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Macronutrient Concentration in Stem, Leaf and Petiole of Wild Grown Water Spinach (*Ipomea Aquatic* Forsk.) and Its Relationship With Pond Water

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Abstract. Water spinach (*Ipomoea aquatic* Forsk.) is a food for human beings and animals. It is rich in minerals, protein, dietary fibre, with high moisture content. The work was undertaken to determine contents of K, Ca, Mg, Na & P in the stems, leaves and petioles of water spinach. Atomic absorption spectrometry (AAS) and Inductive Couple Plasma (ICP) were used to determine concentration of nutrients, where one way ANOVA was applied to analyse if there is any significant differences in the macronutrient contents amongst the leaves, petioles and stems of the water spinach. If any of the results showed significant differences, Turkey post-hoc HSD test ($p < 0.05\%$) was adopted to separate the means. In addition, Pearson's Correlation Coefficient Test was conducted between the plant macronutrients samples (leaves + stem + petioles combined) and water macronutrients data run to determine their relationships. In addition, purpose of this study is to highlight to the public which parts of the plant should be consumed and also to indicate the relationship of Water Spinach with its growing medium. The K concentration was higher than the other elements and maximum concentration was in petioles ($432+27.45$ mg·L-1) and stems ($424.60+14.19$ mgL-1). The element with the least concentration was Na ($3.10+0.40$ mgL-1), in the petiole. There was no difference in Mg content in leaves, petioles and stems (avg. $28.55+1.61$ mgL-1). High amounts of Ca ($150+0.10$ mgL-1) and low amounts of P ($41.11+0.01$ mgL-1) were in pond water. A positive correlation of each nutrient occurred between water spinach and pond water.

1 Introduction

Ipomea aquatic Forsk is a semi-aquatic tropical plant with the stems trailing on mud or floating on water and strives well in moist soil, still water to flowing water and muddy stream banks, freshwater pond, lakes and marches [1, 2]. It is also considered as a laxative [3]. This species is widely found in the waterways of the East and Southeast Asia, requires little human intervention. It is the primary source of nutrients due to its ability to convert nitrogen into edible protein [4, 5]. Water

on earth comprises mostly in saline nature with very small composition in fresh conditions [6]. The over exploitation of natural resources from the earth and pollution from industrial sewage which continuously adds to the water bodies resulted in scarcity of fresh water [7, 8], making it unfit to livestock and other consumers [9, 10].

Water spinach is one of the many plants sensitive to toxic pollutants, and continuous intakewill cause health concerns in humans [11, 12].. Furthermore, the essential nutrients Ca, P, Mg, Na, K and S serve in physiological functions in human [13]. Ipomea aquatic Fork is a favorite crop of the East due to its abundance, low cost (need to find another source of reference here, this one cannot be used) and a source of proteins and carbohydrates [14, 15]. It is necessary to monitor quality of water where this crop strives since it has direct relationship in terms of nutrient uptake, where this finding may offer some pertinent results in nutrient plant water relationship, and elsewhere.

The objectives of the study were to determine and compare nutrients contents in edible stems, leaves and petioles of water spinach and to correlate those parameters with nutrient content of the water source where this plant strived in.

2   Materials and Methods

2.1 Materials
A completely random selection procedure was used to acquire plant and water samples in Lubok Bongor, Kelantan, Malaysia. Composite samples from ten (10) water spinach plants were obtained and tissues separated into petioles, leaves and stems and each used as a replication for macronutrients determination. In addition, ten water samples were obtained at random. Distilled water, deionized water of ASTM grade 1 were derived from in-house laboratory water filtration and processing system. The solvents 37% HCl 1 mol·L-1 (1N) Titripur® from Merck (Darmstadt, Germany) were used. Claritas multi element solution standard, 10 mg·L-1, for Na, Mg, K, Ca and P was used.

