DETERMINING POTASSIUM (K^+) RELEASE, CROP AVAILABILITY AND UPTAKE FROM THREE RED ALGAL (RHODOPHYTA) SPECIES

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By
Chandrappa Gangaiah
Dissertation Committee:
Theodore J. K. Radovich, co-Chair
Amjad A. Ahmad, co-Chair
Nguyen V. Hue
Robert E. Paull
Celia M. Smith
Koon Hui Wang

Dedicated to my late parents

Mr. Gangaiah and Mrs. Sakkamma Gangaiah,

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ABSTRACT

As a result of increasing fertilizer costs associated with rising oil prices, many growers in the Pacific region have become interested in locally available resources that can be used as low cost inputs to improve crop health and productivity. In recent years, use of algae and their extracts have gained in popularity due to their potential use in sustainable farming and may be used as an alternate to synthetic fertilizers. In Hawaii efforts are underway to control the most commonly found invasive algae species; *Eucheuma denticulatum, Gracilaria salicornia,* and *Kappaphycus alvarezii*. These are dominant invasive, non-indigenous species on Hawaiian reefs. The average potassium (K^+) dry matter content in these species is around 14 -20%. Although these seaweeds were used in the past for crop production, not much is understood about application rates, yield performance, species efficacy for crop nutrition, nutrient release pattern and mechanisms of release. To address these gaps, greenhouse and laboratory experiments were conducted to: 1) determine the efficiency of the cardy meter as a rapid, low-cost tool for evaluating the tissue K status of pak choi from fresh sap, 2) describe effect of three invasive algae species on growth (yield) and K nutrition (tissue K^+) of pak choi at different K fertilizer rates, 3) to evaluate algae biomass as a replacement for K^+ synthetic fertilizers, 4) compare the two buffers to minimize ionic interference using a K^+ selective electrode, and 5) to understand the K^+ nutrient release pattern and mechanics of these three species.

Our data show that the invasive algae species increased the yield and growth of pak choi and the response was greater when algae was applied to provide K^+ at the rate of 224-284 kg.ha^{-1}. Results of the algae comparison with synthetic K^+ showed no significant differences between them for yield; e.g. Plants grown with *K. alvarezii,* KCl and KNO_3 had an average dry weight of 7.5 g when K^+ was provided at 280 kg.ha^{-1}. Only K^+ rates, not K^+ source were significant for yields and tissue K concentrations in all trials. Although the values between rapid electrode and ICP measured tissue K^+

values were well correlated, ICP was identified as the preferred method of K^+ quantification in tissue. However, in soil solution, ion strength adjustment buffers (ISAB) were able to significantly mask the other ionic interferences when measuring K^+ and with a selective electrode. No notable difference was observed among the two buffers, $NaClO_4$ and NaCl (slope near 1) with a strong correlation (r =0.99). This is the first report of such a comparison in the literature. Based on the results from the leachate study, we conclude that the total amount of K^+ released from algae was lower than synthetic K fertilizers applied at equivalent rates of K^+. Results from the both the whole plant and polysaccharides leachate studies show that there is a difference in K release pattern among the algae with the cumulative recovery of K^+ from both whole plant *G. salicornia* and extracted agar were significantly lower (10-12%) than whole plant *K. alvarezii* and extracted carrageenan at both K application rates. These differences in release among algal species may be explained in part by differences in cell wall composition, chemical properties including sulfate groups differences and physical properties such as higher gel strength of *Gracilaria* agar.

To our knowledge, this work is the first detailed report of these red algae species as significant sources of K^+. This is also the first report in the literature of the less expensive NaCl buffer being an effective substitute for $NaClO_4$, allowing for cost effective, high-throughput analysis of soil solution K^+. We propose that carrageenan containing species may be more efficient sources of K^+ than those containing agar, but future studies are needed to elucidate the mechanisms for these differences. Our study may also provide the established protocols for further investigation of the functional properties of naturally extracted agar and carrageenan from these species and at different locations.

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CHAPTER 1

INTRODUCTION

1.1 Background

Hawai‘i imports over 85% of the food consumed, has a population over one million residents, is more than 2400 mi away from the closest mainland US port and as a state, has less than two week supply of food on island (Hawaii Farm Service Agency, USDA, 2014). Any improvement in agricultural system that results in higher yield and lower cost production should reduce the negative environmental impact and enhance the sustainability of the system. One such approach is the use of local fertilizers (organic materials), that can bring down the amount of synthetic fertilizers used (Pramanick et al., 2013). The long-term use of synthetic fertilizers invites the crucial problems of soil health and fertilizer use efficiency. Due to these reasons the farmers are being compelled to turn towards options such as organic fertilizers, use of cover crops and etc... And one of such options is the use of seaweeds and seaweed extracts as plant nutrient fertilizer (Russo et al., 1990; Zodape, 2001; Pramanick et al., 2013).

Greater self-sufficiency and to mitigate the costs of commercial fertilizer tied to oil prices, many local growers in the Pacific region are demanding locally available and inexpensive fertilizers that are suitable for use in agricultural production to improve crop health and productivity (Radovich et al., 2012). In recent years, use of seaweeds and their extracts have gained in popularity due to their potential use in organic and sustainable farming and may be used as alternate source for synthetic fertilizers. The rapid growth of invasive algae species in the state of Hawaii currently impose a significant economic burden for control and eradication and threaten native biodiversity (Conservation of Native Biodiversity, 2010). There are continuous ongoing reef remediation efforts by various groups which collects millions of pounds of the wet weight biomass that must be disposed (Franklin, 2010). Preliminary chemical analyses indicate that these invasive algae are rich source of potassium (K) (14-20%) depending on species and location (Table 1).

1.2 Role of potassium in plant growth

Potassium (K) is an essential element in plant growth (Lebaudy et al., 2007). It has many functions in plant nutrition and growth that influence both yield and quality of the crop. Unlike N, P and most other nutrients, K is not incorporated into structures of organic compounds; instead potassium remains in ionic form (K^+) in solution in the cell and acts as an activator of many cellular enzymes (Havlin et al., 2005). K is used as a major active solute to maintain turgor and to drive irreversible and reversible changes in cell volume. These include regulation of metabolic processes such as photosynthesis; activation of enzymes that metabolize carbohydrates for synthesis of amino acids and proteins; facilitation of cell division and growth by helping to move starches and sugars between plant parts. It is known that fruit quality of some plants can be altered with fertilization and in particular by supplying sufficient potassium (K) to plants (Bar-Yosef, 1996 and Ming et al., 1996). Potassium (K) is an essential plant nutrient involved in numerous physiological processes that control plant growth, yield and quality parameters such as taste, texture, nutritional and health properties (Lester, 2005).

The control of cell expansion plays an essential role in plant growth. Cell growth caused by cell expansion is regulated primarily by turgor pressure, which is the physical force against the cell wall, and is maintained by osmotic regulation via osmotically active substances, such as potassium ions (K^+), sugars, and amino acids (Maggio et al., 2006; Zonia and Munnik, 2007). In tomato, higher available K levels around the root zone increased leaf, flower, and fruit formation

(Besford and Maw, 1975), and fruit number (Davies and Winsor, 1967). In a study the researchers observed that total soluble solids and fruit firmness increased at the highest K rates (Demiral and Köseoglu, A. T. 2005). Increases in firmness and total soluble solids are reported to be closely related to increased plant lignification at higher K applications (Aktas, 1991). Bar-Yosef (1996) indicated that high leaf K concentration increased dry matter production in greenhouse-grown muskmelon. Ming et al., (1996) stated that changes in total soluble solids showed a close relationship with potassium in melon. Besford and Maw (1975) stated that blossom and fruit formation and average fruit number per plant were positively affected by higher K application in tomato. K being such an essential nutrient for crop production, but there are limited or fewer options for sourcing K fertilizers in certified organic production.

1.3 LITERATURE REVIEW

1.3.1 Historical use of seaweed in agriculture

In coastal regions, the practice of collecting the seaweeds and using them in crop production was considered as a traditional soil fertility management strategy, especially where agriculture relies on use of local resources (Cuomo et al., 1995; Stephenson, 1968). This readily-available, inexpensive material to improve soil fertility, the application of seaweed biomass is often an integral component of traditional, small-scale, diversified agriculture (Angus and Dargie, 2002). A rotation-intensive system that integrates the application of locally available seaweed biomass was practiced in the past in the Machair region of the Scottish Outer Hebrides Islands (Angus and Dargie, 2002; Kent et al., 2003). Application of manure and seaweeds, primarily the brown alga (*Laminaria digitata*), which is collected and piled onshore for 1-2 weeks prior to application to improve soil fertility was a part of traditional practice (Angus and Dargie, 2002).The practice of seaweed application as a part of sustaining small-scale, diversified agriculture is supported by

Scottish Natural Heritage, a governmental conservation organization, as well as local conservation group efforts (Angus and Dargie, 2002). In the past, historical accounts of using seaweed in agriculture range from the British Isles, to coastal mainland Europe, to Asia and to the pacific and northeastern region of the United States (Smith et al., 1989; Cuomo et al., 1995).

1.3.2 Seaweed in the modern agricultural context

In recent years, use of seaweeds and their extracts have gained in popularity due to their potential use in organic and sustainable farming to avoid excessive fertilizer applications and to improve the mineral absorption (Russo and Beryln, 1990). In organic crop production systems, both in the U.S. and worldwide, seaweed-based agricultural products (e.g. extracts for foliar application and composts) are commonly employed (Khan et al., 2009). In many countries, seaweed and seaweed based fertilizers are still used in both agriculture and horticulture (Verkleij, 1992; Zodape, 2001). Not only providing nutrients, these seaweeds improve soil structure and humus content when they are composted and applied to the soil (Haslam and Hopkins, 1996). Seaweed concentrate prepared from *Ecklonia maxima* (Osbeck) Papenfuss has improved the growth of tomato seedlings when applied as a soil drench (Crouch et al., 1992). Seaweed manure is rich in potassium but poor in nitrogen and phosphorus (Kingman et al., 1982). It was observed that the application of seaweed liquid fertilizer (SLF) of *Ascophyllum nodosum* increased the chlorophyll of cucumber cotyledons and tomato plants (Whaphamem et al., 1993). In another experiment with the soil application of sea weed liquid fertilizer of *Enteromorpha clathrata* and *Hypnea musciformis* increased the growth characteristics of green gram, black gram and rice. Seaweed concentrate (Kelpak) increased the growth and yield significantly in potassium stressed wheat (Kingman and Moore, 1982). It was also observed that the value of seaweeds as fertilizers was not only due to potassium, nitrogen, and phosphorus content, but also because of the presence of trace elements and metabolites (Booth, 1969). Seaweed liquid fertilizer (SLF) of *Dictyota dichotoma* was found to increase the yield, growth of roots and shoots, number of roots and maturity time of *Abelmoschus esculentus L.* (okra) at low concentrations (Sasikumar et al., 2011).

The use of marine macroalgae as fertilizer in crop production is a long-standing practice in many temperate coastal areas around the world (Stephenson, 1968). Synthetic fertilizer as a sole source of fertility is often questioned as a sustainable management strategy, and diversification of inputs is encouraged, particularly inputs that provide not only primary nutrients (i.e. N, P and K), but also organic matter and trace elements (Lal, 2004). Seaweed, which contains primary nutrients, organic C, and other nutrient elements, is thus a good candidate organic amendment material as part of a diversified soil fertility management strategy. Worldwide, many seaweed species are used for agriculture (fertilizer or animal feed), including species that are found in Hawaiʻi (Zemke-White and Ohno, 1999). This practice may be an additional strategy to improve soil fertility and crop quality that addresses the dual problems of reliance on inorganic chemical fertilization and wasting of valuable, nutrient-rich biomass. To meet increasing demand for locally available inputs, the use of seaweed as a source of crop nutrients is a viable option (Pramanick et al., 2014). Many growers in the Pacific region are requesting similar organic fertilizers (Radovich et al., 2012).

1.3.3 Algae in Hawaii

Wild-gathered seaweeds (limu) are a prominent component of Native Hawaiian diet and culture; Seaweeds are one of the traditional wild greens in Hawai’i that continue to be used for food, medicine, and ceremony (Abbott 1978, 1996). Prior to European contact in 1778, the native Hawaiian diet consisted primarily of complex carbohydrates such as taro, sweet potato, yam, breadfruit, and wild-gathered greens such as seaweeds (Fujita et al., 2004). Native Hawaiian seaweeds, also called limu, played a particularly prominent role in the pre-contact Hawaiian diet (Abbott, 1996). The ancient Hawaiians used 60-70 species of seaweed for food, medicine, ceremonies and even for their leis and have used seaweed for everything from sustenance to agriculture (Fujita et al., 2004).

Hawaii is the most isolated group of oceanic islands in the world, and possesses one of the most highly endemic, fragile, and endangered biotas on earth, containing about 40% of the threatened and endangered species in the United States (Cox, 1999). In Hawaii, more than 50 invasive plant species have reached dominance in the natural environment. The non-native invasive seaweed species most common in Hawaiʻi are a result of human introduction. The Hawaii Biological Survey at the Bishop Museum compiled a count of the total number of species in the Archipelago. In 1999, there were 23,150 known species of terrestrial and aquatic algae, plants and animals, including 5047 nonindigenous species (~ 20%). The total number of marine and brackish water alien species in the Hawaiian Islands is 343, including 287 invertebrates, 24 algae, 20 fish, and 12 flowering plants (Hawaii Biological Survey, Bishop Museum, 2002). The foreign seaweeds that have settled along the reefs of Hawaiʻi grow and propagate more readily than Hawaiʻi's native seaweeds (Conklin, et. al., 2009). This rapid propagation is most likely because these seaweeds have less natural predators and herbivorous grazers than they would have in their original habitat. They also may have advantage over the native species because of their nutrient acquisition strategies (Williams and Grosholz, 2007). Potential for complete domination by the foreign seaweeds lies in the possibility of a decreased number of herbivorous grazers and the elevated presence of nitrogen and phosphorous from runoff.

Since the 1950s, around 19 species or more of macroalgae have been introduced to the island of O‘ahu. From that time, their distributions have increased and the algae have spread over much of O’ahu. The alien species from several South Pacific locations Florida, California, and Japan were introduced for commercial, experimental and some accidentally via ship barrages and these have resulted in the larger establishment (Russell, 1992). The increase in the demand for commercial value of carrageenan in the market during 1970s encouraged the introduction of several carrageenan producing species to Hawai’i. *Kappaphycus* spp. and *Eucheuma* spp. were introduced legally to a northwestern reef on the island of O’ahu for growth studies (Glenn, 1981). *Eucheuma* and *Kappaphycus* spp. are both used to produce carrageenan, a gel-forming polysaccharide that forms part of the seaweed cell walls and which has a variety of applications, primarily in the food industry. Presently, *Kappaphycus* spp. has been introduced in 19 countries while *Eucheuma* spp. has been introduced in 13. Annual production of commercially cultivated *Eucheuma* species worldwide (primarily in the Philippines and Indonesia) has increased from less than 1000 tons (dry weight biomass) in 1971 to over 120,000 tons in 2002 with a value of US$ 270 million (Zemke-White and Ohno 1999; Ask and Azanza 2002; McHugh 2002).

The most commonly found macro-size invasive species are *Eucheuma denticulatum*, *Gracilaria salicornia* and *Kappaphycus alvarezii*; all these species are dominant alien algal species in Hawaiian reefs (Smith et al. 2002). The invading plants impose a significant economic burden for control and eradication, and threaten native biodiversity. A sustained effort to remove the algae by various groups has produced millions of pounds of the wet weight biomass that must be disposed. Many growers in the Pacific region are requesting such materials as an organic fertilizer (Radovich et al., 2012). Efforts to compost the material and/or apply it directly to fields indicate that invasive algae can contribute to plant nutrition particularly potassium (K) and growth. Invasive species are high in K and have been routinely applied by local farmers without apparent problems associated with salinity. Rates of application vary significantly across farms. Estimates for application rates of high K species at one farm in a high-rainfall area to long-term crops taro (*Colocasia esculenta*) and sweet potato (*Ipomoea batatas*) are 5.7 $Mg.ha^{-1}$ on a dry weight basis. At an algae concentration of 10% K dry weight; this is equivalent to 570 $kg.ha^{-1}$ of K (Radovich et al., 2012). However, there is very little scientific information available in literature regarding the use of unprocessed biomass and its application rate, yield increase ratio, nutrient release pattern impact on crop nutrition. The Nature Conservancy, Pono Pacific and the University of Hawaii's College of Tropical Agriculture and Human Resources (UH CTAHR) are working together to find the best ways to convert the invasive algae into beneficial nutrient for crop production.

The potential benefit of utilizing this readily available local resource needs to be investigated and over the years, it could be economically beneficial to local growers and may potentially improve the local farms profitability and crop quality. To meet increasing demand for locally available inputs such as organic manures, among many viable options, the use of seaweeds as a plant nutrient bearing fertilizer is an important component.

1.3.4 Study Species:

The three commonly recognized and abundantly available invasive algae species in Oahu coastal waters, *Eucheuma*, *Kappaphycus* and *Gracilaria salicornia* were chosen for this study as they grow predominantly on O'ahu. The genera of *Eucheuma* and *Kappaphycus* belong to the Family *Solieriaceae* of the Order *Gigartinales* whereas the *Gracilaria* belongs to the family *Gracilariaceae* of the order *Gracilariales*.

1.3.5 Kappaphycus **spp. (*K. alvarezii*)**

Kappaphycus alvarezii is one of the largest tropical red algae with extremely high growth rate. Coarse, spiny, invasive seaweed, *K. alvarezii* is usually yellow green in color, but may appear red if shaded. Since their introduction in Kaneohe Bay in 1974, this alga has spread rapidly around O'ahu. Studies of *K. alvarezii spp.* (Glenn and Doty 1981, 1989) at Moku o Lo'e found that water motion was the environmental factor that had the greater influence on growth rates. The high growth rate, plastic morphology, and extremely successful vegetative regeneration make this species potentially destructive invasive in Hawaiian waters (University of Hawaii Manoa Botany Department 2001). The average potassium (K) content based on chemical analysis is around 19-21% (ADSC, Univ.of Hawaii). Carrageenans are the polysaccharides extracted with hot water from certain genera of red seaweeds such as *Kappaphycus* and *Eucheuma* have been used as a natural food additive. Carrageenans are a family of hydrocolloids used to thicken, stabilize and gel solutions. Carrageenan is located in the cell wall and intercellular matrix of the seaweed plant tissue. Kappa carrageenan is extracted from *K. alvarezii* and Iota Carrageenan from *E. denticulatum* species although the concentration and composition of carrageenan found in seaweed varies by the species of plant. Carrageenans global production was approximately 60,000 ton.year^{-1}, with an estimated worth of \$ 626 million in 2014 (Nanna Rhein-Knudsen et al., 2015). *Kappaphycus alvarezii* has been exploited in Asia for several decades and is one of the most important sources of carrageenan in the world. Carrageenan compounds differ from agar in that they have ester sulfate groups (Doty and Norris 1985). About 70% of the world's carrageenan production is based on kappa carrageenan.

1.3.6 Eucheuma spp. (*E. denticulatum*)

This species, much like *K. alvarezii* spp. have characteristics that make them difficult to distinguish. *Eucheuma* attaches to substrate and coral via many attachment points making complete removal without fragmentation difficult. The plant body consists of many terete branches, tapering to acute tips branchlets arranged in whorls, forming distinct "nodes" and "internodes" especially at the terminal portion of the branches. *Eucheuma* populates most of the patch reef systems in Kaneohe Bay and many of the outer portions of fringe reef systems. These species are commonly found on the east shores of the island of O‘ahu, as well as the Waikiki area (Botany Department, University of Hawaii Mānoa, 2001). The average potassium (K) content based on chemical analysis is around 17-19%. The iota carrageenans are predominantly produced from *Eucheuma denticulatum*. The primary differences which influence the properties of kappa and iota carrageenan are the number and position of the ester sulfate groups on the repeating galactose units 6. Current demand for kappa carrageenan are greater than that for iota carrageenan; a situation partly caused by a relatively slower growth rate of the *K. alvarezii* as compared to the *E. denticulatum* (Mtolera et al., 1995).

1.3.7 Gracilaria salicornia

The red alga *Gracilaria salicornia* was introduced intentionally to two reefs on O‘ahu, Hawai‘i, in the 1970s for experimental aquaculture interests to build agar industry. Some 40 years later, this species has spread from the initial sites of introduction and is now competing with native marine flora and fauna (Smith et al., 2004). *Gracilaria salicornia* is successful in calm waters. Cylindrical, the branches are frequently found flattened. This no-indigenous alga is usually not fertile in Hawaiian waters, and was been found to propagate asexually (Nishimura, 2001). Its widespread dispersal is accomplished primarily through fragmentation (Botany Department, University of Hawaii Mānoa, 2001).The average potassium (K) content based on chemical analysis is around 13-15% (ADSC, Univ.of Hawaii).This species is thought to compete with the native reef algae, such as *G. coronopifolia*, for substrate on the reef flat. This species successfully competes with other macroalgae by forming large, intricate mats that cover the substrate and inhibit settlement of other algae. Agars are industrially produced from the agarophytes red seaweed genera *Gracilaria* species. Most agars are extracted from species of *Gelidium* and *Gracilaria* species. Agar is a polymer of agarobiose, a disaccharide composed of D-galactose and 3, 6-anhydro-L-galactose.The global production of agar was approximately 10,600 ton.y-1, with an estimated worth of $ 191 million in 2014 (Nanna Rhein-Knudsen et al., 2015). *Gracilaria*, the most abundant and promising resource of agar production, has probably more than 150 species, distributed mainly in the temperate and subtropical zones.

My research focused on evaluating the three invasive algae species as local source of potassium (K) fertilizer for Pak choi production in Hawaii. Pak choi is an economically important crop for local farmers. A variety of knowledge gaps exist that prevent producers from using locally available inputs in their crop production systems. This study was focused on understanding of factors such as application rates; species efficacy to supply nutrients, yield increase ratio and K release kinetics that contribute to optimizing production, reducing cost and improve quality of produce.

Although these seaweeds are used in the past for crop production, little scientific information is available on use of unprocessed biomass in crop production as most of these studies focused on the seaweed extracts and their byproducts. Quantification of invasive biomass effects on crop quality and crop production is required to validate the positive and/or negative effects of using these species. This research was designed to evaluating the effect of three invasive algae species on growth (yield) and K nutrition (tissue K) of pak choi at different K fertilizer rates, to understand the species efficacy, to evaluate algae biomass as a replacement for K synthetic fertilizers and to determine the K nutrient release kinetics of these three species.

