24] indicated that Ca does not generally accumulate in roots but is translocated into aerial parts of plants.

Levels of Mg in leaves and P in roots increased and then decreased from different levels of Ca in soil.

Table 1 shows the matrix of correlation coefficients describing the relationships between Ca amendment, Al in soil solution and nutrient uptake in roots and leaves of *Hibiscus sabdariffa*. Table 1 showed that Ca amendment had negative significant relationships with Al in soil solution, roots and leaves, and positive significant relationships with Ca in roots and leaves. In other hand, Al in soil solution had positive significant relationship with Al in roots and negative significant relationships with Ca and Mg in leaves and Ca in roots.

Gypsum application is an important amendment for the Al toxic reduction and Ca increasing in soil, without or only slightly altering pH conditions. Thus, Al^{3+}available is reduced into aluminum sulfate ($AlSO_4^+$) considerably less toxic for plants [9]. According with some authors [22,25], the gypsum application resulted in an improved root growth and elevated nutrients uptake in roots and leaves such as S, P, K, Mn and Ca.

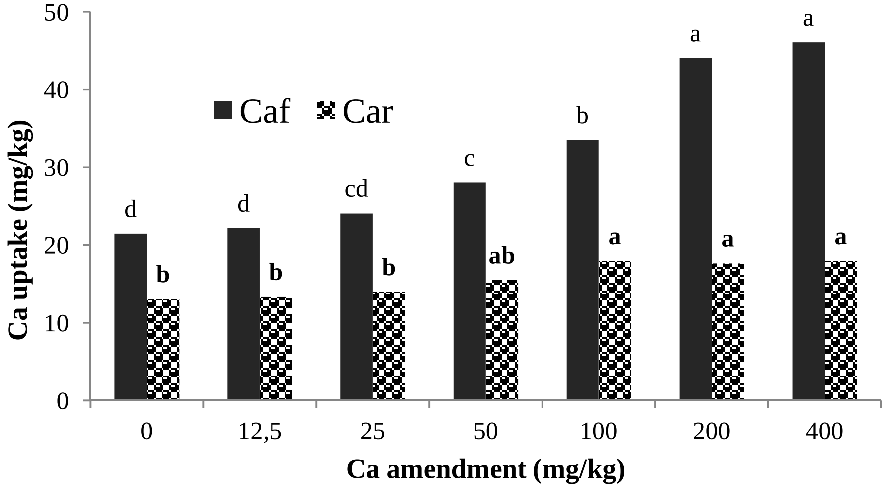

Fig. 3. Ca uptake in roots and leaves as a function of Ca as gypsum amendments
Car: Al level in roots; Caf: Al level in leaves

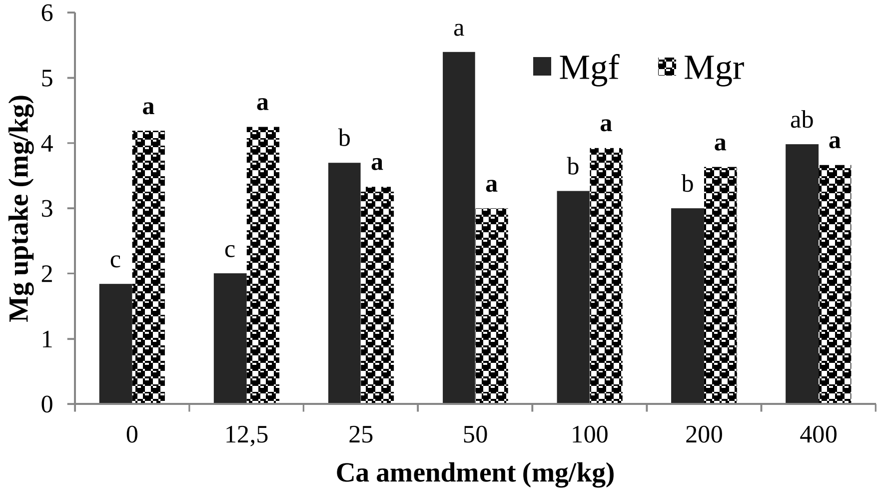

Fig. 4. Mg uptake in roots and leaves as a function of Ca as gypsum amendments
Mgr: Al level in roots; Mgf: Al level in leaves