2.2 Macrominerals Determination
All tissue samples were washed with distilled water to remove contaminants. Each tissue type was ground to fine particles using a blender and passed through 2 mm mesh size. Each composition of petiole, leaf and stem of water spinach were stored in polypropylene bags at room temperature before analysis. Concentrations of Ca, K, Mg and Na in water spinach samples were analysed using Atomic absorption spectrometry (AAS). For P, Inductive Couple Plasma (ICP-MS) of the Forest Research Institute of Malaysia (FRIM) was used. Prior to analysis, pre-treatment of samples occurred where any organic matter present was removed using dry ashing. Samples were dried at 37°C overnight, then placed in a furnace at 500°C for 4 h. Dried samples (1 g) of each tissue was weighed and placed in a porcelain crucible. Ten repetitions for each tissue were used. Samples were placed in a cool muffle furnace and the temperature raised to 500°C for 4 h. After that samples were cooled and ash dissolved in 5 mL of 20% HCl. If necessary, the solution was warmed to facilitate dissolving the residue. The solution was passed through gravity acid washed filter paper into a 50 mL volumetric flask and brought to 50 mL with deionized water. The solutions were mixed and measured as described previously. Blank samples were prepared in the same manner without tissue. The AAS and ICP-MS machines were calibrated, with concentration of stock solution for each mineral as follows: K,500 ppm 500 mgL-1; Na, 50 mgL-1; Ca,,10 mgL-1 P, 50 mgL-1.

2.3 Data Analysis
Data were subjected to analyzed using SPSS (Ver. 16.0), IBM Corp., Armonk, New York. A one way ANOVA was applied to analyze if there is any significant differences in the macronutrient contents amongst the leaves, petioles and stems of water spinach. If any of the results showed significant differences, Tukey post-hoc HSD test ($P<0.05\%$) was adopted to separate the means. In addition, Pearson's correlation coefficient test was conducted between the plant macronutrients samples (Leaves+Petioles+Stems combined) and water macro nutrients data run to determine their relationships.

3   Result and Discussion
Table 1 shows the concentration of macronutrients in the three parts of water spinach which were the petioles, leaves and stems. From Table 1, it can be seen that K is exceptionally high in the petiole (432.92±27.45 mgL-1) & stem (424.60±14.19+ mgL-1) and even relatively high in the leaves

(136.79 ± 21.75 mgL-1), concurring with the findings by Ref. [16] on nine (9) species of vegetables studied in Bangladesh. In addition, Nabila & Amin [17] reported K concentration in the leaves of Ipomea aquatic to be 2.64%, which is exceptionally very high in concentrations based on their study from Bangladesh. This is the most mobile & easily leached nutrients [18] and it is no surprised to find high concentrations of this nutrient in all the plants parts studied. The figure of K in water spinach leaf (136.79 ± 21.75 mg L-1) was lower than the figure found in sweet potato leaf (750 mgL-1) as reported by Ref. [19-21].

Previous study by Umar et al., [22] described that the mineral element contents in the leaves of water spinach were high with remarkable concentrations of K (546 mg/L-1) and Fe (21mgL-1). Ref. [23] noted high amounts of Fe in the leaves (9 mgL-1), stem (3mgL-1) and petiole (3mgL-1) of water spinach. The concentration of K was relatively high, occupying third position in its availability in the pond water of Lubuk Bungor (94 mgL-1), as seen in Figures 1 & 2.

Table 1. Macronutrients concentration of petiole, leaf and stem of *Ipomea aquatic* Forsk.

Sample Part	Mineral Content (mgL^{-1}), P< 0.05				
	Calcium	Potassium	Magnesium	Sodium	Phosphorus
Petiole	43.59 ± 5.74a	432.92 ± 27.45a	28.53 ± 1.08a	3.10 ± 0.40b	51.26 ± 1.45c
Leaf	116.29 ± 8.85b	136.79 ± 21.75b	29.73 ± 1.00a	160.00 ± 10.59c	124.85 ± 6.03b
Stem	170.54 ± 12.38c	424.60 ± 14.19c	27.38 ± 2.74a	154.83 ± 5.31c	169.69 ± 4.17a
Combined	330.42	994.31	85.64	317.93	345.80