1.4 Hypothesis:

The general objective of the study was to determining potassium (K^+) release, crop availability and uptake from three red algae (Rhodophyta) species. To test the hypothesize that invasive species of algae *salicornia Eucheuma denticulatum, Gracilaria salicornia* and *Kappaphycus alvarezii* can be alternate sources of potassium (K) fertilizer to influence on plant growth and K nutritive quality, a series of greenhouse and laboratory experiments were conducted with the following objectives:

1. Characterize relationship between two methods of tissue K measurement using cardy meter and ICP (Inductively Coupled Plasma).

2. Develop a yield response curve of pak choi to application of three invasive algae species as source of K fertilizers at five rates; Comparing the pak choi tissue K uptake to application of three algae species at five rates.

3. Comparing the invasive algae with synthetic K fertilizers KNO_3 and KCl for yield and K nutrition at five application rates.

4. Comparison of two buffers $NaClO_4$ and NaCl in preventing other ionic interferences in leached solutions while measuring K concentrations with ion selective K electrode.

5. Establish potassium (K^+) release pattern and kinetics from three invasive algae species as compared to synthetic fertilizers in three different media in laboratory based leachate column experiments.

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CHAPTER 2

A correlation of rapid Cardy meter sap test and ICP spectrometry of dry tissue for measuring potassium (K^+) concentrations in pak choi (*Brassica rapa* Chinensis group)

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2.1 ABSTRACT

Nutritional status of vegetable crops is often monitored by analysis of dried plant tissues, which is costly and often takes time. Two greenhouse trials were conducted, at the University of Hawaii at Manoa, Magoon facilities, to evaluate the portable cardy ion meter (CIM) in determining K^+ status in fresh petiole sap of pak choi as compared with standard laboratory methods. In the first greenhouse trial, three algae species (*Gracilaria salicornia*, *Kappaphycus alvarezii, Eucheuma denticulatum*) were used to apply 5 rates of K^+ (0, 84, 168,252, and 336 kg.ha^{-1}). The pak choi was directly seeded into 0.0027 m^3 pots and was grown in peat moss. In the 2nd greenhouse trial, K^+ was provided through *Eucheuma denticulatum* and potassium nitrate (KNO_3) at 5 rates (0,112, 168, 224, 280 and 336 kg.ha^{-1}) in peat moss and soil media. At harvest, K^+ concentrations in fresh petiole sap were analyzed immediately with CIM and the dried samples were analyzed by inductively coupled plasma spectroscopy (ICP). The results showed increase in leaf K^+ content at higher rates and the maximum concentration of leaf K^+ at 4500–5300 mg/L for sap and 8-9% for tissue was obtained when K^+ was provided between 224-284 kg.ha^{-1}. There was a close correlation between the CIM readings and the ICP method (*r*= 0.80 and 0.83) from 1stand 2nd GH results, respectively. Although results showing close correlation, we decided to go with the laboratory-ICP measured tissue K values to analyze the data. The data further suggested that 4500-5000 mg K/L for fresh petiole sap and 7.5% K^+ in tissue as critical levels for K^+ concentration in Pak Choi.

Keywords: Algae, cardy meter, *Eucheuma, Gracilaria*, *Kappaphycus*, petiole sap, potassium

2.2 INTRODUCTION

Plant tissue analysis is a valuable tool for evaluating the nutritional status and quality of crops and is widely used for scientific and commercial purposes (Hansen et al. 2013). The majority of plant analyte are now performed by techniques based on inductively coupled plasma optical emission spectroscopy (ICP-OES) or ICP mass spectrometry (ICP-MS) (Hansen et al., 2013). The laboratory methods usually require destructive sampling, either by dry ashing the sample or by dissolving the sample in one or more acids. But, the cost and time delay has led to development of quick tests for plant tissue. Among the recent techniques for NO_3-N and K^+ management in vegetable crops has been the use of petiole sap analysis to determine supplemental fertilizer needs (Hochmuth, 1994; Scaife and Turner, 1984). NO_3-N measurements by the cardy meter were generally well correlated with standard colorimetric techniques for field samples and within certain soil types in a column study (Rebecca et al., 2012). The cardy meter (CM) tests to determine NO_3-N and K^+ concentrations in fresh petiole is a quick procedure as these tests can be performed in a few minutes in the field or lab (Hochmuth, 1994). Fresh tissue or sap may be more representative of living plant tissue than is dried plant material (Szwonek, 1988). Several authors have reported that petiole sap tests using nitrate test strips are well correlated with dry tissue analysis (Scaife and Stevens, 1983; Coltman, 1987; Papastylianou, 1989; Williams and Maier, 1990). Early work with petiole sap testing found that results could be influenced by sampling time during the day (Zhang et al., 1996) or with ionic interferences (Anderson et al., 1999). A cardy meter using non diluted plant sap was well correlated to conventional methods of NO_3-N analysis (Altland et al., 2003). A recently developed portable ion selective electrode can directly measure NO_3-Nin fresh petiole sap, and has been subsequently utilized for field measurements (Westcott et al., 1993). Correlation coefficients were between 0.65 and 0.93 for the relationship between the sap test as measured by the portable electrode and dry tissue analysis for petiole NO_3-N determination of potatoes (Westcott et al., 1993) and broccoli (Hartz et al., 1993). The cardy meter has been used to establish sufficiency levels of K^+ in petiole sap for eggplant (*Solanum melongena* L.) production (Hochmuth et al., 1993). The cardy meter is provided with a specific ion electrode, allowing rapid determinations in the field of soil solution and plant sap ion concentrations. Several authors report some advantages and disadvantages in the use of specific ion electrodes (Jackson, 1980, and Sah, 1994). The main advantage is the short time and low cost required for analysis and the disadvantage is the interference of other ions in the sap. The overall objective of our experiments were to correlate the readings of the cardy ion meter for determining K^+ in petiole sap compared with K^+ in dry tissue analyzed with a conventional laboratory method (ICP spectrometry).

2.3 MATERIALS AND METHODS

Two Greenhouse experiments were conducted at the University of Hawaii’s Magoon Research Facility (lat. 21°18′22″ N, long. 157°48′37″ W). Seaweed species were obtained from the Department of Land and Natural Resources on Oahu Island. The three species of algae were dried in the oven at 70 °C for 72 h. The dried algae were ground into a fine powder using a small coffee grinder. For both greenhouse trials, we used pak choi (*Brassica rapa,* Chinensis group) var. Bonsai, and seeds were obtained from Harris seeds company (Rochester, New York). The pak choi was direct seeded into 0.0027 m^3 pots.

2.3.1 First Greenhouse Trial:

Three algae (*Eucheuma denticulatum*, *Gracilaria salicornia* and *Kappaphycus alvarezii*) were applied at five application rates (0, 84, 168,252, and 336 kg.ha^{-1}) to provide K^+ and other major nutrients such as N and P were provided through tankage, triple super phosphate, urea, and potassium nitrate (KNO_3) at equal rates for all the plants at required rates of 168 kg.ha^{-1}. The experiment was set up as a complete randomized design (CRD) with 5 replicates. Seeds were directly sown to the pots filled with peat-based media (Sunshine Mix #4 Sun Gro Horticulture, Agawam, MA) on 04 /15/2013, and after a week, pak choi germinated seeds were thinned to one plant per pot. Plants were allowed to grow in the greenhouse with overhead sprinklers with a frequency of every 4 h for 5 min throughout the experiments. Plants were harvested at five weeks after emergence. Fresh weights were immediately measured and the plants were placed in an oven (Precision Mechanical Convection Oven, Thermo scientific, Rochester, NY) at 70 °C and were dried for 72 h, and the dry weights were recorded. Petiole plus midrib (hereafter designated as petioles) was collected from each plant immediately after the harvest and pressed in a garlic presser to extract plant sap. For cardy sap test, each 1 mL of sap was diluted with deionized water to a volume of 5 mL and the diluted 1 mL sap was analyzed for K^+ concentrations in the portable ion-specific electrode (B-731 "LAQUAtwin” cardy meter: Horiba Scientific, Irvine, CA). The cardy ion meter was calibrated using standard solutions of potassium chloride (KCl) at 150 and 2000 ppm after every thirty measurements in strict accordance with manufacturer's recommendations. The dried samples were submitted for dry tissue analysis of K^+ by ICP spectrometry using acid digestion procedures at the ADSC laboratory at the University of Hawaii at Manoa.

2.3.2 Second Greenhouse Trial:

At the 2nd greenhouse trial, the experiment set up was much similar to the 1st experiment, but with three replications, two types of growth media (peat and soil) and two fertilizer types *Eucheuma* and KNO_3 at five rates (0,112, 168, 224, 280 and 336 $kg.ha^{-1}$). For soil, an Ultisol from Oahu was used. The soil is well-drained, clayey, oxidic, isohyperthermic, of the Lilehua series characterized by low pH and high aluminum saturation. K^+ was provided at five different rates from Eucheuma and KNO_3 whereas other major nutrients N and P were provided through urea and triple super phosphate at equal rates to all the plants at the required rates at 168 $kgs.ha^{-1}$. Plants were harvested at 5 weeks after emergence and K^+ content of the plants were measured with cardy meter and ICP methods, as described earlier in the 1st trial.

2.3.3 Statistical analysis: Analysis of variance (ANOVA) on K data measured from two methods was performed using Statistix 10 (Analytical Software, Tallahassee, FL). Pearson Correlation Coefficient was performed to statistically measure the relationship between the two methods to measure K data using SigmaPlot 13 (San Jose, CA).

2.4 RESULTS AND DISCUSSION

The K^+ concentrations increased linearly in both sap and dry tissue with increased amount of K^+ in both methods of analysis. Concentrations of K^+ in petiole sap determined by cardy meter were reasonably well correlated with those determined in dry tissue measurements (Fig. 1-4) and the relationships were highly significant ($P < 0.0001$) in both GH experiments. The correlation co-efficient (r) was stronger between the cardy meter and standard laboratory method when means of the reps were used for regression analysis with r values 0.99 and 0.84 from 1st and 2nd GH trials, respectively. The laboratory K^+ measurements from 1st and 2nd GH trials were higher than those determined by the cardy meter from (Fig.1 and 3). Discrepancies observed between the cardy meter and standard method measurements are not only due to the influence of other ions present in solution and sap, may be because cardy meter measures K^+ only in fresh sap, whereas the dry tissue analysis at laboratory measures total K^+ in all tissues. Kallenbach (2000) found that values of r between concentration of K^+ in sap and in dry matter of alfalfa leaf was always below 0.60 and better correlations were obtained when other variables were taken into account (i.e., leaf moisture, temperature, calibration frequency, length and leaf order). The correlation co-efficient (r) were 0.80 and 0.83 for 1st and 2nd GH trials, respectively, when the total numbers of reps were used to analyze the data. The slope and y-intercept did not differ much from each other from both the experiments. Concentrations of K^+ in petiole sap determined by cardy meter were reasonably well correlated with those determined in dry tissue matter and relationships were highly significant for both the methods in the two trials (Fig.1-4).The values of r for the concentrations in the petiole sap and in dry tissue matter from our trials were close to those observed by other authors in their research. The r values for NO_3-N and K^+ varied from zero to 0.80 for cultivated tomato depending on the location and crop age (Locascio et al., 1997). The r values improved further in the 2nd GH trial when the data from individual treatments (*Eucheuma* and KNO_3 were separately analyzed with r values reading at 0.85 and 0.84 for *Eucheuma* and KNO_3 using total samples and 0.98 and 0.96 with mean values respectively (Fig. 5-8). The testing of the plant sap by specific ion electrode methods is usually considered less expensive and faster than spectrophotometric or other standard laboratory methods. The results, though close, are not as accurate as measurements from an analytical laboratory (Brust, 2008).

2.5 CONCLUSIONS

Cardy ion meters are easy to use and less expensive than standard lab analysis, making them an appealing substitute for analyzing large number of samples. The user of the quick sap test electrodes need to evaluate the need for speed versus accuracy for the nutrients to be determined. With significant correlation between the measurements from these two methods in this study and the relationship between the two methods is of adequate strength to conclude that cardy meter is a valuable tool for on-site monitoring of nutrient status of pak choi, particularly for detecting K deficiencies. With results showing close correlation, we decided to go with the laboratory-ICP measured tissue K values to analyze the data. Various factors need to be considered such as ionic interference, sample size, sampling method, temperature, calibration frequency, and leaf order while using the cardy meter to get better results and these should be examined in future research.

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List of Figures:

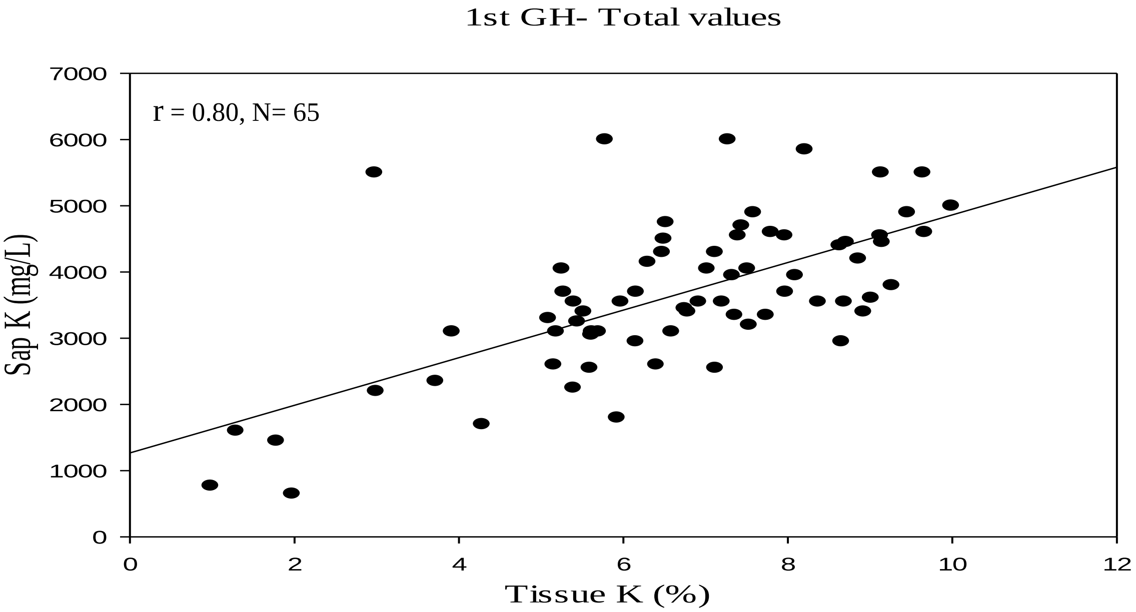

Fig 2.1: Relationship between K$^+$ concentrations measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 1st greenhouse trial using totals (n = 65)

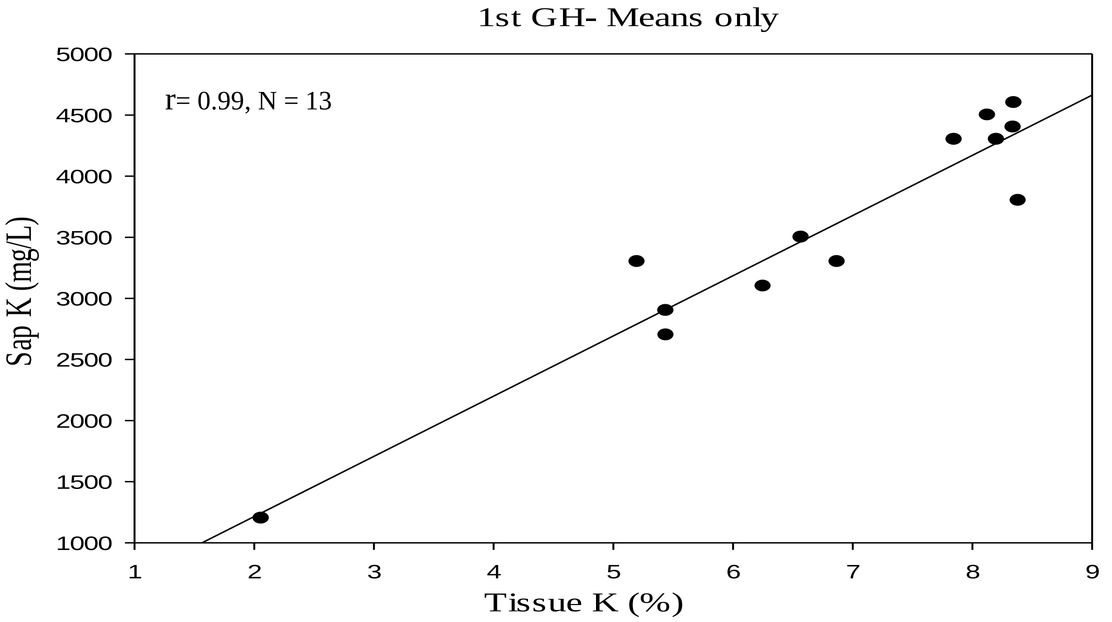

Fig 2.2: Relationship between K$^+$ concentrations measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 1st greenhouse trial using mean (n = 13).

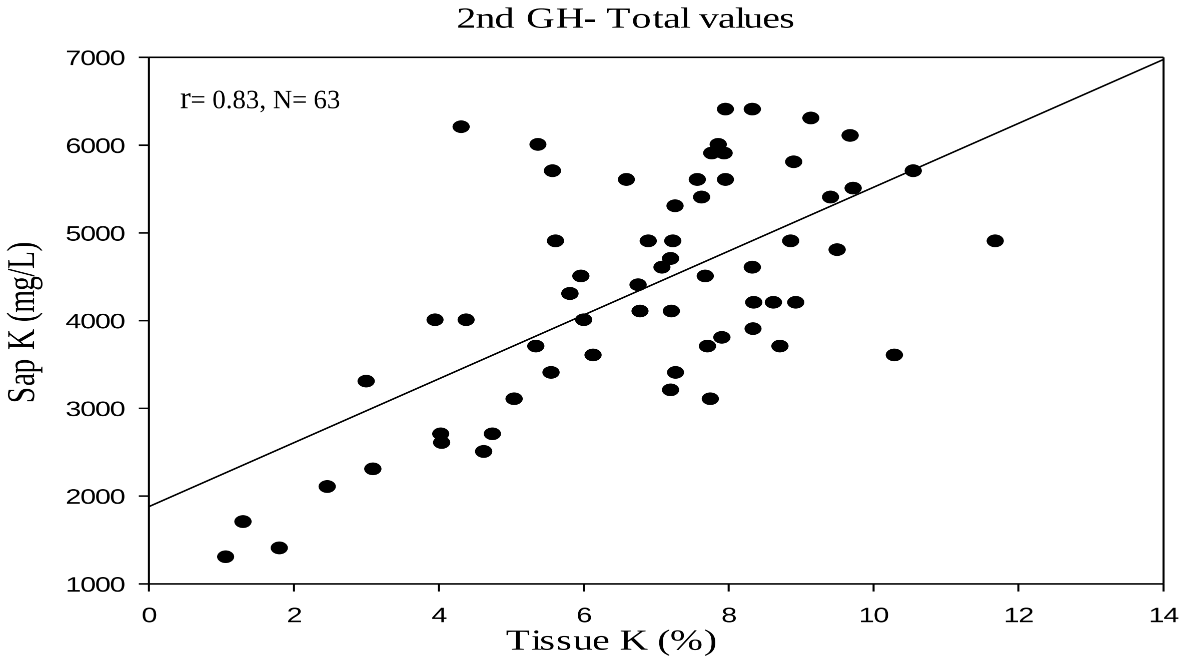

Fig 2.3: Relationship between K^+ concentrations measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 2nd GH trial using totals (n=63).

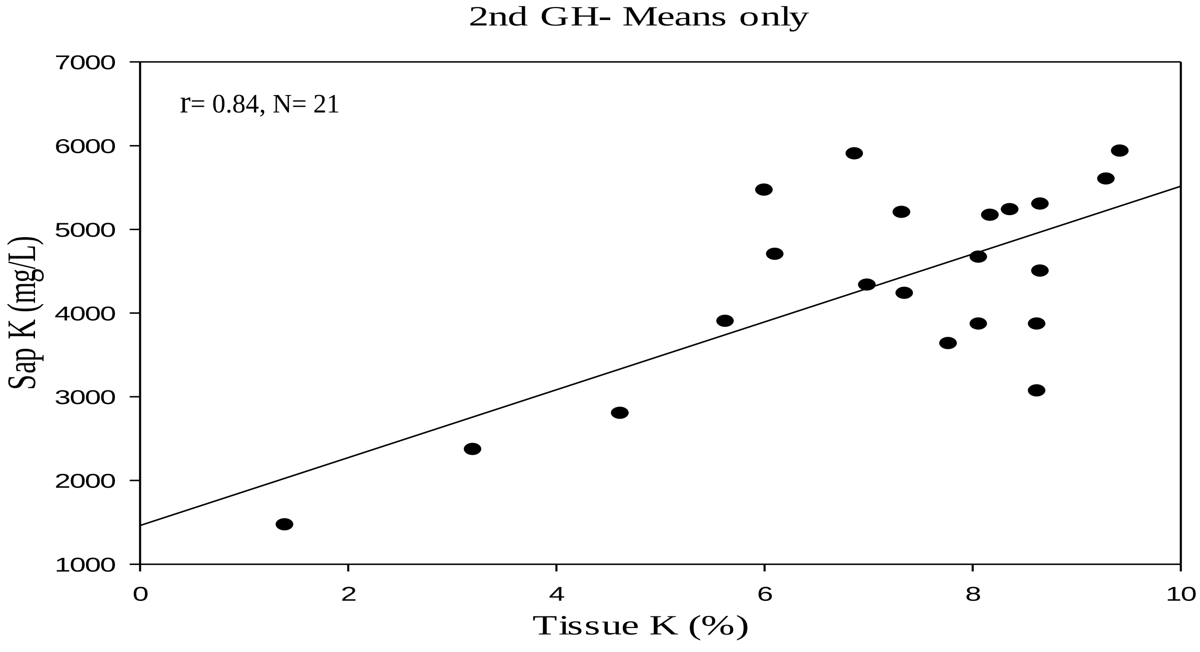

Fig 2.4: Relationship between K^+ concentrations measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 2nd GH trial using means (n=21).