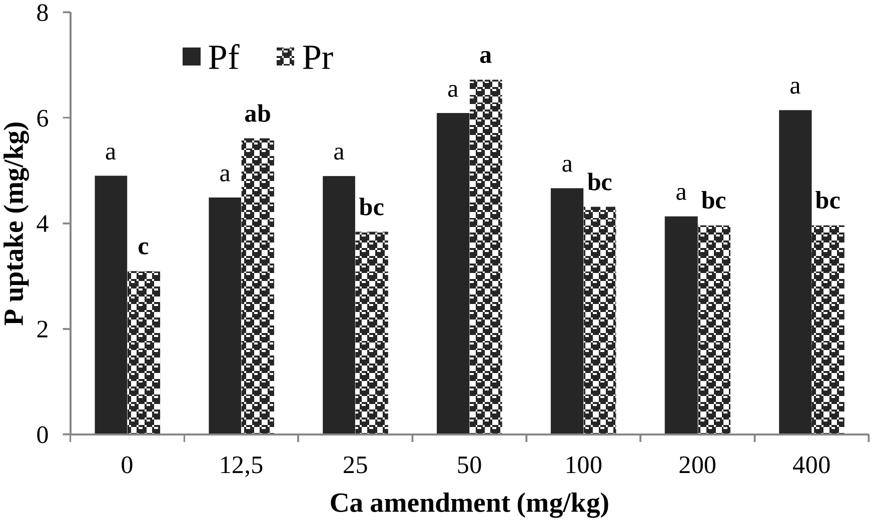

Fig. 5. P uptake in roots and leaves as a function of Ca as gypsum amendments
Pr: Al level in roots; Pf : Al level in leaves

Ca and Mg adsorption increased and Al adsorption decreased with decreased Al concentration in soil solution. This result is according with others studies on other crops [26,27]. Free ion Al^{3+} affects cell membrane structure and permeability by obstructive the Ca^{2+} channels. It may obstruct the arrival of divalent cations as Ca^{2+} and Mg^{2+} into cells, but it excites the anion cell arrivals. Binding of Al^{3+} to cell membrane phospholipids and transport proteins, reduces the net negative membrane surface charge, permitting the movement of anions and restricting that of cations. Thus, the alleviation of Al^{3+} level through Ca^{2+} addition causes a reduction in the negative potential of the plasma membrane, leading to a drop in the electrostatic attraction of the toxic Al^{3+} cation [9]. The decreased Al level in soil solution had a much stronger implusion effect on uptake and translocation of Ca than it had on Mg. This result is not according with van Praag et al. [28] which observed that Al in soil solution had a much stronger effect on Mg than on Ca in Norway spruce.

A multiple regression analysis (Ca supply and available Al as qualitative variables) revealed that available Al and Ca amendment could be explained significantly Al, Ca and Mg uptake in the leaves, and Al, Ca, Mg and P in the roots ($R^2 > 0.804$, $P < 0.001$; $R^2 > 0.973$, $P < 0.001$; $R^2 > 0.880$, $P < 0.001$;$R^2 > 0.972$, $P < 0.001$; $R^2 > 0.827$, $P < 0.001$; $R^2 > 0.774$, $P < 0.001$; $R^2 > 0.819$, $P < 0.001$, respectively).This result is particularly interesting for Mg uptake in roots. Indeed, the ANOVA showed that gypsum amendment only did not change significantly Mg level in roots. Murata [29] also observed that at physiological maturity of groundnut, gypsum had no effect on the Mg status in roots. But the combined effect of gypsum application and Al in soil solution was significant ($R^2 > 0.774$, $P < 0.001$) and showed a decreased of Mg in roots.

4. CONCLUSION

A pot experiment was led by calcium as gypsum amendments in different levels. Al concentration in soil solution rapidly decreased significantly below the toxicity threshold value. The gypsum application was a good amendment for Al level decreasing, particularly in roots, and an increase of Ca, Mg and P levels in *Hibiscus sabdariffa*, exception of Mg in roots and P in leaves. The effect of both gypsum application and Al in soil solution showed a significant decrease of Mg in the roots of *Hibiscus sabdariffa*. However, gypsum amendment with other organic or inorganic fertilizer could be studied to alleviate toxic Al in soil solution in the long-term and provide a good source of nutrients such as Ca, S, P and Mg.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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