Means in the vertical column followed by the same letter are not significantly different at 5% (p<0.05)
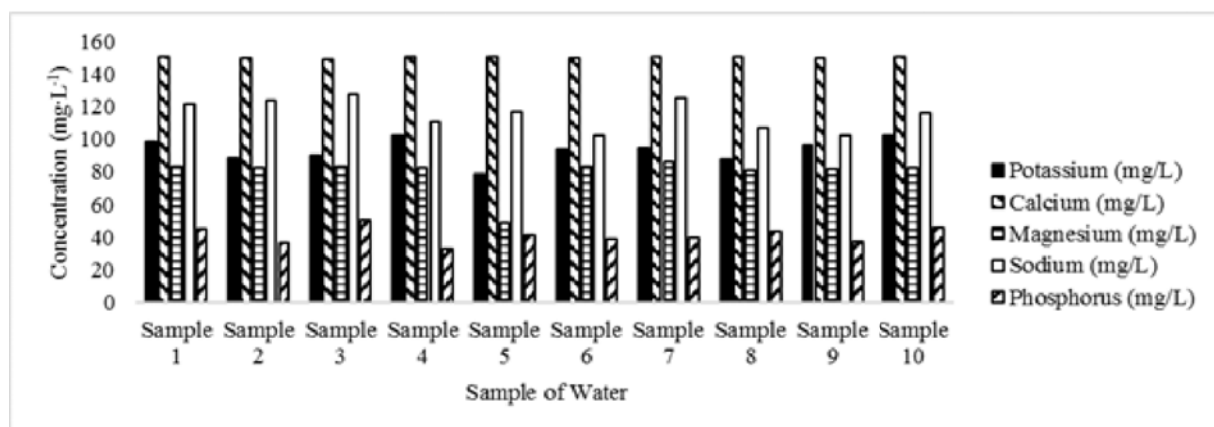

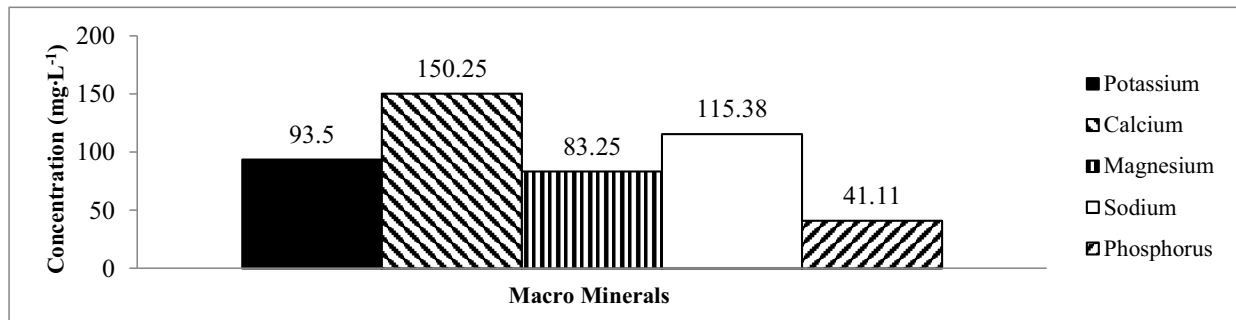**Fig. 1**. Macrominerals concentrations of water samples.

Fig. 2. Average macrominerals concentration of water sample

Relatively, the stem of water spinach was rich in all the macronutrients studied, with the exception of lower Mg content found in all the three parts, with 30 mgL-1 in the leaf, 27 mgL-1 in the stem and 29 mgL-1 in the petiole (with no significant difference amongst them). This was much expected since this element is practically a mobile nutrient [18] and capable of well distribution amongst the leaves, stems and petioles. The three samples (leaves, petioles & stems), which were green in colour should be capable of manufacturing food since they are expected to be rich in chlorophyll, where high Mg is always expected in the leaves [24, 25]. The amounts of Mg in the pond water was of relatively average (83 mgL-1) and rank fourth in its availability in the pond of Lubuk Bungor (Figures 2).

Macronutrient P is relatively highest in stem (170 mgL-1), relatively moderate in the leaves (125 mgL-1) and lowest in the petiole (51 mgL-1), with significant difference noted at $P<0.5$ %. [17], recorded 3665 mgL-1 of P concentrations in the leaves of Ipomea aquatic in their study from Khuna, Bangladesh which is exceptionally high, whilst Umar et al., [22], noted a figure of 10.9 mgL-1 in their study in Nigeria, which is ten folds lower than the current study. This nutrient was also reported to be largely translocated prior to leaf abscission, indicative of its importance [26, 27, 28]. This similar strategy was observed to overcome the deficient supply of P in tropical trees through translocation [29, 30] and in this study, the lowest P concentration (41 mgL-1) in pond water of Lubuk Bungor recorded (Figure 2).

It is interesting to note the lowest amounts of macronutrient found in this study was Na with 3.1 mgL-1, in the petiole (Table 1), but high in the leaf (160 mgL-1) and the stem (144 mgL-1), where both the latter two showed no significant different at $P<0.5$% level. The Na content of Ipomea aquatic leaves reported by Nabila & Amin [17], from Bangladesh is 242 mgL-1 and Umar et al., [22] noted a figure of 13.5 mgL-1 in their study. Na is a mobile nutrient [31], which moves readily from the roots to the stems and the leaves as supported in this study cautions the intake may vary between species. This is true, for water spinach and vegetables crops, where the former distribution order of leaf>stem>petiole and the reverse is true for the latter [16].