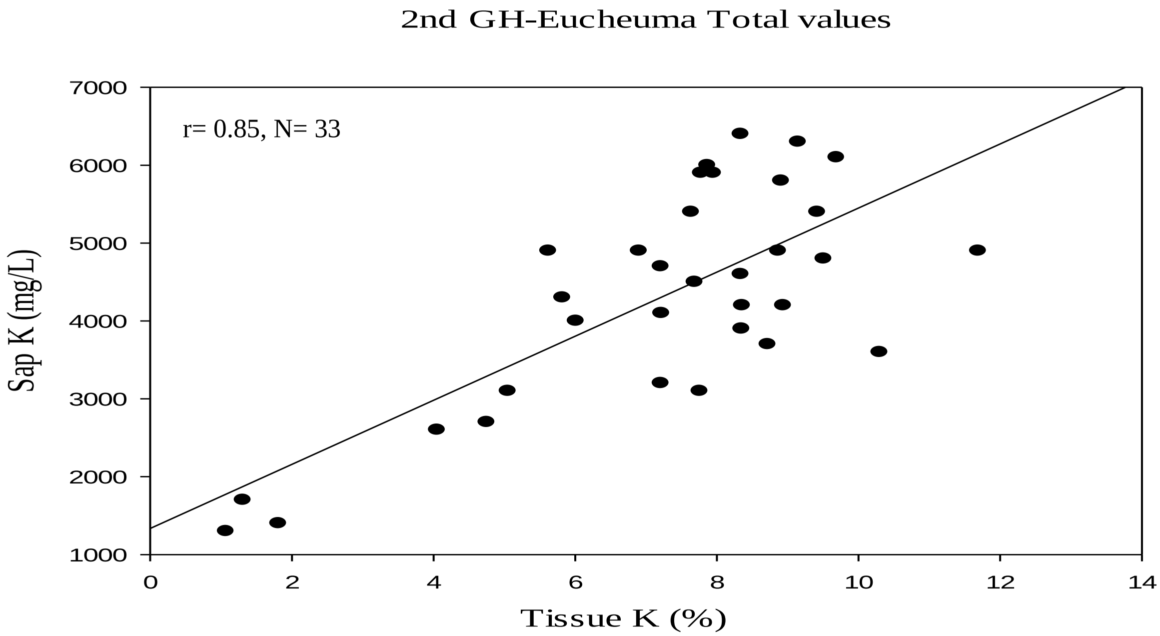

Fig 2.5: Relationship between K^+ concentrations in *Eucheuma* treated plants as measured by cardy meter using fresh petiole sap and dry tissue measurements from CP methods for 2nd greenhouse trial (n = 33).

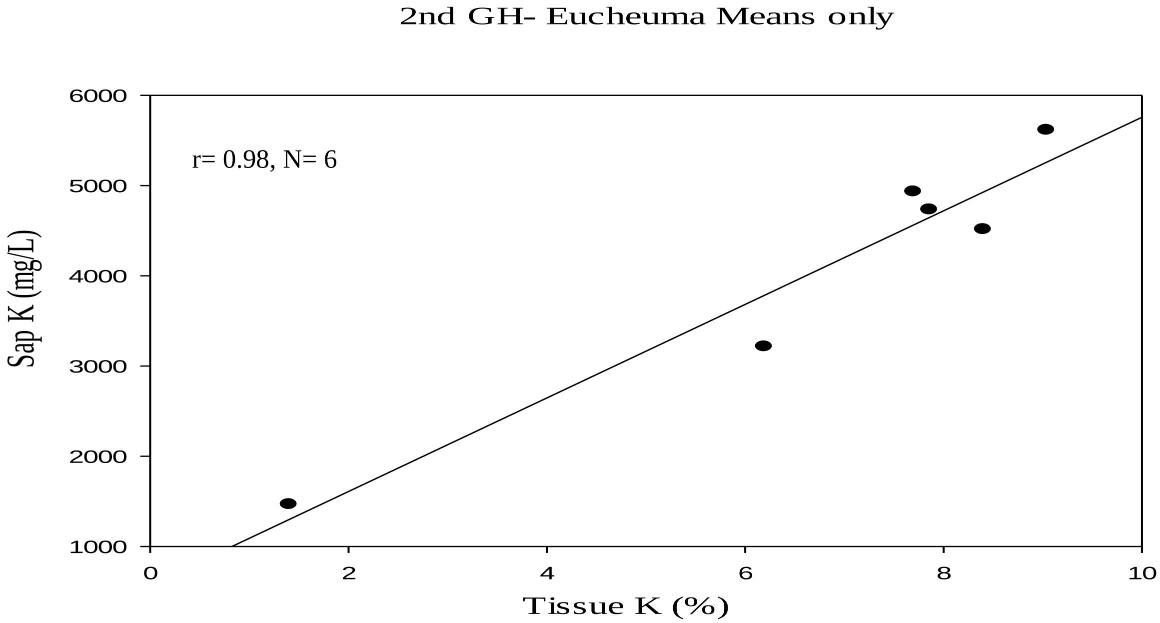

Fig 2.6: Relationship between K^+ concentrations measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 1st greenhouse trial (n = 6).

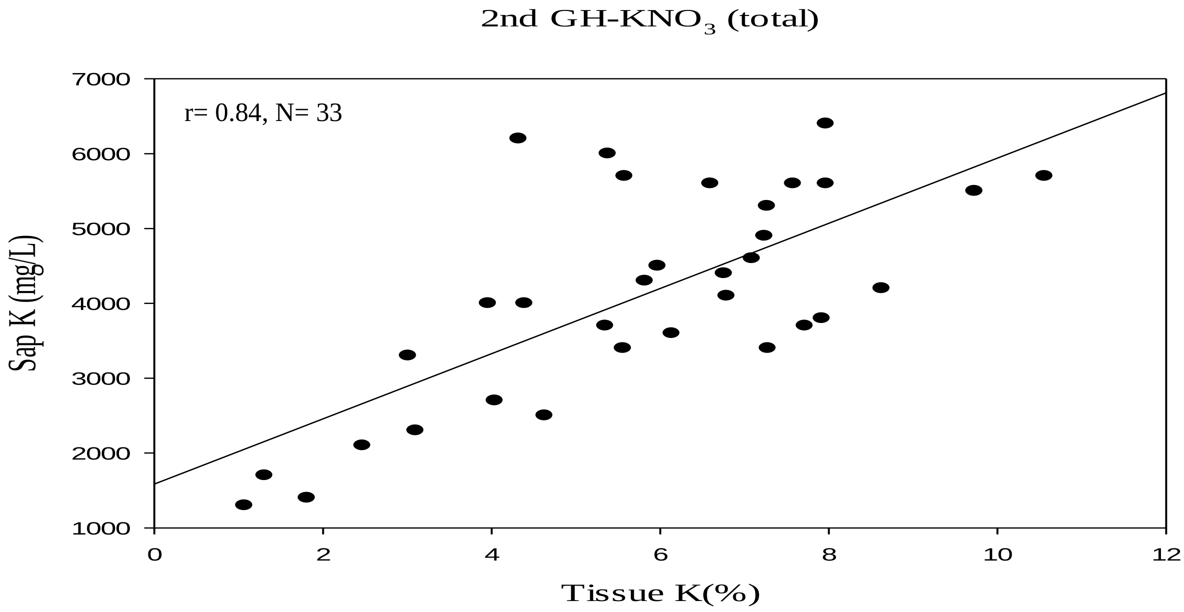

Fig 2.7: Relationship between K^+ concentrations in KNO_3 treated plants as measured by cardy meter using fresh petiole sap and dry tissue measurements from ICP methods for 2nd greenhouse trial (n = 33).

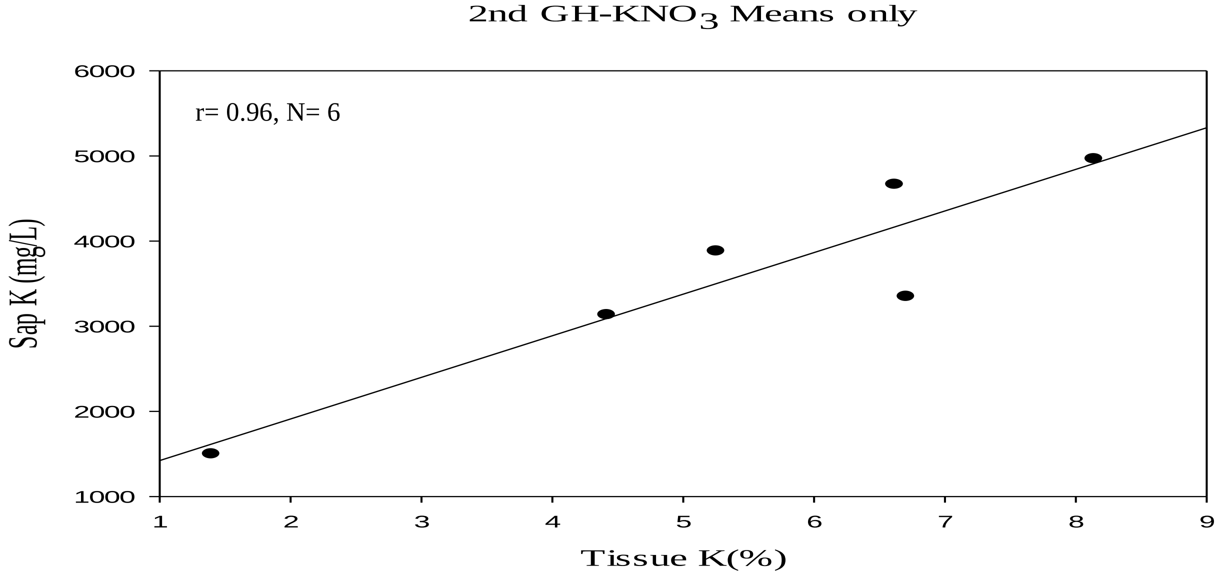

Fig 2.8: Relationship between K^+ concentrations KNO_3 treated plants as measured by cardy meter using mean values of fresh petiole sap and dry tissue measurements from ICP methods for 2nd greenhouse trial (n = 6).

CHAPTER 3

Evaluating Three Invasive Algal Species as Local Organic Sources of Potassium (K) for Pak Choi *(Brassica rapa,* Chinensis group) Growth

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3.1 ABSTRACT

The application of locally available invasive algae biomass as a fertilizer for crop production in Hawaii is being investigated as a substitute for imported chemical fertilizers. Three closely related greenhouse trials were conducted to determine if the algae served as a source of potassium (K) on growth, yield, and K mineral nutrition in pak choi (*Brassica rapa*, Chinensis group). In the first trial, three algae *Eucheuma denticulatum*, *Gracilaria salicornia*, and *Kappaphycus alvarezii* were applied at five rates of K each to evaluate their effects on growth and K nutrition of pak choi plants. The pak choi was direct seeded into 0.0027 m^3 pots containing peat moss based growth media. In trial 2, pak choi was grown in peat media at 5 rates of K provided by algae *(E. denticulatum*) or by potassium nitrate (KNO_3). In trial 3, the 5 rates of K were provided through algae (*K. alvarezii*), KNO_3 and potassium chloride (KCl) and were compared for growth and K nutrition. Results from the first greenhouse trial showed no significant differences among the 3 algal species in yield or tissue K content of pak choi. However, plant yield and tissue K concentration were increased with application rates. The maximum yield and tissue K was observed when K was provided within the range of 250-300 $kg{\cdot}ha^{-1}$. Similarly, in experiments 2 and 3 there were no significant differences between commercial K fertilizers and algal K species for yield. Only K rates were significant for yields and tissue K concentrations. It was concluded that K in the invasive algae was similarly available as K in commercial synthetic fertilizers for pak choi growth in terms of yield and tissue K content under our experimental conditions.

Keywords: Agroecology, Vegetables

3.2 INTRODUCTION

To meet increasing demand for locally available inputs, the use of seaweed as a source of crop nutrients is a viable option (Pramanick *et al.*, 2014). Many growers in the Pacific region are requesting such an organic fertilizer (Radovich *et al.*, 2012). Marine algae are used in agricultural and horticultural crops, resulting in many beneficial effects on yield and quality (Dhargalkar and Pereira, 2005). In many countries, seaweed and seaweed based fertilizers are still used in both agriculture and horticulture (Verkleij, 1992; Zodape, 2001). Not only providing nutrients, these seaweeds improve soil structure and humus content when they are composted and applied to the soil (Haslam and Hopkins, 1996). Seaweed liquid fertilizer (SLF) of *Dictyota dichotoma* was found to increase the yield, growth of roots and shoots, number of roots and maturity time of *Abelmoschus esculentus L.* (okra) at low concentrations (Sasikumar *et al.*, 2011). Seaweed concentrate prepared from *Ecklonia maxima* (Osbeck) Papenfuss has improved the growth of tomato seedlings when applied as a soil drench (Crouch *et al.*, 1992). It was observed that the application of SLF of *Ascophyllum nodosum* increased the chlorophyll of cucumber cotyledons and tomato plants (Whaphamem *et al.*, in 1993). In Hawaii, the most commonly found macro-size invasive species are *Gracilaria salicornia*, *Kappaphycus alvarezii* (*Kappaphycus*) and *Eucheuma denticulatum* (*Eucheuma*); all are dominant alien algal species in O’ahu reefs (Smith *et al*., 2002). They show potential for use as a locally available source of potassium (Radovich et al., 2012). Several farmers in Hawaii have been using the algal species for some crops, but there is a lack of information about the availability and optimal rates of K for crop growth from these algal species. The overall objective of this research was to evaluate three invasive algal species on yield and K mineral nutrition of pak choi, and then to compare one algae species with KNO_3 and/or KCl.

3.3 MATERIALS AND METHODS

Three greenhouse experiments were conducted at the University of Hawaii’s Magoon Research Facility (lat. 21°18′22″ N, long. 157°48′37″ W). The three algae were obtained from the Department of Land and Natural Resources on Oahu Island and were dried in the oven at 70°C for 72 h. The dried algae were ground into a fine powder using a coffee grinder. The required amounts of K provided from each algal species and for each application rate were calculated based on the K analysis report received from the Agricultural Diagnostic Service Center (ADSC) at University of Hawaii, Manoa (Table1). The amount of K required from algae per plant based on rates was calculated based on pak choi plant density of 71659 per ha with a spacing of 0.30 m between plants and 0.45 m between rows. The required amount of K per plant was divided by the % of K present in each species. For trials 1 and 2, we used pak choi (*Brassica rapa,* Chinensis group) cv. Bonsai and for the trial 3, cv. Mei Qing choi was used. All the seeds were obtained from Harris Seeds of Harris Seeds Company (Rochester, New York). Seeds were planted in 0.0027 m^3 size pots.

3.3.1 Trial 1: Three algal species (*Gracilaria salicornia*, *Kappaphycus alvarezii*, and *Eucheuma denticulatum*) were applied to supply 5 rates of K at 0, 1.17, 2.35, 3.51, and 4.70 $g.plant^{-1}$ (which is equivalent to 0, 84, 168, 252, and 336 $kg{\cdot}ha^{-1}$), with “0” being the control treatment without K fertilizer. The other major nutrients nitrogen (N) and phosphorus (P) were provided through tankage (bone meal) (10-3.7-0.8) and triple super-phosphate (0-45-0) at constant rates for all the plants at the normal required rates at 168 and 112 $kg{\cdot}ha^{-1}$, respectively (Hemphill, 2010). The actual amounts provided were 22.72 $g.plant^{-1}$ of bone meal and 3.74 $g.plant^{-1}$ of triple super-phosphate. The experiment was set up as a complete randomized design (CRD) with 5 replicates. Three to four seeds were directly sown to each pot filled with peat-based media (Sunshine Mix # 4, SunGro, Agawam, MA) on 15 Apr. 2013. A week after seedling emergence in all pots, plants were thinned to one per pot. Overhead irrigation was provided twice a day for 10 minutes. Plants were harvested on 27 May 2013, 6 weeks after emergence.

3.3.2 Trial 2: This trial also set up as a CRD with three replications and two fertilizers, *Eucheuma denticulatum* and KNO_3. Based on the results of trial 1, in which no significant differences were found between the three invasive algal species, *E. denticulatum* was randomly selected and used as a source of K fertilizer and compared to KNO_3 synthetic fertilizer. The two K fertilizers were applied to supply 5 rates of K at 0, 1.56, 2.35, 3.12, 3.91 and 4.70 $g.plant^{-1}$ (which is equivalent to 0, 112, 168, 224, 280, and 336 $kg·ha^{-1}$), with "0" being the control treatment without K fertilizer. The other major nutrients nitrogen (N) and phosphorus (P) were provided through urea (46-0-0) and triple super-phosphate (0-45-0) at constant rates for all the plants at the normal required rates at 168 and 112 $kg·ha^{-1}$, respectively. Urea was provided at 5.10 $g.plant^{-1}$ to provide N at 2.35 $g.plant^{-1}$ for all pots except the pots treated with KNO_3 which received 3.93, 3.37, 2.79, 2.22 and 1.63 $g.plant^{-1}$ of urea. The triple super-phosphate was provided to all the pots to provide 1.56 g of P per plant. The same procedures were followed as in trial 1 with the following exception; Plants were watered to excess of pot capacity (150 ml) through a drip system once a day. Seeds were sown on 28 Jan. 2014 and were harvested on 11 Mar. 2014, 6 weeks after emergence.

3.3.3 Trial 3: This was also CRD with four replications of three fertilizers, *Kappaphycus alvarezii*, KNO_3, and KCl to supply 5 rates of K 0, 1.56, 2.35, 3.12, 3.91 and 4.70 $g.plant^{-1}$ (which is equivalent to 0, 112, 168, 224, 280, and 336 $kg·ha^{-1}$). Three to four pak choi seeds were sown into each pot filled with peat moss on 6 Mar. 2015. The same amount of N and P were provided from urea and triple super-phosphate as used in trial 2 with adjustments made in KNO_3 treated plants. The same procedures were followed as in trials 1 and 2 with the following exceptions. A Deionized (DI) Water System (US Water system, Indianapolis, IN) filter was fitted in the greenhouse connecting to the regular greenhouse irrigation system producing DI water at 0.0075 m^3 per minute. The collected DI water was used to irrigate the pots using a watering can, 4 times per week. Plants were harvested on 17 Apr. 2015, 6 weeks after emergence.

3.3.4 Plant harvest and measurement: For trials 1 and 2, above ground fresh weights (tops) were immediately measured after harvest and data were recorded. The plants were then placed in an oven (Precision Mechanical Convection Oven, Thermo scientific) at 70°C and were dried for 72 h to constant weight, and the dry weights were recorded. The dried tissue samples were analyzed for K and other nutrients at the ADSC laboratory, University of Hawaii at Manoa using an inductively coupled plasma spectrometer (Optima 7000DV, Waltham, MA). The fresh petioles harvested from trials 1 and 2 were also analyzed for leaf sap K concentrations using the portable ion-specific electrode (B-731"LAQUAtwin" Cardy meter: Horiba Scientific, Irvine, CA). The 4th leaf plus midrib were collected from each plant immediately after the harvest and pressed in a garlic presser to extract plant sap. Each 1 mL of sap was diluted with DI water to a volume of 5 mL and the diluted 1 mL sap was analyzed for K concentrations in the Cardy ion meter which was calibrated after every thirty measurements using a potassium chloride (KCl) standard calibrating solution. For trial 3, the same procedures were followed as in trials 1 and 2 with the exception that sap K was not measured.

3.3.5 Statistical analysis: Analysis of variance (ANOVA) on data from plant tissue K content, fresh and dry weights were performed using general linear model (GLM) on main treatment effects and their interactions. Data were analyzed using PROC GLM in SAS v.9.2 statistical software (SAS Institute Inc., 2003). Regression analysis was conducted using SigmaPlot v.13 (San Jose, CA) to examine the relationship between the rates of K fertilizers and yield or tissue K.

3.4 RESULTS

3.4.1 Trial 1: There was no significant difference in fresh or dry weights of tops or tissue K concentration due to algal species (Table 2). A significant effect due to K fertilizer rate was found in which increasing rates resulted in increased fresh or dry weight and tissue K. The highest yields (dry wt. mean 8.34 g.plant^{-1}) and tissue K (mean 8.2%) were recorded when K was provided in the range of 250 to 300 kg·ha^{-1} (Fig. 1A and 2A). The interactions of species and rates were not significant.

3.4.2 Trial 2: The results showed that main treatment effects of K rates from both fertilizers had significant, positive effects on fresh or dry weights of tops ($P < 0.05$) and tissue K ($P < 0.01$) (Fig. 1B and 2B; and Table 3). There were no significant differences due to the two fertilizer types, *E. denticulatum* and KNO_3 with regards to fresh or dry weights of tops. The two fertilizer types differed significantly ($P < 0.022$) in tissue K concentrations; with algae-treated plants (8.38%) having greater tissue K than KNO_3-treated plants (7.38%). The interaction of fertilizer type and rates were not significant with regards to yield or tissue K content. The highest tissue K (8.9%) was recorded when K was provided at 284 kg·ha^{-1}.Based on quadratic equation formula, the highest dry weight (5.22 gm) was observed when K was provided at 395 kg·ha^{-1}.

3.4.3 Trial 3: Fertilizer rates significantly increased fresh ($P<0.0001$) or dry weights of tops ($P<0.001$), and tissue K concentrations ($P<0.001$; Figs. 1C, 2C). These results were consistent with those of trials 1 and 2. No significant differences in fresh or dry weights of tops were found among the 3 fertilizer types; however the source of fertilizer significantly ($P<0.018$) affected tissue K with KCl resulting in higher tissue K than KNO_3 and algae *K. alvarezii* (Fig.1C). No significant interactions were found among the main treatments. The highest dry weight (7.61 gm) and tissue K (3.87%) were recorded when K was provided at 350 kg·ha^{-1}.

3.5 DISCUSSION

These three very similar trials all address whether these invasive algae serve as a potassium source to a *Brassica* model crop under a range of experimental conditions. In all three trials, increasing rates of K application significantly increased fresh or dry weights of tops of plant and tissue K. In the 1st greenhouse trial, no significant differences were found among three algae species with regards to fresh or dry weights of tops or tissue K. No significant interactions were found. The 2nd trial was modified to compare the organic source of K (algae species) with synthetic source (KNO_3) of K. However, since the 1st trial showed no significant difference between the 3 algae species, one species was utilized. Both algae and synthetic sources of K were applied at the same application rates. In the 2nd trial, we compared one algal species *E. denticulatum* with KNO_3 and found no significant differences between these two sources of K with regards to fresh or dry weights of tops. Fertilization with algae (8.38% K) resulted in significantly higher tissue K than plants fertilized with KNO_3 (7.38% K). No significant interactions were found. The results from 1st and 2nd trials showed that pak choi yield was declining at some point (K application rate at 336 $kg.ha^{-1}$). We suspected the decline might be related to K toxicity or salt effects that may occur with high application of algae. To address both possibilities, a different species of algae (*K. alvarezii*) was used and an additional synthetic K source (KCl) was included to ensure that the chloride content may indicate possible toxic effects. No significant differences were found between these three sources of K with regards to fresh or dry weights of tops. Source of K fertilizer significantly affected tissue K with KCl resulting in higher tissue K (3.7%) than KNO_3 (3.59%) and algae *K. alvarezii* (3.16%). No significant interactions were found. No significant differences among three algae species were detected in total fresh or dry weights of tops or tissue K (Table 2). Our results confirm those previously reported by Crouch et al., (1990) who noted increased uptake of K in lettuce with seaweed concentrate application. The possible reason for lower yields in trial 2 can be attributed to the change in the method of irrigation switched from overhead sprinkler to drip system. The change of pak choi cultivar from Bonsai to Mei Qing choi in the trial 3 may have contributed to some extent for lower tissue K concentrations. Chemical analysis of algae and their extracts made by other researchers showed the presence of a whole range of various plant growth regulators such as auxins, cytokinins, gibberellins, abscisic acid, etc (Prasad et al., 2010 and Yokoya et al., 2010) which at high concentrations may either inhibit or stimulate plant growth depending on the concentration (Crouch and van Staden, 1993). Seaweed constituents include macro and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that affect cellular metabolism in treated plants, may have enhanced pak choi growth and yield. Another possible contribution to increased yield is the presence of polysaccharides in some of the red algae as sugars that are known to improve plant growth in a similar way to hormones (Rolland et al., 2002).

3.6 CONCLUSION

The invasive algae used in these studies positively influenced the growth and K nutrition of pak choi. The consistent results from these studies suggest that these invasive algae species of Hawaiian Islands have potential to be used as a replacement for synthetic K fertilizer in vegetable crop production and are particularly beneficial when used for crops with high K requirements. The algae waste biomass could be mixed with commercially available fertilizers to enhance the plant growth. These seaweeds may provide an effective approach to nutrient management in places where inorganic fertilizers are expensive and limited. Further studies on specific mechanisms attributed to plant growth including K availability over the time are required to determine the potential of these algae species as a commercially viable product.

3.7 Acknowledgements

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Table 3.1: Mineral nutrient concentration in *K. alvarezii*, *E. denticulatum*, and *G. salicornia* used in these trials.

Nutrient	*K. alvarezii*	*E. denticulatum*	*G. salicornia*
N (%)	0.26	0.57	0.8
C (%)	18.12	18.54	22.05
P (%)	0.04	0.07	0.16
K (%)	20.34	18.02	14.1
Ca (%)	0.21	1.55	4.62
Mg (%)	0.41	0.76	0.78
Na (%)	3.35	3.82	3.88
Fe ($\mu g.kg^{-1}$)	173	72	375
Mn ($\mu g.kg^{-1}$)	10.5	14	210

Mean values with n = 2. The tissue samples were analyzed by the ADSC lab at the University of Hawaii, Manoa).

Table 3.2: Effect of three invasive algae species on plant growth and tissue K from trial 1. Mean values represent 5 replicates from each treatment with SE in parenthesis. NS = Not significant at $\alpha = 0.05$.

Species of Algae	**K rates** ($g \bullet plant^{-1}$)	**Fresh wt.** (above ground) ($g \bullet plant^{-1}$)	**Dry wt.** (above ground) ($g \bullet plant^{-1}$)	**Tissue K** (%)
K. alvarezii	4.70	144.7 (10.5)	7.7 (1.2)	8.2 (0.4)
	3.51	179.8 (14.1)	9.4 (0.8)	8.3 (0.4)
	2.35	138.6 (15.8)	6.3 (0.6)	6.5 (0.6)
	1.17	125.78(10.4)	6.1 (0.4)	5.4 (0.4)
E. denticulatum	4.70	133 (15.4)	5.9 (0.6)	8.3 (0.4)
	3.51	169.2 (27.8)	10 (2.8)	7.8 (0.4)
	2.35	136.9 (11.9)	6.7 (0.5)	6.8 (0.4)
	1.17	127.8 (4.9)	6.5 (0.2)	5.2 (0.6)
G. salicornia	4.70	144.8 (8.6)	7.3 (0.2)	8.03 (0.7)
	3.51	123.6 (16.1)	6.2 (0.3)	8.0 (0.4)
	2.35	135.5 (10)	6.1 (0.3)	6.2 (0.3)
	1.17	103.2 (10.6)	5.1 (0.6)	5.4 (0.5)
Control	0	95.5 (1.7)	4.6 (0.4)	2.1 (0.5)
Rates		$P < 0.0111$	$P < 0.0131$	$P < 0.0001$
Species		NS	NS	NS
Rates*Species		NS	NS	NS

[z] The amounts of K rates ($g.plant^{-1}$) shown in the table are calculated from the fertilizer application rates of 84,168,252 and 336 kg of $K.ha^{-1}$.

Table 3.3: Effect of *Eucheuma denticulatum* algae and potassium nitrate on plant growth and tissue K from trial 2.Mean values represent 3 replicates from each treatment with SE in parenthesis. NS = Not significant at α = 0.05.

Fertilizer Types	**Fertilizers K Rates** (g•plant^{-1})	**Fresh wt**. (above ground) (g•plant^{-1})	**Dry wt**. (above ground) (g•plant^{-1})	**Tissue K** (%)
E. denticulatum	4.70	61.4 (1.8)	3.8 (0.03)	8.9 (1.4)
	3.91	70.2 (4.5)	4 (0.08)	9.4 (0.1)
	3.12	74.6 (5.2)	3.7 (0.3)	8.6 (0.3)
	2.35	66.5(2.3)	3.8 (0.08)	8.1 (0.3)
	1.56	58.7 (3.0)	3.1 (0.2)	6.7 (0.3)
Potassium nitrate	4.70	71.2 (3.9)	3.8 (0.2)	9.2 (0.8)
	3.91	67.6 (1.3)	3.8 (0.1)	8.0 (0.4)
	3.12	75.5 (2.1)	3.6 (0.1)	7.1 (0.3)
	2.35	63.3 (4.9)	3.0 (0.06)	6.4 (0.7)
	1.56	68.3 (1.6)	3.3 (0.3)	5.9 (0.5)
Control	0	45.3 (0.8)	2.2 (0.1)	1.4 (0.2)
Rates		$P< 0.0156$	$P< 0.0198$	$P<0.0024$
Fertilizer types		NS	NS	$P<0.0022$
Rates*Fert. Types		NS	NS	NS

^{z}The amounts of K rates (g.plant^{-1}) shown in the table are calculated from the fertilizer application rates of 112,168,224, 280 and 336 kg of K.ha^{-1}.

Table 3.4: Effect of *Kappaphycus alvarezii*, potassium nitrate (KNO_3) and potassium chloride (KCl) on plant yield and tissue K from trial 3. Mean values represent 4 replicates from each treatment with SE in parenthesis. NS = Not significant at $\alpha = 0.05$.

Fertilizer Types	**Fertilizers K Rates** (g•plant^{-1})	**Fresh wt.** (above ground) (g•plant^{-1})	**Dry wt.** (above ground) (g•plant^{-1})	**Tissue K** (%)
K. alvarezii	4.70	90 (0.9)	5.4 (0.2)	3.3(0.2)
	3.91	131 (9.9)	7.8 (0.5)	3.4(0.4)
	3.12	106 (8.6)	5.9 (0.9)	3.6(0.4)
	2.35	100 (10.7)	6.4 (0.8)	3.2(0.3)
	1.56	89.7 (7.3)	5.6 (0.7)	2.1(0.1)
Potassium nitrate (KNO_3)	4.70	94.3(11.6)	5.9 (0.5)	3.7(0.3)
	3.91	133(7.8)	7.5 (0.4)	3.9(0.3)
	3.12	110(13.4)	6.1 (0.5)	3.8(0.1)
	2.35	104(16.4)	6.1 (0.9)	3.4(0.1)
	1.56	97.4(6.3)	5.6 (0.2)	3.1(0.2)
Potassium chloride (KCl)	4.70	108 (5.1)	6.1(.08)	4.0(0.3)
	3.91	121 (19.1)	6.8(0.6)	4.0(0.5)
	3.12	126 (12.6)	6.7(0.8)	3.6(0.3)
	2.35	86.5 (11.2)	4.8(0.4)	3.4(0.1)
	1.56	89.6 (7.1)	5.1(0.3)	3.2(0.3)
Control	0	66.8 (2.4)	4.1(0.1)	1.9(0.1)
Rates		$P< 0.0001$	$P< 0.0015$	$P<0.0026$
Fertilizer types		NS	NS	$P<0.0185$
Rates*Fert. Types		NS	NS	NS

^{Z}The amounts of K^{+} rates (g.plant^{-1}) shown in the table are calculated from the fertilizer application rates of 112,168,224, 280 and 336 kg of K.ha^{-1}.

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Fig 3.1: Effect of K fertilizer rates on tissue K levels (%) of Pak choi from trials 1 (A), 2 (B) and 3(C).

Fig 3.2: Effect of K fertilizer rates on dry weight (g) of Pak choi from trials 1 (A), 2 (B) and 3(C).

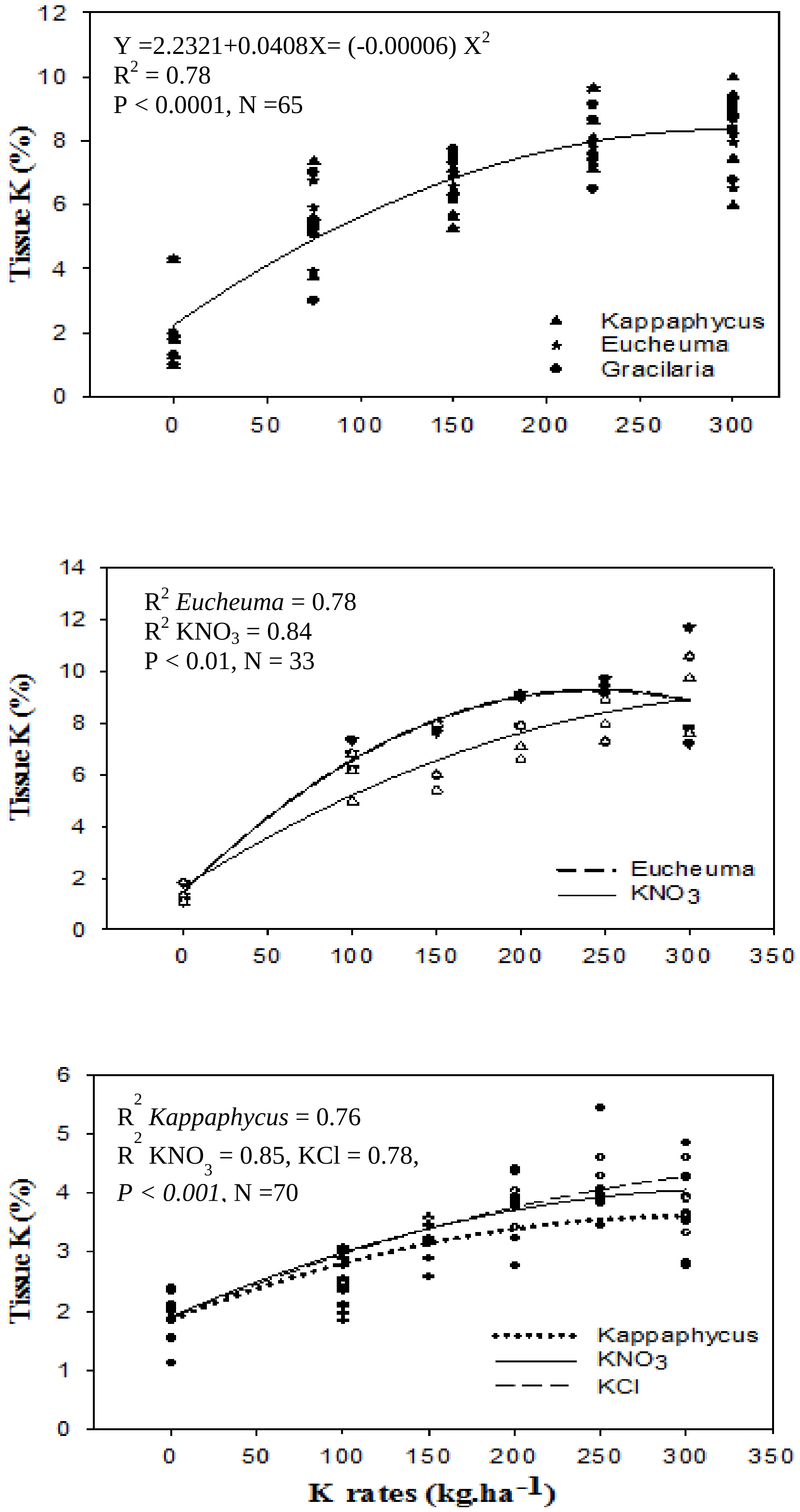

Fig 3.1: Effect of K fertilizer rates on tissue K levels (%) of Pak choi from trials 1 (A), 2 (B) and 3(C).

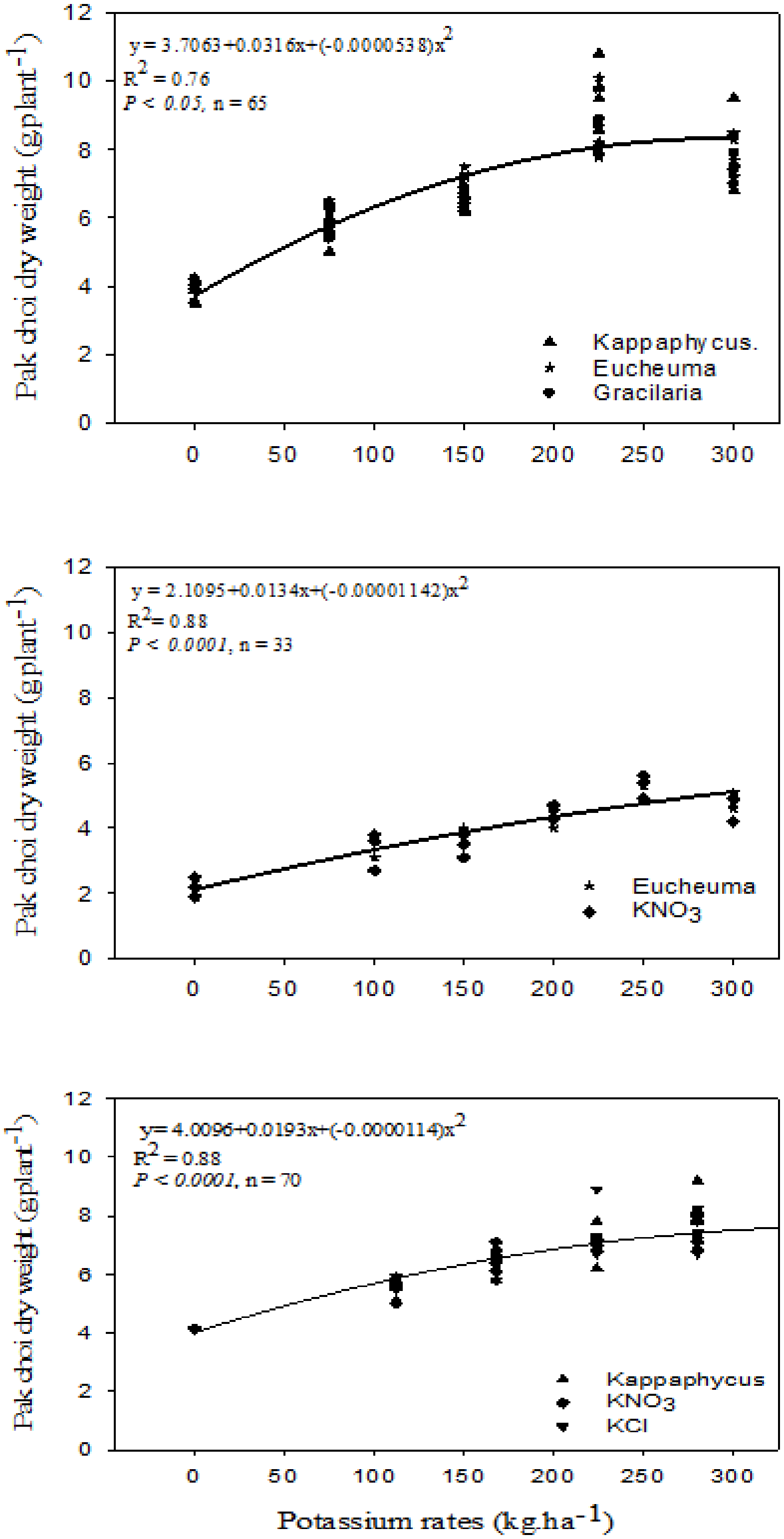

Fig 3.2: Effect of K fertilizer rates on dry weight (g) of Pak choi from trials 1 (A), 2 (B) and 3(C).

CHAPTER 4

A Comparison of Two Ion Strength Adjustment Buffer's Accuracy and Efficiency in Measuring Potassium (K^+) in Aqueous Solutions Using an Ion-Selective Electrode

4.1 ABSTRACT

The practice of using ionic strength adjustment buffers (ISABs) such as NaCl and $NaClO_4$ to maintain a constant ionic strength while measuring the potassium concentrations in aqueous solutions using ion selective electrodes (ISE) is recommended. NaCl is significantly less expensive than $NaClO_4$; however there is no report in the literature comparing the relative efficacy of these two ISAB. These two standard ISABs solutions essentially mask most chemical interferences in the analyte solution and hence increase the accuracy of the reading. The intent of this study was to compare and correlate the potential of these two ISABs to rapidly and accurately measure K^+ concentrations in range of solutions leached from different media types. Three laboratory leachate column studies were conducted to study the correlation and efficiency of two buffers, 2.5 M of sodium perchlorate ($NaClO_4$) and sodium chloride (NaCl) in measuring potassium (K^+) concentrations in the leachate collected from 3 types of media. 3 species of algae (dried ground) and synthetic fertilizers (KCl and KNO_3) were applied to provide K at 112 and 336 $kg.ha^{-1}$ into peatmoss, Oxisol and Mollisol in 1^{st}, 2^{nd}, and 3^{rd} leachate column trials, respectively. At each trial, 400 g of media were placed inside the Polyvinyl chloride (PVC) columns and deionized water added at half pore volume every week before collecting the leachate. A total of 16 leachate samples were collected over 4 month period and measured for K using a Potassium Ion-Selective Electrode (Vernier Software & Tech, OR). Inductively coupled plasma (ICP) measurements of K were also conducted on randomly selected samples. The K data from the 3 trials and at both low and high K application rates showed strong correlation between

the two buffers measurements with correlation coefficient value ranging from *r* = 0.99-0.99. The two buffers were also well correlated to the unbuffered (original) readings with correlation coefficient value ranging from *r* = 0.95-0.99. This is the first report in the literature of the less expensive NaCl buffer being an effective substitute for $NaClO_{4,}$ allowing for cost effective, high-throughput analysis of soil solution K^+.

4.2 INTRODUCTION

With recent advances in using the ion-selective electrodes (ISEs), nutrients in plant fresh sap such as nitrate (NO_3–N) and potassium (K^+) can be measured directly, rapidly and at low cost. There is also much progress and interest in developing best analytical methods to use ISEs for quick nutrient analysis in soil leachate and soil solutions (F. Di Gioia et al., 2010). In the past, there are many studies where ISEs have been used to measure NO_3-N and K^+ concentrations in soil solution and soil leachate samples, but the results were much inconsistent when compared with spectrometry methods. It was reported in a study that when NO_3-N-ISE measured data were compared with laboratory method, there were different correlation coefficients (r) for different soils. The *r* value ranged from 0.48-0.98 for mineral soils and from 0.07-0.97 for organic soils (Westerveld et al., 2003). There were reports of both good and poor agreement when ISE measured concentrations were compared with reference methods (F. Di Gioia et al., 2010). The NO_3-N concentrations measured in soil solution extracts using ISEs and colorimetric methods showed slightly smaller numerical differences (Onken and Sunderman 1970).

There are studies in the past where the NO_3-N and K^+ measurement from ISEs and reference methods are compared using both correlation and regression analysis. But not many studies have focused on the influence of ionic interferences in these solutions and the accuracy of the measurements. The electroanalytical sensors in these ISEs convert the activity of a specific ion dissolved in a solution into an electrical potential and measured by a voltmeter according to the Nernst equation (Guilbault, 1981). Because most of these soil solutions contain both companion cations and anions, they are electrically neutral. This will affect the chemical activity of other ions and which may ultimately affect the readings of the ISES (Paul and Carlson 1968; Milham et al., 1970). When certain amount of chloride (Cl^-), bicarbonate (HCO_3^-), and sulfates (SO_4^{2-}) were added to solutions containing different ranges of NO3-N concentrations, SO_4^{2-} had least interference in higher NO_3-N concentrations with ISE- NO_3-N readings, whereas at lower NO_3-N concentrations, Cl^- and HCO_3^- had least interference on ISE- NO_3-N readings (Onken and Sunderman, 1970). In a laboratory experiment with nine different K^+ concentrations in solutions of potassium chloride (KCl), potassium nitrate (KNO_3) and potassium sulfate (K_2SO_4), the K^+ readings by K-ISE were very accurate when Cl^- was present in the solution compared to the presence of NO_3^- or SO_4^{2-} (Kallenbach, 2000). However, in other studies it was found that the main interfering ions were reported to be NH_4^+ and Na^+ in the determination of exchangeable K^+ in soil using K-ISE (Wang and Scott, 2001; Brouder et al., 2003). The use of ion strength adjustment (ISA) solution for masking interferences from NH_4^+ and Na^+ in measuring K^+ from K-ISE is currently recommended (Davenport and Jabro, 2001).