From this study it is of worthy to note relatively high amounts of Ca in the stem (170 mgL-1), this is very much expected since this element is least mobile [18, 28, 32, 33] and expected to be deposited mainly in the stems than the leaves, even though Brown et al., [34] noted the Ca mobility may vary between species. This is true for Ca content in vegetables of Bangladesh, where petiole>leaf>stem [16], 2015) and the reverse is true for this study. The relatively high amount of Ca in the leaves (116 mgL-1) compared to the petioles (44 mgL-1), is an indication of the ability of this element to be deposited in the leaves via movement through the xylem petiole or through atmospheric deposits from terrestrial sources or from the high concentrations of Ca (150 mgL-1) in the pond water coming in direct contact with the leaves and stems (Figures 1 & 2). Umar et al., [22] on the other hand, recorded 41.6 mgL-1 of Ca in Ipomea aquatic leaves, which is 3 folds lower that the current study.

The macronutrient concentrations in the pond water of Ipomea aquatica at Lubok Bungor, for Ca, Na, K, Mg and P were 150 mgL-1, 115

mgL-1, 102 mgL-1, 83 mgL-1 and 46 mgL-1, respectively (Figures 2) and even each individual of the ten water samples display the same phenomenal pattern for all the nutrients studied (Figure 2).

One of the objectives of this research was to examine whether there is a significant relationship between combined petioles, leaves and stems against pond water in terms of macronutrients concentration of K, Ca, Mg Na and P. Simple Pearson Correlation Analysis was adopted on water spinach and pond water samples, where significance positive correlations were established (Table 2).

Based on Table 2, strong positive correlation were shown (0.964) between K and Ca, (0.970) between K and Na, (0.988) between K and P. It is clearly shown that the increase in macronutrients in water spinach corresponded with the high availability of these nutrients in the pond (Table 2) and this study is in congruent with the study by Nida et al., [23], but with micronutrients. The strong positive correlation also was noted (0.983) between Ca and Na, (0.982) between Ca and P, and (0.991) between Na and P. It is clearly shown the increasing Ca as well when the increase of K, Na and P (Table 2). The positive correlation was shown (0.970) between Na and K, (0.983) between Na and Ca, and (0.991) between Na and P (Table 2). It is clearly shown that the Na increases as well when K, Ca and P increase (Table 2). Lastly, positive correlation (0.988) between P and K, (0.982) between P and Ca, and (0.991) between P and Na also noted in Table 2. It is clearly shown the increasing of P as well when the K, Ca and Na also increase (Table 2). It can be concluded that all macronutrients in combination of petioles, leaves and stems showed had correlation with the macronutrients in the pond water. Macronutrients of K, Ca, Na and P were observed to have synergistic effect when they uptake each other. Interestingly, K, Ca, Na and P in the combination of petiole, leaf and stem showed no relationship with macronutrient Mg in the pond water (Table 2).

Table 2. Correlation coefficients (Rs) of macro minerals of combined petiole, leave and stem of *Ipomea aquatic* Forsk. of K., Ca, Mg, Na, and P versus pond water of K, Ca, Mg, Na, and P where n= 10 at P≤ 0.01

Mineral	Potassium	Calcium	Magnesium	Sodium	Phosphorus
Potassium	1	0.964**	0.141	0.970**	0.988**
Calcium	0.964**	1	0.276	0.983**	0.982**
Magnesium	0.141	0.276	1	0.168	0.124
Sodium	0.970**	0.983**	0.168	1	0.991**
Phosphorus	0.988**	0.982**	0.124	0.991**	1

**Correlation is significant at the level 0.01 level (2-tailed)

4   Conclusion

Water spinach in this study contains high concentrations of K compared to other elements and high concentration of K was found in the petiole and stem. The least amounts of nutrient was Na, found in the petiole, whilst on the average basis Mg was found to be low in all the parts of water spinach studied. Furthermore, there was positive correlation each elements found between water spinach and water pond, suggesting a synergistic relationship amongst them, except for Mg. On combined basis of stems, petioles and leaves, K>P>Ca>Na>Mg, with 995 mgL-1, 346 mgL-1, 33 mgL-1, 318 mgL-1 and 86 mgL-1, respectively.

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Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.