Ion-selective electrodes (ISE) have been widely used for determining fluoride since Frant and Ross first constructed a fluoride-ISE (Front and Rose, 1966). Due to the formation of stable fluoroaluminate complexes interference of aluminum has been common in accurate determination of fluoride by ISEs (Siqingaowa Borjigin et al., 2009). Fluoride must be separated from aluminum by steam distillation prior to its determination by ISEs (American Society for Testing and Materials, 2004; and Japanese Industrial Standard, 2003).This difficulty can be overcome to some extent by adding a suitable masking agent to a total ionic strength adjustment buffer (TISAB). Past studies have investigated the efficiency of four kinds of TISABs which have been reported to possess high capabilities of reducing the interference from aluminum (Corbillon et al., 1995). Corbillon et al., 1995 reported that TISAB-IV could be used at a pH of 8.4 to complex aluminum ions. Although TISAB-IV has been adopted by the American Society for Testing and Materials (ASTM), the practical applications of TISAB-IV are currently limited due to a lack of published data (Billington et al., 2004).

Increased precision measurement of K with electrode in soil solutions added with ISABs requires methodologies that use less expensive approaches and that has led to renewed interest in alternatives in commercial expensive ISABs such as $NaClO_{4.}$ However, despite the promise of the technology for rapid, inexpensive soil-K assessment as demonstrated in limited studies, the ISE-K has received little further examination with use and comparison of ISABs for their potential uses in routine soil testing. Their performances have not been comparatively evaluated (Wang et al., 1990). The ionic strength adjustment buffers (ISAB) are added to sampling solutions to eliminate the ionic effects and thereby giving a uniform ionic strength (NICO, 2000). Some of the commercial suppliers of ISE also supply ISABs. These vary in their composition depending on the detected ion and may contain other components which may suppress interfering ions (Brouder et al., 2003). The compositions of these solutions are maintained as trade secret and some of these ISABs are expensive too when using these on regular basis. There are not many studies or efforts in the past in comparing the NaCl as an alternate ISAB to replace the commercial company recommended and high cost $NaClO_{4.}$ The intent of this study was to compare the potential of two ion strength adjustment buffers (sodium perchlorate ($NaClO_4$) and sodium chloride (NaCl) in minimizing ionic interferences in aqueous solutions while measuring K^+ measurements using K- Ion Selective Electrode.

4.3 MATERIALS AND METHODS

Three laboratory trials, each incubated for 16 weeks, were conducted during 2015 and 2016 at the University of Hawaii at Manoa. In the 1st trial, a well dried peatmoss (Sunshine Mix # 4, SunGro, Agawam, MA) was used as media and during the 2nd and 3rd trials, two soils, a Hawaiʻian Mollisol (Waialua soil series) pH 5.7 and Oxisol (Wahiawa soil series) pH of 6.3 were used after air dried and sifted through a 6 mm screen. The Polyvinyl chloride (PVC) columns (cylindrical shape) were constructed with measurements of 8cm diameter and 30cm in length. A 0.5 mm mesh suspended in each column at the bottom on a 3.0cm gravel layer. At each trial, 400 g of media (peatmoss, Oxisol and Mollisol soil) were placed inside the PVC columns with 300 g directly poured into the column and the remaining 100 g media mixed thoroughly with K fertilizer sources (algae and synthetic fertilizers) and added on to the top layer of the 300g media. A total of 5 potassium (K) fertilizer sources selected were 3 algae species (*Kappaphycus alvarezii*, *Eucheuma denticulatum*, and *Gracilaria salicornia*) and 2 synthetic fertilizers (potassium chloride (KCl) and potassium nitrate (KNO_3). There were 3 application rates of K (0, 112 and 336 $kg.ha^{-1}$) and the actual rates added were: 0, 1.56, and 4.7 g of K per incubation column, respectively. The total amount of each fertilizer to provide these actual rates of K varied depending upon each fertilizer's K content (Table 1). Each treatment was replicated 3 times for a total of 33 columns. Two analytical grade reagents, sodium perchlorate ($NaClO_4$) and sodium chloride (NaCl) (Sigma-Aldrich Co) were used to prepare buffer solutions of 2.5 M $NaClO_4$ and NaCl. Deionized water (DI) was added at half pore volume every week before collecting the leachate. A total of 16 leachate samples collected over 4 month period, each sample collected were subdivided into 3 sub-samples: a) unbuffered, b) buffered with 0.5 ml $NaClO_4$, and c) buffered with 0.5 ml NaCl. All the 3 sub-samples from each treatment were measured for K using a Potassium Ion-Selective Electrode (Vernier Software & Tech, OR). The electrode was calibrated using the standard solutions (KCl) of 10 mg/L, 250 mg/L, 500 mg/L and 1000 mg/L before the samples were analyzed and calibration curve was developed and the readings were adjusted using the calibration equation. Random subsamples were also submitted to the University of Hawaii's Agricultural Diagnostic Service Center (ADSC) for quality assurance and were correlated with K measurements from unbuffered and buffered sample solutions.

4.3.1 Statistical analysis: Analysis of variance (ANOVA) on K data measured from two buffers was performed using Statistix 10 (Analytical Software, Tallahassee, FL). Pearson Correlation Coefficient was performed to statistically measure of the strength of linear relationship between the two buffers and two buffers with unbuffered K data using SigmaPlot 13 (San Jose, CA).

4.4 RESULTS AND DISCUSSION

In the 1st leachate trial with peatmoss, the correlation coefficient between the K readings reported by these two ISAB buffers, NaCl and $NaClO_4$ was very strong with *r* =0.99 in both low (112 kg.ha^{-1}) and high (336 kg.ha^{-1}) K application rates (Fig 4.1a) and (4.1d). The relationship between K measurements from the unbuffered samples and $NaClO_4$ added samples was very strong with a correlation coefficient of *r* = 0.99 and 0.99 in both low and high K application rates (Fig 4.1b) and (4.1e). The correlation co-efficient was also stronger when K readings from NaCl added samples were compared with K measurements from unbuffered samples (*r* = 0.99 and 0.99) in both low and high K application rates, respectively (Fig 4.1c) and (Fig 4.1f).

In the 2nd leachate trial with Oxisol soil, the K readings from $NaClO_4$ and NaCl had strong correlation coefficient of r =0.99 and 0.99 in low and high rates, respectively (Fig 4.2a) and (Fig 4.2d). The K readings from these two buffers ($NaClO_4$ and NaCl) had a strong correlation with unbuffered readings with an average r value of 0.96 from both low and high rate K application categories (Fig 4.2b - 4.2f). In the 3rd trial with Mollisol soil, strong correlation co-efficient were observed from K concentrations reported from within two buffers and between the two buffers and unbuffered K readings as seen in the previous 1st and 2nd trials (Fig 4.3a – 4.3f). The correlation co-efficient for all the 3 leachate trials were calculated using the total number of reps and treatments in each high and low K application categories (N=48 for each comparison).

Concentrations of K^+ in aqueous solutions measured after adding two buffers ($NaClO_4$ and NaCl) were highly correlated and the ANOVA analysis showed no significant differences between them in the 1st, 2nd, and 3rd trials. The findings from these 3 leachate column trials showed that these two ISAB buffers ($NaClO_4$ and NaCl) had a significant stronger correlation in their K measurements (r= 0.99). The correlation coefficient was constant and stronger in all the 3 trials and at both high and low K application rates. These findings show that there was hardly any notable difference between the K measurements from adding two buffers, $NaClO_4$ to NaCl. When the K concentrations measured from unbuffered solutions were correlated with two buffers, the correlation coefficient was stronger with an average r value of 0.96; this may be attributed to the similar pattern existing between the readings, although the unbuffered K readings were numerically much higher than the buffered K measurements. The table 4.2 shows that the K measured from buffer $NaClO_4$ were always numerically slightly lower than the K readings from NaCl, whereas the K measured from unbuffered solutions were numerically much higher (almost double) than the K measured from $NaClO_4$ and NaCl (Table 4.2). In the process of quality assurance check, when the ADSC analyzed K numbers were correlated with two buffers $NaClO_4$ to NaCl, they had a significantly strong correlation of r = 0.94 and 0.95, respectively, whereas the unbuffered K with ADSC readings had a correlation of r = 0.86 (Table 4.2). The lower K numbers reported from sample solutions added with these two buffers show that these buffers have significantly contributed to minimize the other ionic interferences in the solutions during the reading.

4.5 CONCLUSIONS

The ion standard adjustment buffer $NaClO_4$ is a standard recommended buffer for accurate K reading in soil solutions. The potential benefit of using less expensive and readily available NaCl as a buffer in routine laboratory tests for K analyses in soil solutions is a possibility. We conclude that both these buffers have significantly reduced the other ionic interference in the soil leachate solutions, although the K readings from $NaClO_4$ were always slightly lower than the NaCl reading, but were both significantly correlated to ADSC measured K values. Further studies to assess the accuracy of this buffer NaCl in different soil nutrient concentrations and at different dilutions are therefore recommended.

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Table 4.1: Amount (grams) of fertilizer provided from each K fertilizer source at each application rate in the 3 leachate column trials.

K fertilizer types	**Actual K per column based on application rates**(Kg. ha^{-1})		**Total amount of fertilizer per column** (Kg. ha^{-1})	
	112	**336**	**112**	**336**
Kappaphycus alvarezii	1.56	4.7	7.8	23.4
Eucheuma denticulatum	1.56	4.7	8.7	26.1
Gracilaria salicornia	1.56	4.7	11.11	33.33
Potassium chloride (KCl)	1.56	4.7	3.0	9.1
Potassium nitrate (KNO_3)	1.56	4.7	3.41	10.21

Table 4.2: Comparing the K concentrations measured from ADSC-ICP analysis with K concentrations measured in unbuffered and buffered sample solutions. (Values in mg/L)

Sample #	**K ICP**	**K Electrode measures**			**Sample #**	**K ICP**	**K Electrode measures**		
		Unbuffered	**$NaClO_4$**	**NaCl**			**Unbuffered**	**$NaClO_4$**	**NaCl**
1	5069	8570	5050	5085	21	248	385	225	230
2	4824	9850	4200	4235	22	2288	4715	1980	1995
3	7027	9880	6950	6940	23	1035	3210	1020	950
4	288	530	310	325	24	6013	9975	5740	5710
5	2987	7200	2980	3035	25	6096	9560	5120	5100
6	3515	7195	3420	3510	26	1998	2250	1250	1310
7	299	565	345	350	27	971	1560	975	1040
8	1095	2450	1150	1195	28	698	1350	650	665
9	3215	5740	3210	3240	29	7836	9540	6545	6555
10	2394	4250	2410	2490	30	8417	9875	6210	6250
11	3151	5750	3210	3280	31	623	1650	610	675
12	2581	6785	2610	2705	32	4052	7610	3150	3210
13	3176	4905	3280	3325	33	3378	7240	3050	3120
14	873	2540	870	950	34	401	920	305	315
15	2994	5065	3210	3295	35	701	1420	665	685
16	4162	6020	4100	4155	36	841	1620	740	780
17	1986	3685	2210	2255	37	788	1845	785	820
18	93	405	105	120	38	422	3510	1355	1400
19	2624	5750	3210	3280	39	384	955	325	360
20	1385	2980	1450	1475	40	2315	5610	2165	2190
	Correlation coefficient ‘*r*’ values						**0.8688**	**0.9435**	**0.9581**

*ADSC measured K was correlated to unbuffered, $NaClO_4$, and NaCl measured K respectively.

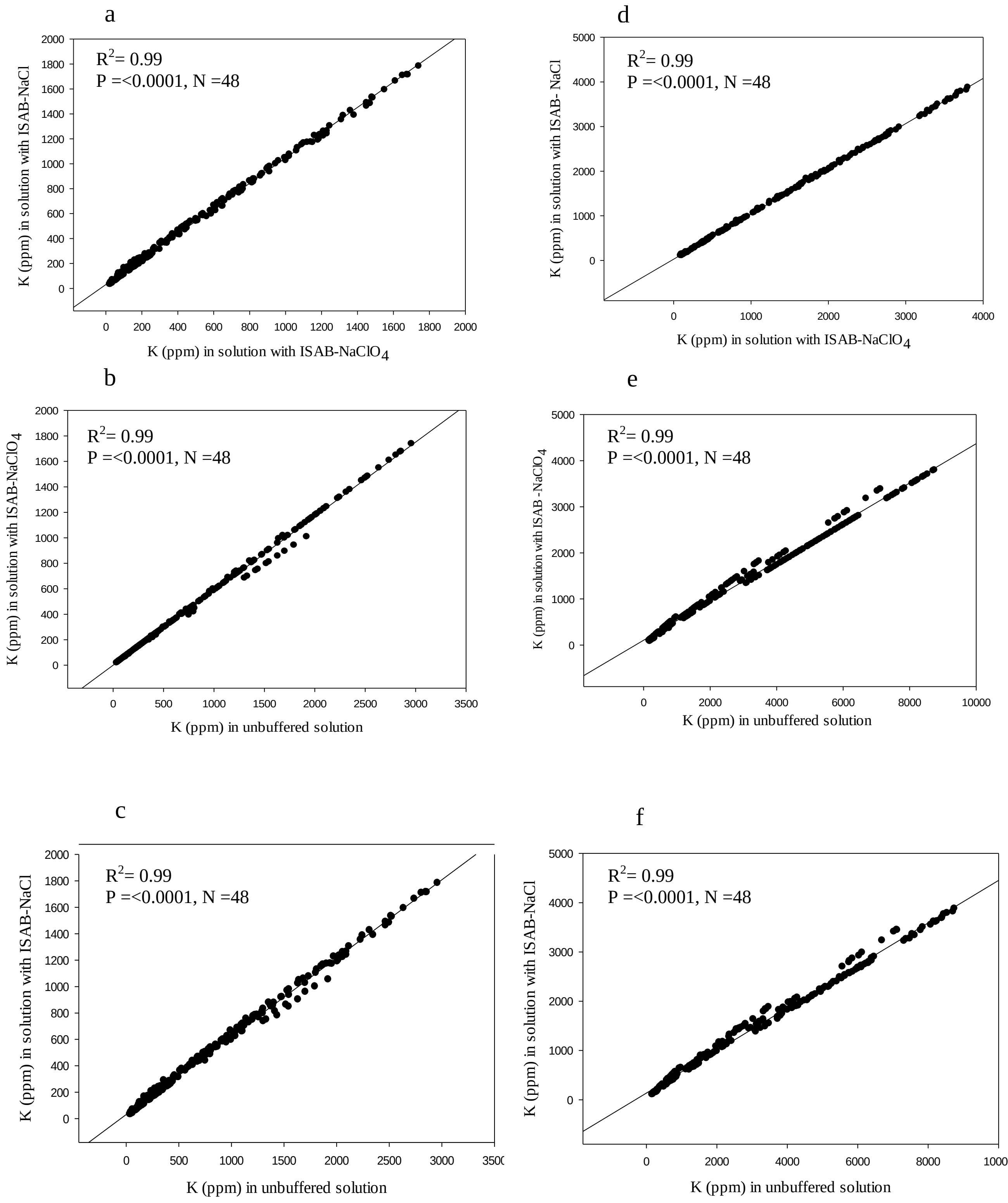
a
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB-NaCl
K (ppm) in solution with ISAB-NaClO4
0 200 400 600 800 1000 1200 1400 1600 1800 2000
d
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB- NaCl
K (ppm) in solution with ISAB-NaClO4
0 1000 2000 3000 4000 5000
b
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB-NaClO4
K (ppm) in unbuffered solution
0 500 1000 1500 2000 2500 3000 3500
e
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB -NaClO4
K (ppm) in unbuffered solution
0 2000 4000 6000 8000 10000
c
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB-NaCl
K (ppm) in unbuffered solution
0 500 1000 1500 2000 2500 3000 3500
f
R2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB-NaCl
K (ppm) in unbuffered solution
0 2000 4000 6000 8000 10000

Fig 4.1(A-F)

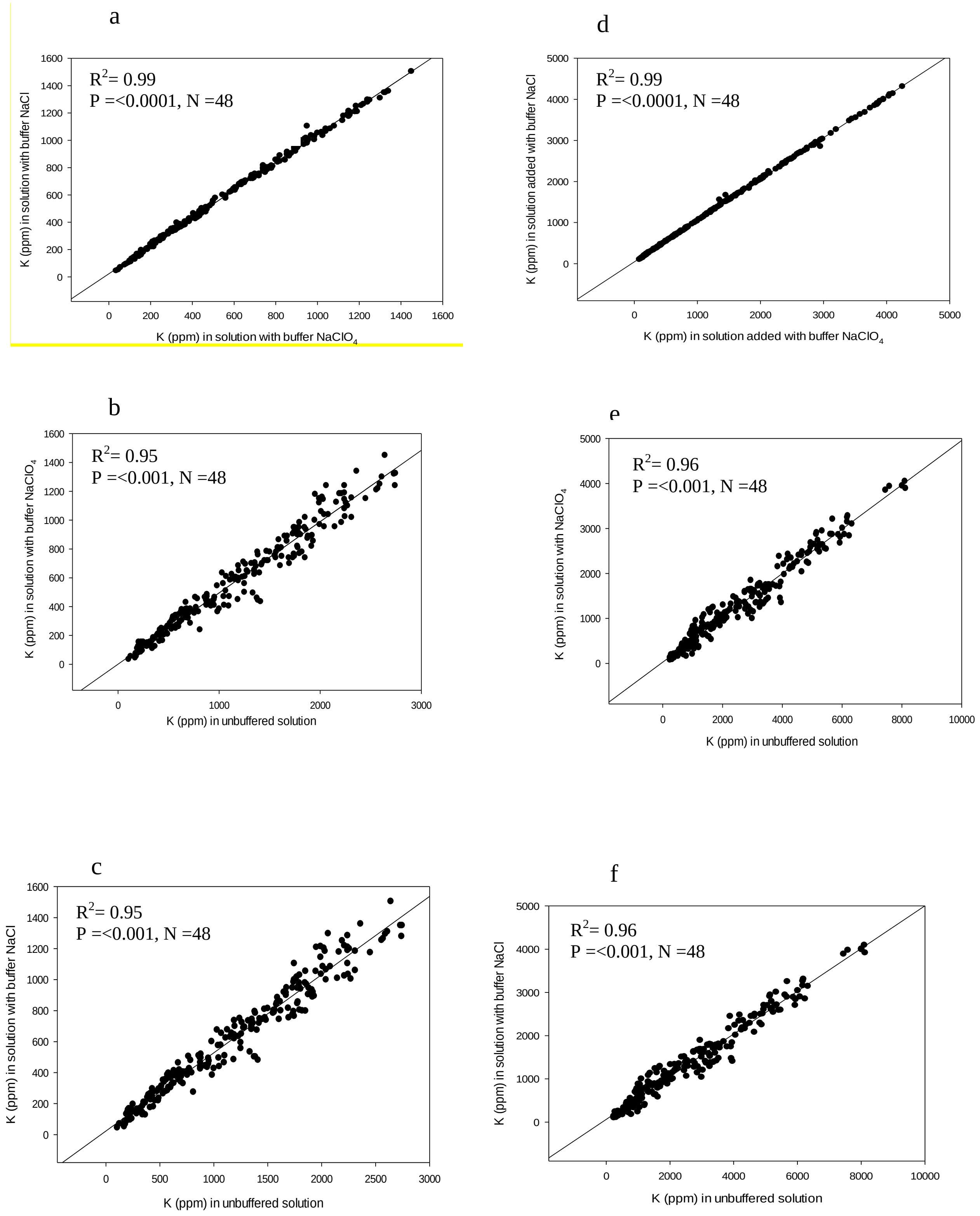
a
R^2= 0.99
P =<0.0001, N =48
K (ppm) in solution with buffer NaCl
K (ppm) in solution with buffer NaClO$_4$
0 200 400 600 800 1000 1200 1400 1600
d
R^2= 0.99
P =<0.0001, N =48
K (ppm) in solution added with buffer NaCl
K (ppm) in solution added with buffer NaClO$_4$
0 1000 2000 3000 4000 5000
b
R^2= 0.95
P =<0.001, N =48
K (ppm) in solution with buffer NaClO$_4$
K (ppm) in unbuffered solution
0 1000 2000 3000
e
R^2= 0.96
P =<0.001, N =48
K (ppm) in solution with NaClO$_4$
K (ppm) in unbuffered solution
0 2000 4000 6000 8000 10000
c
R^2= 0.95
P =<0.001, N =48
K (ppm) in solution with buffer NaCl
K (ppm) in unbuffered solution
0 500 1000 1500 2000 2500 3000
f
R^2= 0.96
P =<0.001, N =48
K (ppm) in solution with buffer NaCl
K (ppm) in unbuffered solution
0 2000 4000 6000 8000 10000

Fig 4.2 (A-F)

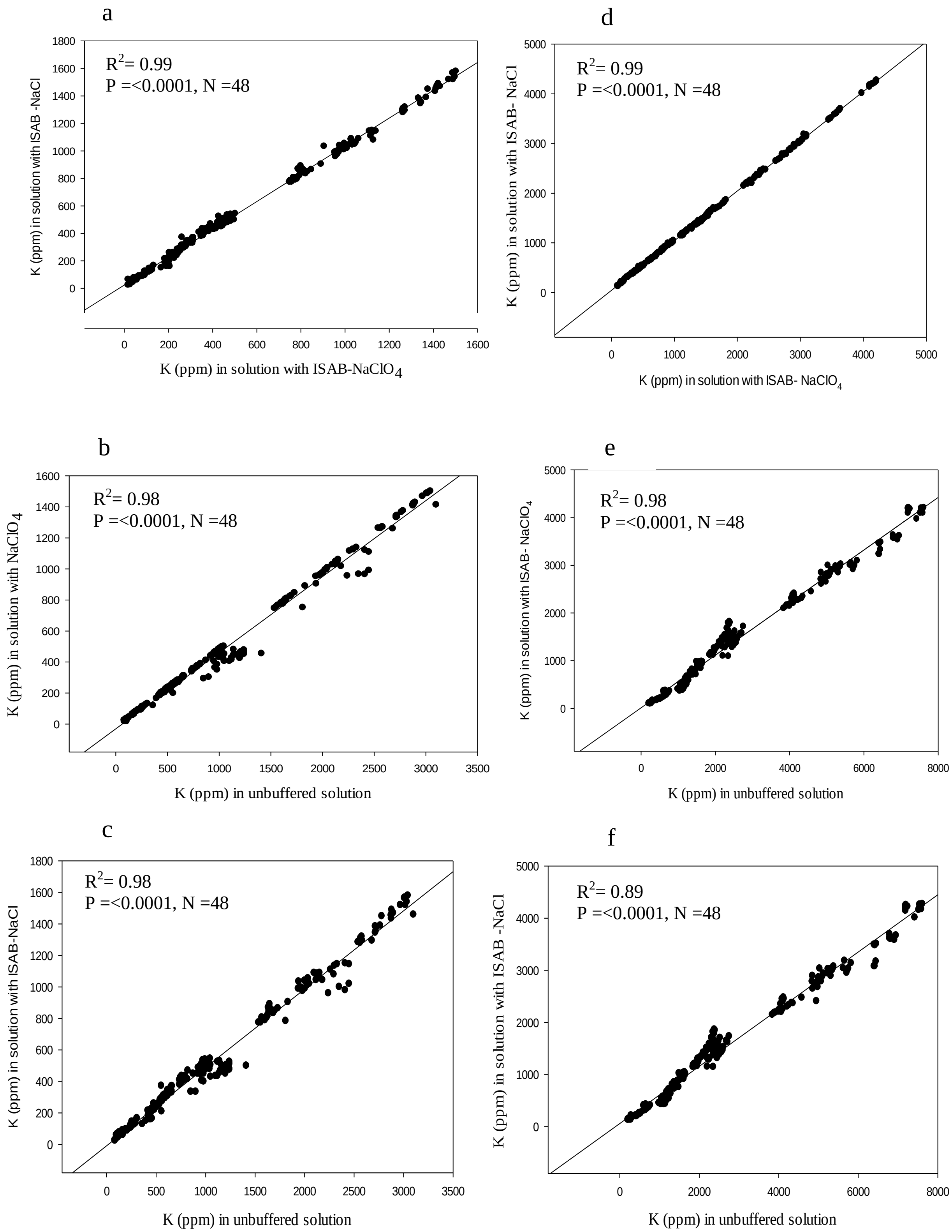
a
R^2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB -NaCl
K (ppm) in solution with ISAB-NaClO$_4$
1800
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
1200
1400
1600
d
R^2= 0.99
P =<0.0001, N =48
K (ppm) in solution with ISAB- NaCl
K (ppm) in solution with ISAB- NaClO$_4$
5000
4000
3000
2000
1000
0
0
1000
2000
3000
4000
5000
b
R^2= 0.98
P =<0.0001, N =48
K (ppm) in solution with NaClO$_4$
K (ppm) in unbuffered solution
1600
1400
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
3000
3500
e
R^2= 0.98
P =<0.0001, N =48
K (ppm) in solution with ISAB- NaClO$_4$
K (ppm) in unbuffered solution
5000
4000
3000
2000
1000
0
0
2000
4000
6000
8000
c
R^2= 0.98
P =<0.0001, N =48
K (ppm) in solution with ISAB-NaCl
K (ppm) in unbuffered solution
1800
1600
1400
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
3000
3500
f
R^2= 0.89
P =<0.0001, N =48
K (ppm) in solution with ISAB -NaCl
K (ppm) in unbuffered solution
5000
4000
3000
2000
1000
0
0
2000
4000
6000
8000

Fig. 4.3 (A-F)

CHAPTER 5

Potassium Release kinetics from Three Invasive Algae mixed with Different Media in Leachate Column Studies

5.1 ABSTRACT

Understanding the nutrient release pattern from organic residues and fertilizers will help better manage nutrient efficiency in agricultural crop production, specifically, in organic production. The two objectives of this work were to: 1) study the pattern of potassium (K) release from three invasive red algae (*Eucheuma denticulatum, Gracilaria salicornia, and Kappaphycus alvarezii*) as compared with synthetic potassium fertilizers (potassium chloride (KCl) and potassium nitrate (KNO_3), and 2) better understand potential mechanisms underlying differences in K release among the materials. Three leachate column studies were undertaken in the laboratory with three media, peat moss, Oxisol, and Mollisol soils. Columns were leached over 16 weeks with one leaching every week. The experimental design consisted of five K fertilizers with three replicates per treatment, including controls. In all the three studies, two rates of K (112 and 336 $kg \cdot ha^{-1}$) were provided through three algae as well as KNO_3 and KCl were placed into the upper part of the soil, within the first 3 cm. De-ionized water was percolated through the columns every week and the collected leachates were analyzed for K^+ concentration using a K^+ ion selective electrode. Results from the three studies showed the amount of K^+ released was much higher in the leachate treated with synthetic K in the first 6 weeks (4100-4200 mg/L) and sharply declined at later weeks. In contrast, the release of K^+ from algae treated leachate was slower in the first three weeks and optimized at 8-9 weeks (2800-3400 mg/L). Rates steadily declined over subsequent weeks. Algae samples did not perform similarly however. Release of K from *G. salicornia* was consistently lower than from *Eucheuma denticulatum* and *Kappaphycus alvarezii* in all studies.

The amount of K released as a percentage of the total applied was in the order of KCl (81%) > KNO_3 (80%) > *K. alvarezii* (71%) >*E. denticulatum* (70%), > *G. salicornia* (62%), respectively. To better understand the slow release of K from *G. salicornia*, a leachate study was conducted with Oxisol soil and dried gels of agar extracted from *G. salicornia* and carrageenan from *K. alvarezii* at two rates. A total of 12 leachate samples collected over 6 weeks, measured for K. Cumulative recovery of K from agar was significantly lower (10-12%) than from carrageenan . We conclude that the rate and total amount of K released from algae was lower than synthetic K fertilizers applied at equivalent rates of K, and that differences in release among algal species may be explained in part by differences in cell wall composition.

5.2 INTRODUCTION

Organic residues or plant biomass play a vital role in maintaining soil fertility; and knowledge of the nutrient release from these organic residues will help optimize nutrient efficiency in crop production (Villegas-Pangga et al., 2000). Present agricultural production depends heavily on use of chemical fertilizers and pesticides whereas the consumers' demand for organic foods produced with reduced chemical use is increasing. Meeting this demand requires efficient use of crop inputs (Owen et al., 2015). The total nutrient contents present in plant biomass or organic residues will impact on the possible enrichment of the soil with nutrients, but the uptake of nutrients by plants depends on the rate at which these are available to roots (Nishanth and Biswas, 2008). The decomposition and nutrient release of plant biomass and their residues are time-dependent and related to chemical composition of organic residues (Berendse et al., 1987; Comelissen 1996; Aerts and De Caluwe, 1997). It is important to know and understand the nutrient release patterns of organic based plant residues as it helps in optimizing nutrient efficiency in agricultural crop production systems (Mafongoya et al., 1998). The decomposition process may be influenced by many factors, such as temperature, moisture, and type of residue, and its chemical compositions (Palm and Sanchez, 1991; Porter et al., 1997). The rates of decomposition and mineralization of plant materials depend to a large extent on the chemical composition of the plant tissues and also to their structural and chemical characteristics (Palm et al., 2001). The rate of decomposition and nutrient release of an organic residue depends on its chemical and biochemical characteristics and some past studies have shown that K is easily leached from the organic residues at the initial stage of release. Many researchers have indicated that K is leached quickly from decomposing residues (Kolahchi and Jalali, 2012).

Potassium (K) is an essential element in plant growth, and K^+ uptake and efflux affect plant productivity that influences both yield and quality of the crop (Lebaudy et al., 2007). K is taken up as much as N by many crops, so its supply is critical. Often, the ability of many soils to supply adequate amounts of K for many years leads to its under-application (Johnston et al., 2001). The supply of acceptable forms of K to organic systems is a particular problem (Watson et al., 2004).With increasing costs of inorganic fertilizer, the use of seaweed application may be an alternative source of soil fertility management for sustainable crop production, particularly, in coastal areas (Wosnitza and Barrantes 2005). There is a growing trend in recent years on low-input agriculture and use of locally available inputs to increase the production efficiency and for ensuring the sustainability of agriculture and to improve yields without compromising environmental integrity. One such local input in Hawaii is harvested invasive algae biomass which has high K content (14-20%) in the tissue analysis (Radovich et al., 2012). It is still unclear whether the 'slow release' of nutrients from organic compost or residues can be well managed to match crop demand with nutrient supply to increase nutrient-use efficiency in crop production (Drinkwater et al., 1998). More research on improving efficiency and minimizing loses from both inorganic and organic nutrient sources are needed. However, there is very little scientific information available in literature regarding the nutrient release pattern of K from these algae species.

Different Rhodophyta contain a wide group of polysaccharides whose chemical composition and amount vary among species (Rhein-Knudsen et al., 2015). Red algae are known to synthesize unique sulfated galactans, such as carrageenans which function in complex composite cell walls made of cellulose and the extensive matrix polysaccharides. Commercial sources of carrageenans are mainly from large plants native to warm water regions, *Kappaphycus*, *Eucheuma*. Carrageenans are made up of repeating disaccharides of D-galactopyranose units bound together with alternating alpha-1, 3 and beta-1, 4 linkages (De Ruiter and Rudolph, 1997). A second polysaccharide, agar is largely from cooler water species of *Gelidium* and *Gracilaria* (McHugh, 1987). Agar is made up of galactopyranose dimers, connected by alternating alpha-1, 3 and beta-1, 4 linkages, but with the important difference that in agar the 3,6-anhydro-a-galactopyranose units are in the L configuration and not in the D-configuration as is the case for carrageenan (Usov, 2011). A comparison of the physical-chemical properties of agar and carrageenan shows that the gel strength of agar is 2–10 times higher than that of carrageenan, and that the melting point of agar is close to the boiling point of water, whereas the melting point of a carrageenan gel is 50–70 °C (Rhein-Knudsen et al., 2015). The levels and extent of structural differences vary in different red seaweed species, and this variation obviously affects the functional properties of the hydrocolloids (Rhein-Knudsen et al., 2015). Our hypothesis is that there would be differences in K^+ release among the different fertilizer materials. A series of leachate column experiments were conducted to provide an overview of the K nutrient release pattern from these species and explore potential reasons for these differences.

5.3 MATERIALS AND METHODS

5.3.1 Invasive Algae Leachate Trials. Three column experiments were conducted during 2015-2016 in the Sustainable and Organic Farming Systems Laboratory, Department of Tropical Plant and Soil Sciences, University of Hawaii, Manoa. Each column experiment was leached over a period of 16 weeks between temperatures ranging from 20 to 28 °C in the laboratory. In the 1st trial, a well dried peatmoss (Sunshine Mix # 4, SunGro, Agawam, MA) was used as media and during the 2nd and 3rd trials, two soils, a Hawaiian Mollisol (Waialua soil series) pH 5.7 and Oxisol (Wahiawa soil series) pH of 6.3 were used after air dried and sifted through a 6 mm screen. The polyvinyl chloride (PVC) columns were constructed with measurements of 8 cm diameter 30 cm in length. A 0.5 mm mesh suspended in each column at the bottom on a 3.0 cm gravel layer. At each trial, 400 g of media (peatmoss, Oxisol and Mollisol soils) were placed inside the PVC columns with 300 g directly poured into the column and the remaining 100 g media mixed thoroughly with K fertilizer sources (algae and synthetic fertilizers) was added as a top layer. A total of five K fertilizer sources selected for this experiment. There were three algae of *Eucheuma denticulatum, Gracilaria salicornia, Kappaphycus alvarezii*, and 2 synthetic fertilizers, potassium chloride KCl and potassium nitrate (KNO_3). There were three application rates of K based on 0, 112 and 336 $kg.ha^{-1}$ and the actual K amounts added were: 0, 1.56 and 4.7 g. The total amount of each fertilizer to provide these actual rates of K varied depending upon each fertilizer's K content (Table 1). There were 3 replicates for each treatment and altogether there were 33 columns per run.

5.3.2 Agar and Carrageenan Leachate Trials the experiments started on November 15, 2016 as before using PVC columns and a study period of 6 weeks with temperatures ranging from 20 to 22 °C in the laboratory. Fine powders of Agar extracted from *Gracilaria* (Modernist Pantry, York, Maine) and Kappa carrageenan extracted from *Kappaphycus* (Landor Trading Co, USA) were used in this experiment. The actual agar amounts added were: 0, 4.49 and 13.33 g and actual carrageenan amounts were 0, 3.1 and 9.5 g per each treatment rate. Gels were prepared by using actual amounts of agar and carrageenan powders diluted in 200 ml of DI water along with K provided through 2 rates of 112 and 336 kg.ha^{-1}(the actual K amounts were 1.56 and 4.7 g and actual KCl added was 3.0 g and 9.1 g) and heated on a hot plate at low to medium flame till the solution starts boiling. Later the gel cubes were cooled for two hours and poured in to separate glass beakers and dried in oven in 50-60 C for 72 hours. Dried gels ground to fine powders before mixed with soil media. Dried 400 g of Oxisol (Wahiawa soil series) with pH of 6.3 were placed inside the PVC columns with 300 g directly poured into the column and the remaining 100 g soil mixed thoroughly with agar and carrageenan dried gel powders and added on to the top layer of the 300 g media.

5.3.3 Collection and Analyses of Leachate DI water was added at half pore volume (80 ml with 40% pore spacing) to each column every week before collecting the leachate. A Total of 16 leachate samples collected over 3 month period. A standard buffer sodium perchlorate ($NaClO_4$) of 0.5 ml from (Sigma-Aldrich Co) was added to each collected leachate sample to mask any other ionic interference in the analyte solution and to increase the accuracy of the reading. A total-ionic strength adjustment buffer (TISAB) like $NaClO_4$ is used to adjust samples and standards to the same ionic strength; this allows the concentration, rather than the activity, to be measured directly with the electrode. Then the samples were measured for K using a Potassium Ion-Selective Electrode (Vernier Software & Tech, OR). The electrode was calibrated using the standard solutions (KCl) of 10 mg/L, 250 mg/L, 500 mg/L and 1000 mg/L before the samples were analyzed and calibration curve was developed and the readings were adjusted using the calibration equation. Random subsamples were also submitted to the University of Hawaii's Agricultural Diagnostic Service Center (ADSC) for quality assurance as a check to measure the accuracy of the K measured from samples with using ISABs. The leachate collection with agar and carrageenan experiment were similar to the above except the leachate were collected twice a week to the total of 12 leachate samples collected over 6 weeks period.

5.3.4 Statistical analysis the measured K data from leachate solutions from 16 weeks were subjected to ANOVA for repeated measures modeling with PROC MIXED–SAS. Means separation was conducted using the significant difference test of Tukey–Kramer.

5.4 RESULTS AND DISCUSSION

5.4.1 Algae Leachate Column Trials: Mean concentrations of K (mg/L) released from invasive algae and synthetic K fertilizersin Peat moss, Oxisol, and Mollisol soils in weekly eluate over 16 week period at two application rates (kg.ha^{-1}) are shown in Table 2. The pattern of K release (mg/L) per week from five fertilizers in three media types with K provided at two application rates over 16 week period are shown in Fig 1(A-B), 2(A-B) and 3(A-B).

In peat moss media with K added at 112 kg.ha^{-1}, the cumulative recovery was 77.8% from KCl, 76.7% from KNO_3, 62.15% from *K.alavrezii*, 61.5% from *E.denticulatum* and 55.4% from *G.salicornia* whereas at K at 336 kg.ha^{-1} the cumulative recovery was 90.10% from KCl, 89.5% from KNO_3, 72.97% from *K.alavrezii*, 71.6% from *E.denticulatum* and 62.11% from *G.salicornia* (Fig 4-A).

In Oxisol soil, with K added at 112 kg.ha^{-1}, the cumulative recovery was 84.4% from KCl, 82.8% from KNO_3, 70.6% from *K.alavrezii*, 70.9% from *E.denticulatum* and 61.08% from *G.salicornia* whereas at K at 336 kg.ha^{-1}, the cumulative recovery was 87.6% from KCl, 86.5%

from KNO_3, 71.56% from *K.alavrezii*, 70.4% from *E.denticulatum* and 60.7% from *G.salicornia* (Fig 4-B). In Mollisol soils with K added at 112 $kg.ha^{-1}$, the cumulative recovery was 81.56% from KCl, 80.8% from KNO_3, 68.2% from *K.alavrezii*, 67.9% from *E.denticulatum* and 58.3% from *G.salicornia* whereas at K at 336 $kg.ha^{-1}$, the cumulative recovery was 93.7% from KCl, 92.4% from KNO_3, 81.8% from *K.alavrezii*, 80.9% from *E.denticulatum* and 65.18% from *G.salicornia* (Fig 4-C).

The ANOVA shows the fertilizers, rates, weeks, and Fert*week interactions were all significant for both low and high rates in all the three experiments ($p<0.0001$). The K release pattern or the measured recovery of K was similar among the synthetic fertilizers in all the three leachate column experiments. Two synthetic fertilizers KCl and KNO_3 had a cumulative recovery of K at an average of 76% in the first eight weeks, while an average 24% was recovered in the last eight weeks from the three experiments in both K application rates. The cumulative K release from invasive algae species of *K.alvarezii* and *E.denticulatum* were similar in all the three experiments but were lower than KCl and KNO_3 with difference ranging from 11-17% in both K application rates. The K release pattern of invasive algae species of *K.alvarezii* and *E.denticulatum* were similar in all the three experiments with cumulative K recovered in the first eight weeks had an average of 60-63 % and the rest was recovered in the last 8 weeks from the three experiments in both K application rates. The cumulative K release from *G. salicornia* species was significantly different from*K.alvarezii* and *E.denticulatum* species and was consistently lower than these in all the three experiments with an average difference of 13 % at 112 $kg.ha^{-1}$ rate and 19 % at 336 $kg.ha^{-1}$rates. The cumulative recovery of K from *G.salicornia* after eight weeks had an average of 47-50%, with the remaining K recovered at the last eight weeks of consistently in the three experiments. The overall cumulative recovery of K from

G.salicornia was consistently lower than *K. alvarezii*, *E. denticulatum* in all the 3 experiments and at both application rates. In the experiments with peat moss as media, the differences of least square means in high rate category shows KCl and KNO_3 had highest K release with mean of 1961 and 1948 mg/L respectively and were not significant from each other, but were significant from other algae fertilizers *Kappaphycus* (1596 mg/L) *Eucheuma* (1567 mg/L) and *Gracilaria* (1352 mg/L). But, *Kappaphycus* and *Eucheuma* were significant from *Gracilaria*. Similar trend was observed in low rates, with KCl (700 mg/L) and KNO_3 (691 mg/L) not significantly different from each other, but were different from all other fertilizers; *Kappaphycus* (555 mg/L) and *Eucheuma* (546 mg/L) were not different from each other but different from *Gracilaria* (499 mg/L). The least square means of K release from two synthetic fertilizers (KCl and KNO_3) in all the three experiments was significantly higher than the three algae species (Table 2). Among the three algae, *K.alvarezii* and *E.denticulatum* were significantly different from *G.salicornia* consistently in the three experiments (Table 2). The K recovery pattern in both low and high was similar in KCl and KNO_3, as K recovery was high in the first 5-7 weeks with both low and high rates and then going down quickly, maxing out at 7th week. The outcomes of *K. alvarezii* and *E.denticulatum* were similar in release pattern at both low and high rates with the numbers going up from 3rd week till reaching optimum at 7-8 weeks, but the K recovery was lower as compared to the synthetic fertilizers. Thus it can be suggested that invasive alga supply K to crops in the long term, whereas KCl and KNO_3 supply K in the short term. The synthetic fertilizers observed a rapid release of K during the initial weeks of leaching as compared to the algae species.

5.4.2 Agar and Carrageenan Leachate Trial: Average K (mg/L) released from agar and carrageenan at two application rates ($kg.ha^{-1}$) from 12 measurements over a six week period is shown in Table 3. The K release pattern and averegare (mg/L) per week from agar, carrageenan and control (KCl) in Oxisol soil with K provided at two application rates over six week period are shown in Fig 5(A-B). The cumulative potassium release from *Gracilaria* agar and *Kappa* carrageenan in Oxisol soil at two application rates over a period of six weeks are shown in Fig 6(A-B). When dried agar gel powder was added to Oxisol soils with K added at 112 $kg.ha^{-1}$, the cumulative recovery of potassium was 20.87% whereas carrageen recovery was at 30.72% and in the control treatment with no agar or carrageenan added the cumulative recovery was at 50.16%. When K was added at 336 $kg.ha^{-1}$, the cumulative recovery was 19.78% from agar, 29.31% from carrageenan, and 49.25% in control Fig 6 (A-B).

In the leachate experiment with dried gels of agar extracted from *G. salicornia* and kappa carrageenan from *K. alvarezii*, the ANOVA shows the two treatments of agar and carrageenan gel powders and weeks were highly significant when data was analyzed for both low and high K application rates ($p<0.0001$). The cumulative recovery of K from *Gracilaria* agar was significantly different from Kappa carrageenan and was lower with a difference of 10-12% in both K application rates. The K recovery pattern of agar (*G. salicornia) and carrageenan (K. alvarezii*) in both low and high were much similar to the pattern observed in the leachate column studies in Oxisol soil as media. This supports our findings from the leachate column experiments where the K release from *Gracilaria salicornia* species was significantly different and lower in all the three experiments as compared with *K. alvarezii* and *E. denticulatum* species. The lower K release from *Gracilaria* species may be attributed to several reasons; differences are seen in physical properties which include gel strength, boiling temperature, and viscosity and in chemical properties such as structure of polysaccharides and cell wall composition.

The consistent results from experiments with whole plant algae species of *G.salicornia* and *K.alvarezii* and from extracted products from these algae species connects us the cell wall chemistry of these species which might be causing the differences in K release among these species. Agar occurs as structural carbohydrate in the cell walls of red algae and is a complex mixture of polysaccharides composed of two major fractions - agarose, a neutral polymer, and agaropectin, a charged, sulfated polymer. Agars are usually composed of α-1, 3 linked D-galactose and β-1, and 4 linked 3, 6-anhydro-L-galactose units (Arvizu-Higuera et al., 2008). The backbone structure of carrageenan polysaccharide is based on linear chains of repeating galactose units in D configuration (D-sugar) and 3, 6-anhydro-galactose copolymer, joined by alternating α-(1→3) and β-(1→4) linkages, containing 15%–40% ester sulfate (Percival E. 1979). The major difference between agars and carrageenan is that the agar contains D and L galactose units whereas the carrageenan consists entirely of the D-sugar. Besides being substituted by half ester sulphate groups some of the galactose units in all these polysaccharides are present as 3, 6-anhydrogalactose (Percival 1979).

The polysaccharides of different genera vary in the proportions and site of the sulphate groups and the amount of 3, 6-anhydrogalactose units. In agars, an actual agarose contains a significant amount of the sulphated form, sometimes referred to as agarose sulphate, and sometimes agaropectin (Stanley, 2006; Usov, 2011; and Usov and Zelinsky, 2013).The location and its sulphate content affect the shape and structure of agar-polysaccharides and the interaction with other biomolecules, and thus may influence other properties (Fidelis et al., 2014).The shape of the macromolecules with twofold helices, determines the physical properties and they may in be affected by the point of attachment of the sulphate groups and as well with the ratio of 3,6-anhydrogalactose in the molecules (Percival, 1978b).

When the physical-chemical properties of agar and carrageenan were compared, it showed that the gel strength of agar is 2–10 times higher than that of carrageenan, and that the melting point of agar is close to the boiling point of water, whereas the melting point of a carrageenan gel is 50–70 °C (Rhein-Knudsen et al., 2015).

Since, the structure is different in each agar polysaccharide depending on the species and the extraction methods, most papers pointed out the necessity for further in-depth studies. The position of sulfate groups in these species chemical structure is so important, but with limited knowledge about the topic, a great deal of research is still required to fully understand these properties. In general agar is a complex mixture of polysaccharides composed of a charged, sulfated polymer, whereas the carrageenan are polysaccharides made up of both sulfated and nonsulfated groups, mainly ester sulfate group. It was observed in a study that the higher sulfate content in the extracted agar samples was due to the probable presence of sulfate in other positions in addition to 6-position of L-galactose (Marinho-Soriano, E. 2001). The high sulfate content in red alga *Melanothamnus somalensis* samples was due to different environmental conditions at the area of seaweed collection especially under elevated temperatures (27-28 °C) which might have made the agar to contain more sulfates and less 3,6 AG (Heydari, et al., 2014 and MODIS 2013). In previous study in glycosaminoglycans, where binding of calcium to sulfated polysaccharides was studied, it was observed that the sulfate groups are capable of binding calcium with a stronger affinity than expected for simple salt formation (Hunter et al., 1988). The higher amount of K release from kappa carrageenan with higher ester sulfate content may have to do with its lower binding and chelating properties as compared to sulfated polymers present in agar which may have the higher ability to bind cat ions such as K^+ and causing the slow release of potassium from agar as compared to carrageenan. However, further studies are needed to explore the amount of sulfate in *G.salicornia* and *K.alvarezii*, which affects the binding properties and thereby showing differences in the release of K^+ ions.

5.5 CONCLUSIONS

The results from the leachate column studies with algae whole plant and their polysaccharides show that there is a difference among the species with respect to K release. Agar (*G.salicornia*) and carrageenan (*K.alva*rezii), have complex hybrid galactans in their composition.The physical (gel strength), chemical (sulfate groups) properties and polysaccharides structure (Sugar units and α and ß linkages) are the major factors for lower K release from *G.salicornia.*The findings strongly support our hypothesis that *G.salicornia* is different from and *K.alva*rezii in the K release pattern. The K release pattern and the difference among the species can be better understood with more knowledge on the structural and bio-chemical properties of these polysaccharides which not only improves the efficiency of application, but also reduces the probability of nutrient leaching and loss to the environment.

However, to take the maximum benefit from the potential of these materials, there is a strong need to dedicate research to a deeper knowledge on the cell wall chemistry of the polysaccharides of these species and at different locations and temperature, which will help us to better understand the mechanism and the functions associated with it. More importantly, the uncertainty about the cell wall structures and their differences in the same genera and species harvested from different locations around the globe are definitely the strongest limitations. Our research on the three invasive alga species can be used as a modelby other researchers with the potential to expand the research to explore other invasive speciesin Hawaiian shores and elsewhere as a source of K nutrient. However, the local and state regulations, environmental concerns and other factors may affect the availability of invasive algae in huge quantities for larger scale commercial applications.

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LIST OF TABLES:

Table 5.1 Amount (grams) of K fertilizer provided from each fertilizer source at each application rate (kg. ha^{-1}) in the 3 leachate column trials.

K fertilizer types	**Actual K (g) per column based on application rates**		**Total amount of each fertilizer (g) per column**	
	112	**336**	**112**	**336**
Kappaphycus alvarezii	1.56	4.7	7.8	23.4
Eucheuma denticulatum	1.56	4.7	8.7	26.1
Gracilaria salicornia	1.56	4.7	11.11	33.33
Potassium chloride(KCl)	1.56	4.7	3.0	9.1
Potassium nitrate(KNO_3)	1.56	4.7	3.41	10.21

Table 5.2 Mean concentrations of K (mg/L) released from invasive algae and synthetic K fertilizers in Peat moss, Oxisol, and Mollisol soils in weekly eluate over 16 week period at two application rates (kg.ha^{-1}).

K fertilizer source	**Peat moss**		**Oxisol**		**Mollisol**	
	112	**336**	**112**	**336**	**112**	**336**
Kappaphycus alvarezii	555^b	1596^b	636^b	1566^b	614^b	1791^b
Eucheuma denticulatum	546^b	1567^b	638^b	1531^b	531^b	1774^b
Gracilaria salicornia	499^c	1352^c	565^c	1301^c	430^c	1401^c
Potassium chloride (KCl)	700^a	1961^a	759^a	1922^a	734^a	2050^a
Potassium nitrate(KNO_3)	691^a	1948^a	742^a	1901^a	728^a	2021^a
Fertilizer types	***	***	***	***	***	***
Weeks	***	***	***	***	***	***
Fert. Type * Weeks	***	***	***	***	***	***

[z] Least square means followed by the same letter are not significantly different ($P < 0.05$) within each application rate based on Tukey-Kramer t test.

Mean values represent 3 replicates from each treatment measured at weekly interval (N= 48).

Table 5.3 Mean concentrations of K (mg/L) released from agar and carrageenan at two application rates (kg.ha^{-1}) from 12 measurements over 6 week period.

Types of algae gel	**K rate provided to Oxisol**	
	112	**336**
Gracilaria Agar	469^{c}	999^{c}
Kappa Carrageenan	691^{b}	1473^{b}
Control	1068^{a}	2452^{a}
Gel types	***	***
Weeks	***	***
Gel Types * Weeks	***	***

[z] Least square means followed by the same letter are not significantly different ($P< 0.05$) within each application rate based on Tukey-Kramer t test.
Mean values represent 5 replicates from each treatment measured at weekly interval (N= 60).

LIST OF FIGURES:

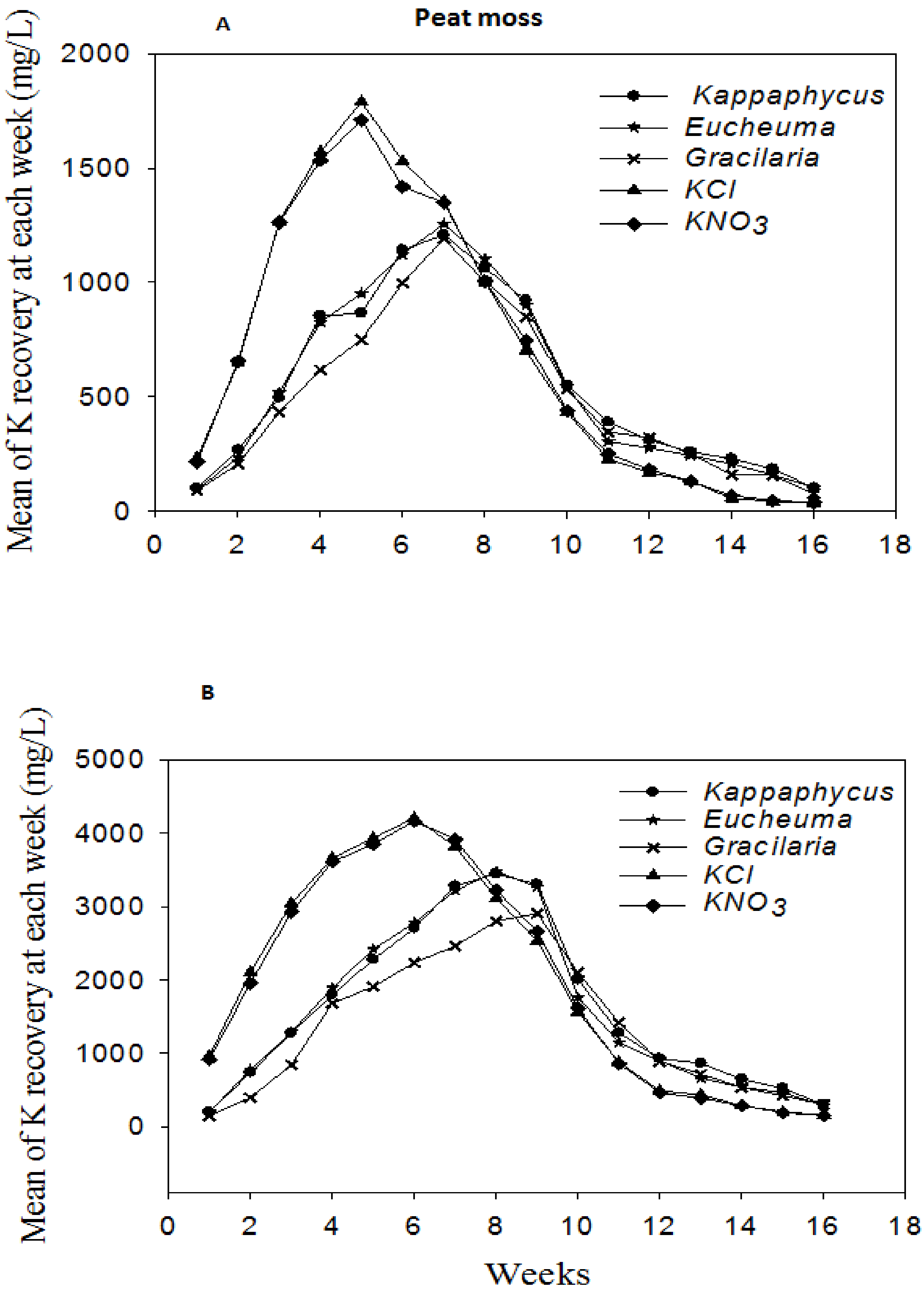
A
Peat moss
Mean of K recovery at each week (mg/L)
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
Kappaphycus
Eucheuma
Gracilaria
KCl
KNO_3
B
Mean of K recovery at each week (mg/L)
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
16
18
Weeks
Kappaphycus
Eucheuma
Gracilaria
KCl
KNO_3

Fig 5.1 (A-B)

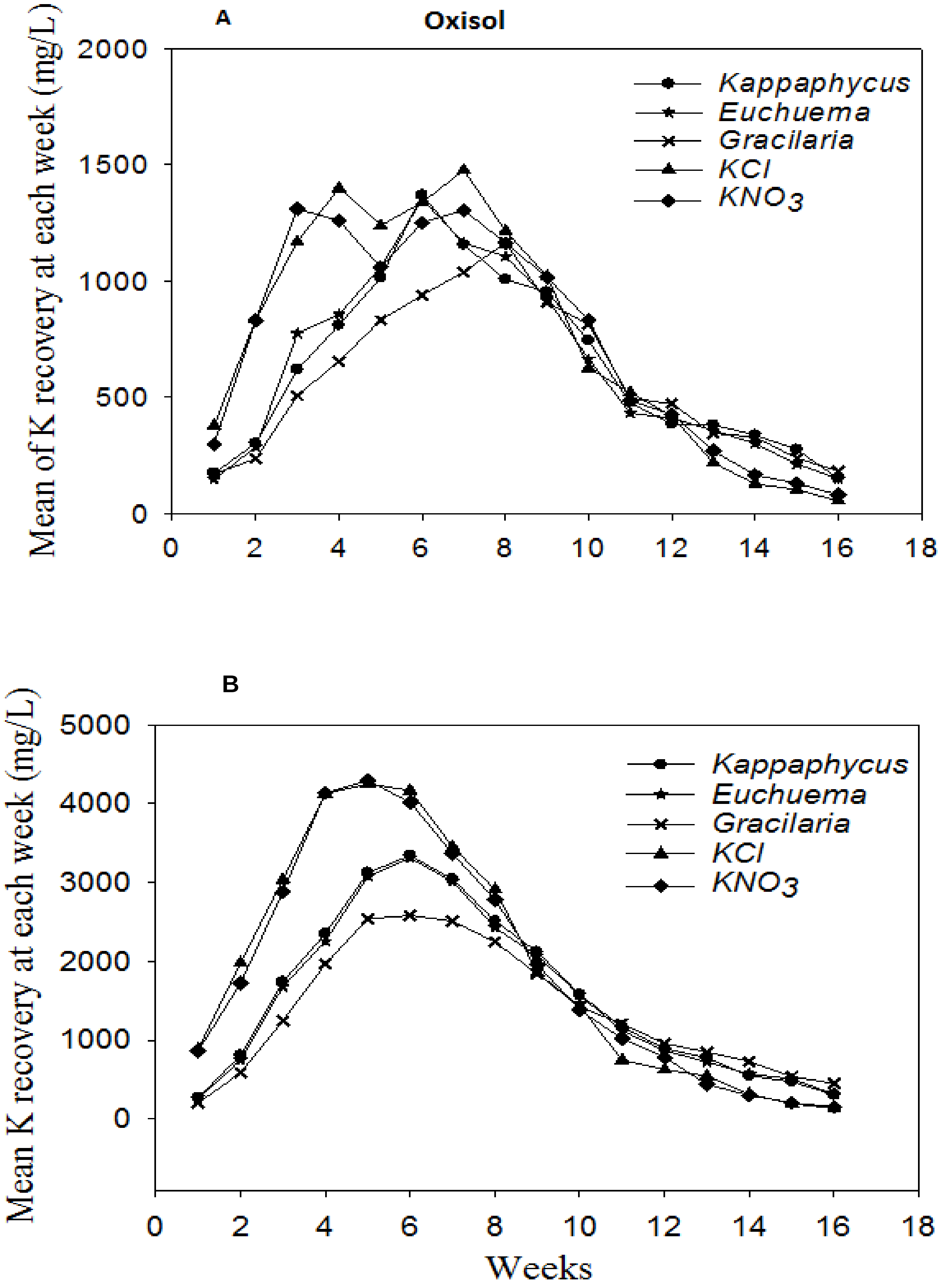
A
Oxisol
Mean of K recovery at each week (mg/L)
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
Kappaphycus
Euchuema
Gracilaria
KCl
KNO_3
B
Mean K recovery at each week (mg/L)
5000
4000
3000
2000
1000
0
Weeks

Fig 5.2 (A-B)

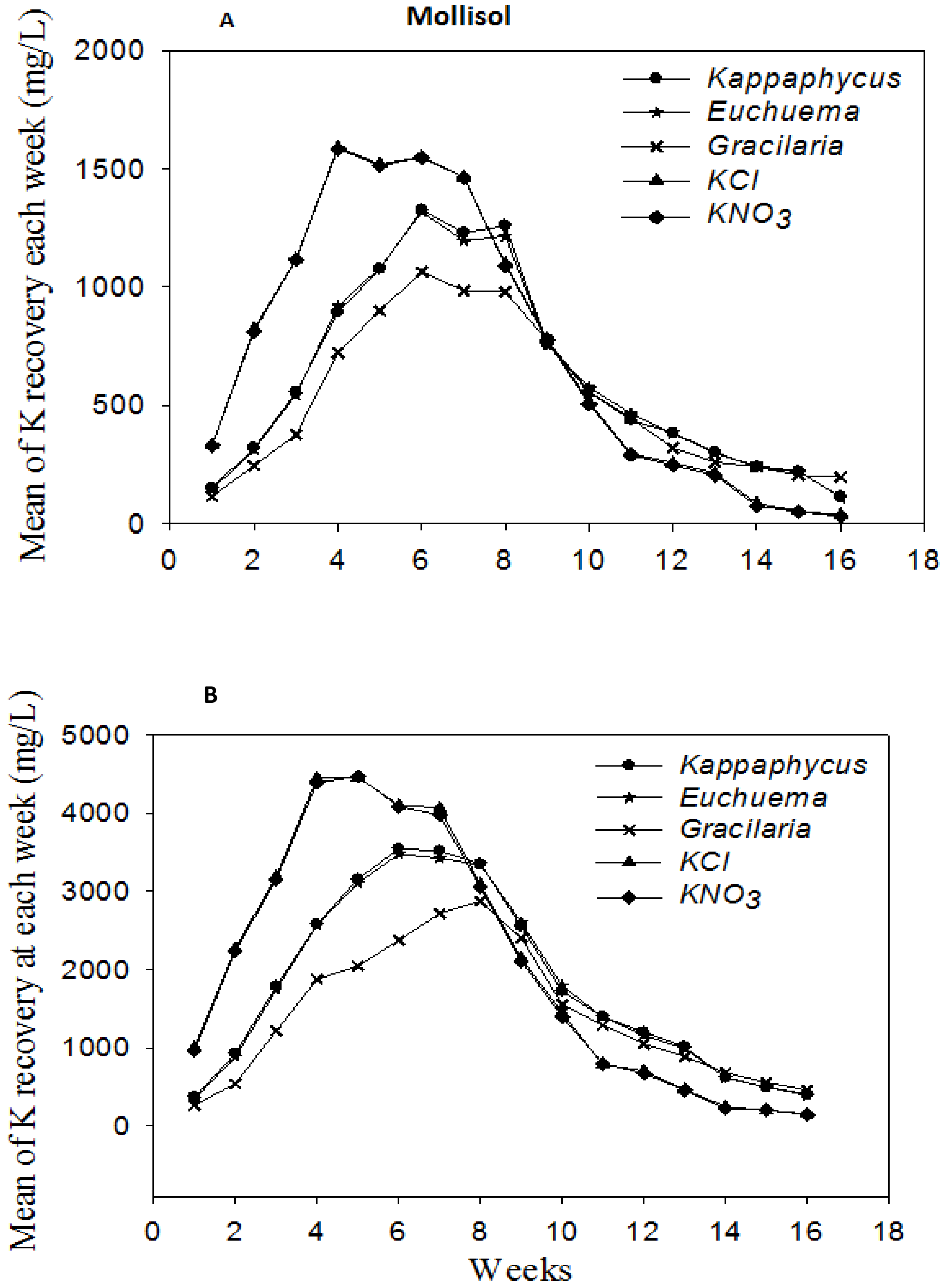
A
Mollisol
Mean of K recovery each week (mg/L)
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
Kappaphycus
Euchuema
Gracilaria
KCl
KNO_3
B
Mean of K recovery at each week (mg/L)
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
16
18
Weeks
Kappaphycus
Euchuema
Gracilaria
KCl
KNO_3

Fig 5.3 (A-B)

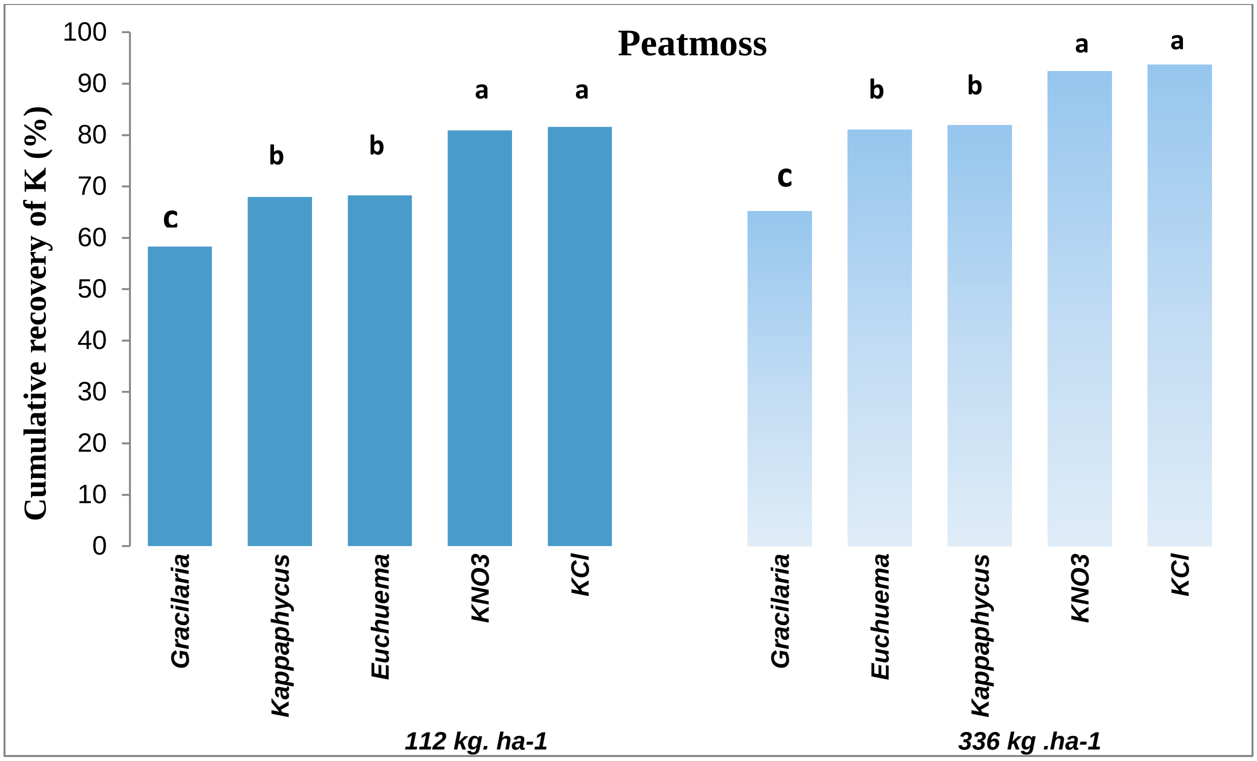

Fig 5.4 (A)

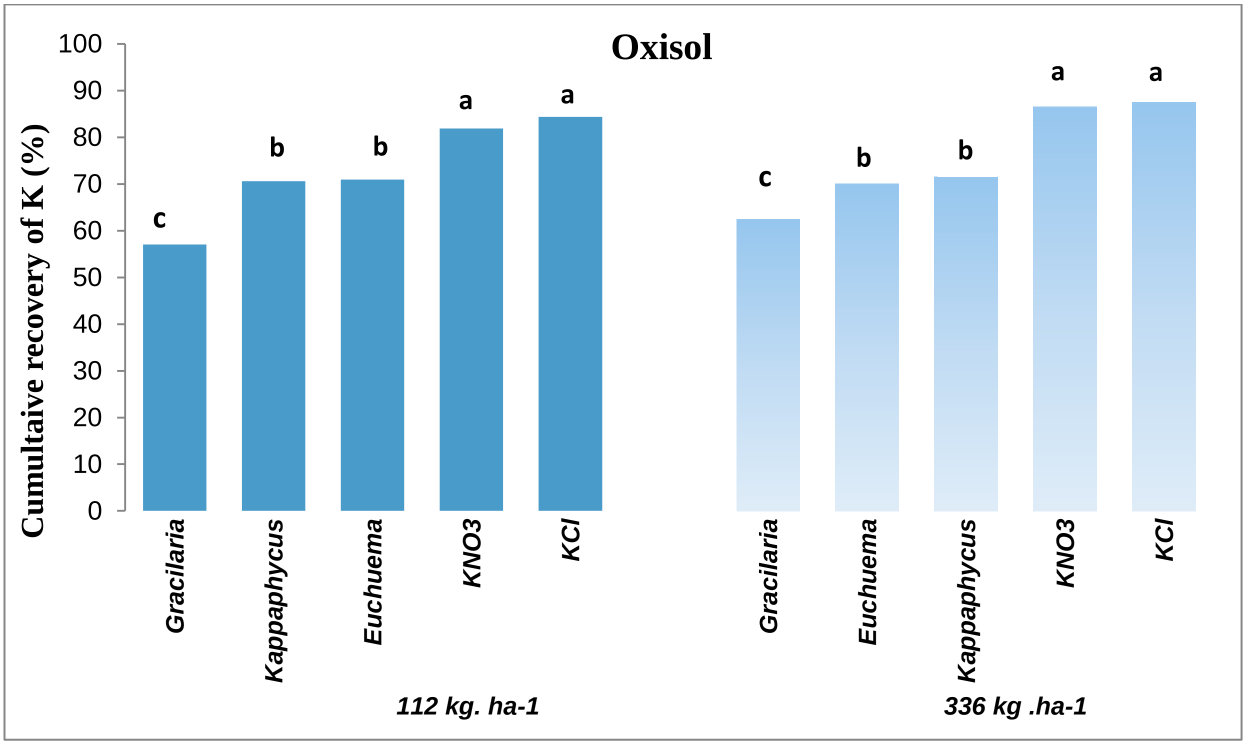

Fig 5.4 (B)

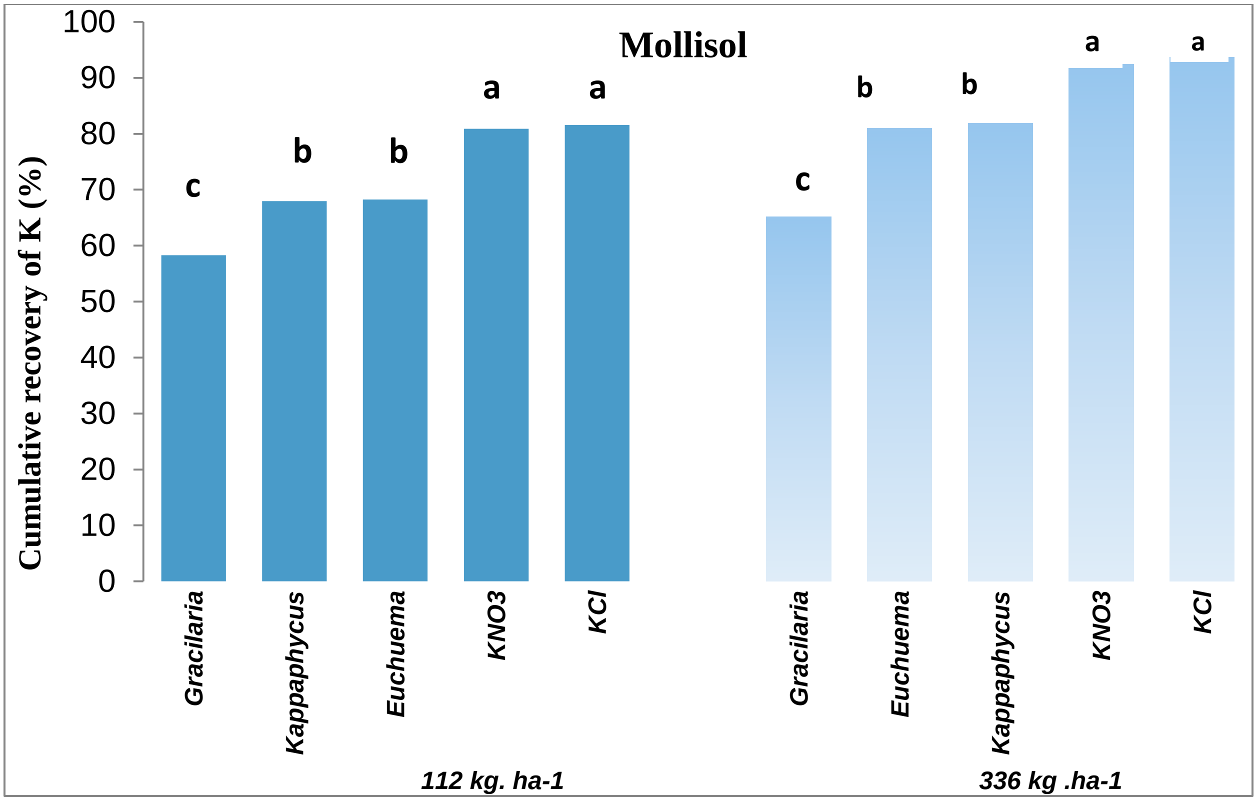
Mollisol
Cumulative recovery of K (%)
100
90
80
70
60
50
40
30
20
10
0
c
b
b
a
a
c
b
b
a
a
Gracilaria
Kappaphycus
Euchuema
KNO3
KCl
Gracilaria
Euchuema
Kappaphycus
KNO3
KCl
112 kg. ha-1
336 kg .ha-1

Fig 5.4(C)

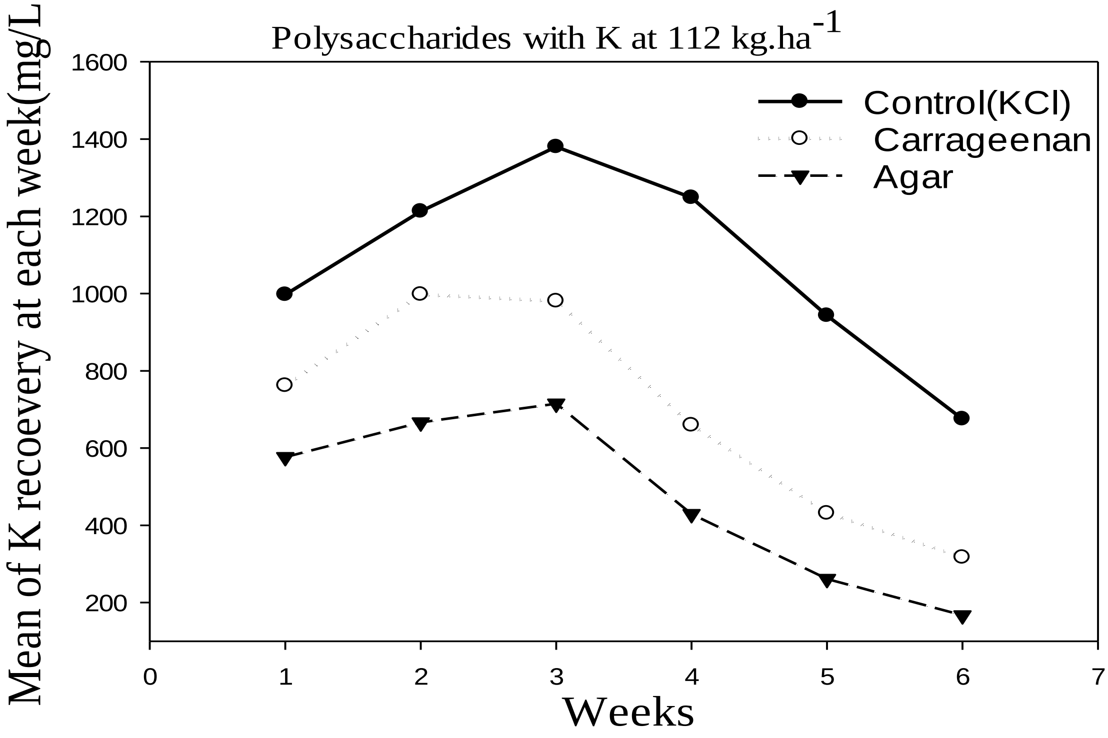

Fig 5.5(A)

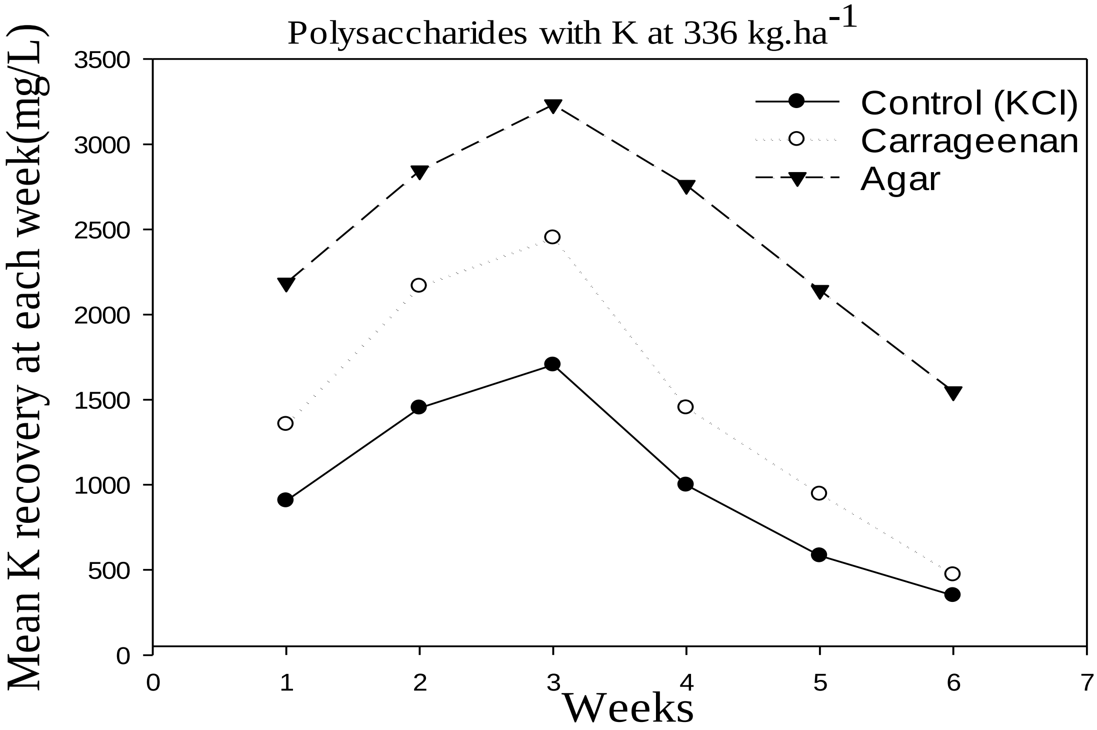

Fig 5.5 (B)

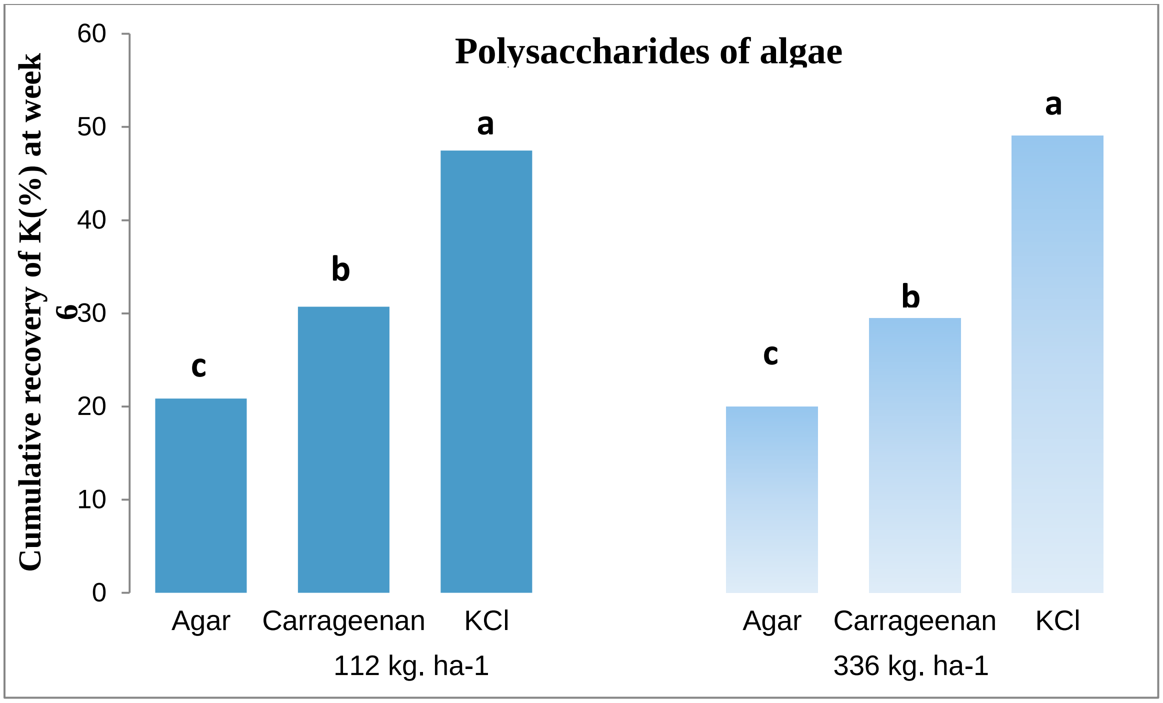

Fig 5.6(A)

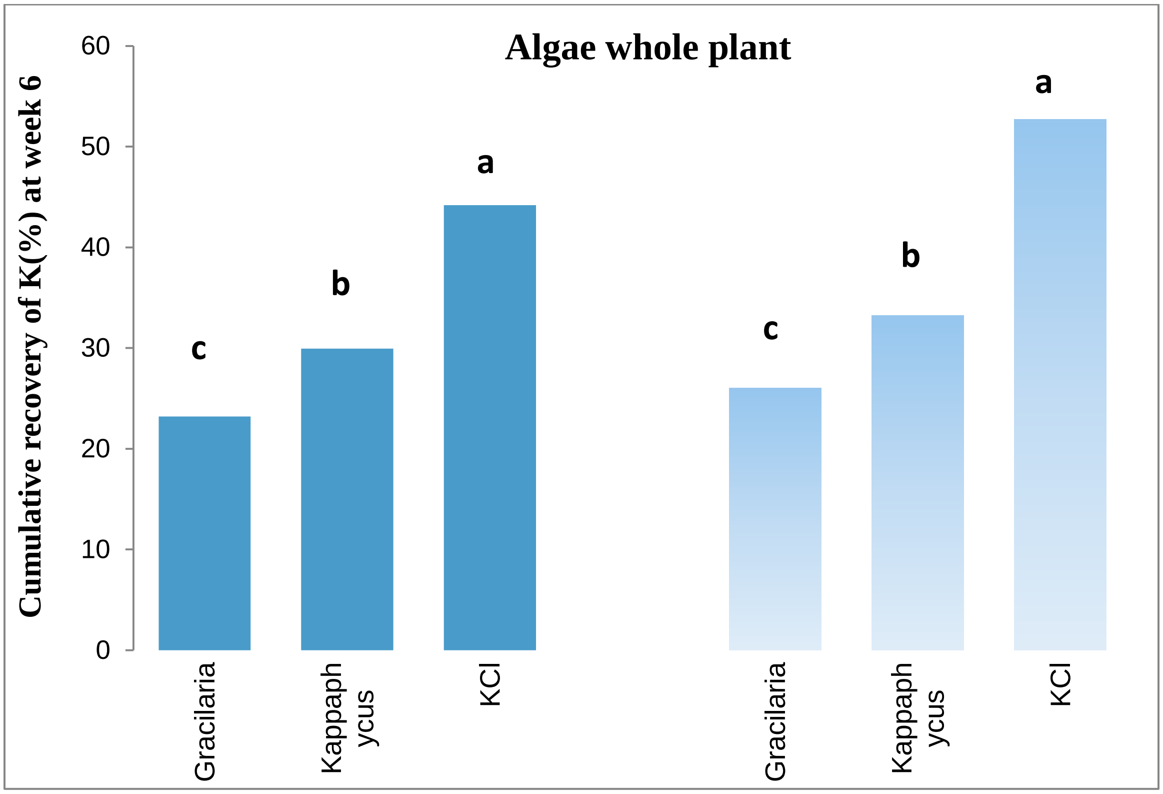

Fig 5.6 (B)

CHAPTER 6

SUMMARY AND CONCLUSION

Prior to this research, the majority of studies related to use of seaweeds in agriculture or horticulture have been focused on investigating the effects of seaweed extracts on crop growth and yield and predominantly as a foliar application (see Chapter 1). Limited data and understanding existed on the use of direct application of seaweed plant biomass as major source of nutrients such as N, P and K and to evaluate the use of these red seaweeds for improving yield and nutritional quality of vegetable crops. Previous research focused on the potential of these seaweed products and their extracts as plant growth hormones or plant bio stimulants and disease control with diverse results. A number of knowledge gaps exist that prevent growers in efficiently utilizing these inputs in their crop production. In this research, multiple experiments were conducted with the over-arching theme of investigating influence of three invasive algal *Eucheuma denticulatum*, *Gracilaria salicornia* and *Kappaphycus alvarezii* on growth, yield and K nutrition content in pak choi (*Brassica rapa* Chinensis group) as well as evaluating their potential as a replacement for commercial synthetic K fertilizers, such as KCl and KNO_3. The overall goals of these efforts were two-fold: (i) to validate the yield response curve and tissue K uptake with application of these species at different rates as compared to synthetic K fertilizers, (ii) to elucidate the K release pattern and mechanisms of these species. This improved understanding would be expected to both answer basic questions regarding mechanisms of K availability as well as help to deliver practical information and techniques that farmers could benefit from the use of these three invasive algae as a source of K in vegetable production.

6.1 Plant growth and K nutrition

Multiple greenhouse trials with different K fertilizer application rates have demonstrated that application of three invasive algae species increased yield and mineral nutrient (K) concentration of pak choi positively influenced the growth of pak choi (Chapter 3). The consistent results from these studies suggest that the these invasive algae species of Hawaiian Islands have potential to be used as a replacement for synthetic K fertilizer in vegetable crop production and are particularly beneficial when used for crops with high K requirements. The algae waste biomass could be mixed with commercially available fertilizers to enhance the plant growth. These seaweeds may provide an effective approach to nutrient management in places where inorganic fertilizers are expensive and limited.

6.2 Cardy meter vs ICP methods

Plant tissue analysis is a valuable tool for evaluating the nutritional status and quality of crops which helps farmers to better manage crop nutrient requirements. In this study, the two methods of K measurement from ICP and cardy meter had a significant (p<0.01) and strong (r^2=0.80) correlation suggesting that the cardy meter is a valuable tool for on-site monitoring of nutrient status of pak choi, particularly for detecting K deficiencies (Chapter 2). Although the data shows close correlation, the K readings from cardy meter are not as accurate as measurements from an analytical laboratory and the K measured from ICP was much higher than the cardy meter measured. Discrepancies observed in the cardy meter readings are probably due to the influence of other ions present in and these instruments are sensitive to environmental conditions and can give inaccurate or inconsistent readings.

6.3 K release from algae species in leachate column

Although GH results showed no statistically significant difference among the three algae we noted that *G. salicornia* had numerically lower yield and tissue K numbers as compared to *K.alvarezii* and *E. denticulatum* (Chapter 3). The three leachate columns lab studies validated these results from GH studies and the findings showed that the K release from *G. salicornia* was consistently lower in all the three trials with three media types (Chapter 5). Based on these data and subsequent literature review, I hypothesized that a probable mechanism for these differences is in the complexity in cell wall structure of these algae species and their physical and chemical properties.

6.4 K release from extracts of algae (agar and carrageenan) in leachate column

The findings from agar and carrageenan trials show that there are clear differences with K release pattern and mechanics from polysaccharides of agar and kappa carrageenan. The difference in the physical, chemical properties and polysaccharides structure of these invasive algae species which we believe are the factors for causing slow release of K from *G. salicornia*. The K release from agar was lower than the kappa carrageenan and this may be attributed to several reasons such as differences in the structure of their polysaccharides, physical and chemical properties of these species (Chapter 4). These differences in release among algal species may be explained in part by differences in chemical composition and particularly with types of sulfate groups, such as *K. alvarezii* consisting mostly of ester sulfates and *G. salicornia* consists mainly of agaropectin, a highly charged sulfated polymer. Secondly, the gelling strength of agar from *G. salicornia* is 7-10 times stronger than carrageenan extracted from *K. alvarezii.*

The three GH trials strongly supported our hypothesis that the three algae can be alternative sources of K, whereas the leachate column studies with whole plant biomass and the algal polysaccharides provided us the chemical and structural differences among the 3 species. Before recommending these species to local farmers, research needs to verify that high K requiring crops can be grown with invasive seaweeds on a large scale, probably in open field conditions. However, to take the maximum benefit from the potential of these materials, there is a strong need to dedicate research to a deeper knowledge on the cell wall chemistry of naturally extracted polysaccharides of these species and at different locations and temperature, which will help us to better understand the mechanism and the functions associated with it. Since commercial extraction process of agar and carrageenan involves disturbing and/or displacing the original chemical composition of these species. More importantly, the uncertainty about the cell wall structures and their differences in the same genera and species harvested from different locations around the globe are definitely the strongest limitations.

As a whole, the potential of these invasive algae as source of K nutrient in crop production has been studied and the results are quite promising. Basic characteristics remain, however, to be clarified and characterized on the differences in cell wall chemistry and other bio-chemical and physical properties among these species so that the usage of these algae biomass as a fertilizer in nutrient management in crop production can be made more efficient and effective. Lastly, the research that is presented only describes three species of invasive alga species, the potential to expand the use of other invasive species present in Hawaiian shores should be the priority, and one interesting species will be *Hypnea muscifera* rapidly establishing in Maui Island. Another issue is the city and state regulations, environmental concerns and other factors which may affect the availability of invasive algae in large quantities. Overall, these three invasive algae may be used to improve plant yield and K nutrient status of crops with higher K needs, particularly with pak choi crop.