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Pre-treatment extraction of silica and other nutrients from seagrass (*Cymodecea serrulata*)

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Abstract

Silicon has been found to improve plant resistance to stresses such as pests, diseases, lodging and drought. Thus, methods of utilizing plant materials as a source of silicon in the form of silica (SiO_2) is now being studied worldwide. This study was conducted to determine seagrass potential as a source of silica and other nutrients for plant uptake and identify the most effective silica extraction technique. The four treatments arranged in a completely randomized design (CRD) with three replications include yeast fermentation (control), enzyme, acid, and enzyme and acid pre-treatments combined. After the fermentation process all extracts from the different treatments were subjected to nutrient analysis which includes the determination of total macronutrients (nitrogen, phosphorus and potassium), total micronutrients (copper, zinc, manganese and iron) and availability of silica. Significant differences between the treatments were observed in total K and silica content. Highest total K (1.77%) was noted in control treatment, however, it was comparable with the combined enzyme and acid pre-treatment (1.71%). Silica content in acid pre-treatment (103.7mg L^{-1}) was significantly highest among the treatments, followed by enzyme pre-treatment (82.3mg L^{-1}). In terms of silica extraction, acid pre-treatment is shown to be the most efficient; however, further study on the efficiency of the extracts in the form of liquid fertilizer on field crops should be conducted.

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Introduction

Seagrass is known to be one of the most abundant marine resources and it plays a very important role in the entire coastal environment. They provide vast food source for many herbivores, and act as a natural filter by reducing the available levels of phosphate and nitrogenous wastes. It also stabilizes and holds bottom sediments, maintaining balance, biodiversity and a good symbiosis among the living aquatic organisms (Fortes, 1989). But it is now being studied as one the sources of nutrients for plant growth especially as a source of one the most debakg element on Earth which is Silicon (Jana and Jeong, 2014).

Silicon (Si), a micronutrient is one element ever present in some plants' leaves yet its function is not fully understood. As a result it is routinely omitted from formulations of culture solutions and considered a non-entity in much of plant physiological research. However, ample evidence is presented that silicon, when readily available to plants, plays a large role in their growth, mineral nutrition, mechanical strength, and resistance to fungal diseases, herbivory, and adverse chemical conditions of the medium. Recently, studies in Japan have associated Si with resistance to fungal and insect attacks. Si tends to harden rice leaves that serve as a barrier against fungi, insects and mites (Yoshida *et al.*, 1969). Likewise, an increased absorption of silicon maintains erect leaves which are very desirable traits in a high yielding rice variety. Moreover, in China's hybrid rice breeding, large spikelet grains has been attributed to the addition of Si slag during the plants vegetative stage. Taken together, the evidence is overwhelming that silicon should be included among the elements having a major bearing on plant life. This study therefore aimed to (1) determine the potential of seagrass as a source of silica and other nutrients required for plant growth, and (2) identify the most efficient pre-treatment method for extracting silica and other nutrients from seagrass.

Materials and methods

Sample collection and extraction

Seagrass samples were collected in Sta. Ana, Cagayan, Philippines. The extraction experiment was conducted in Bio-Laboratory, Cagayan State University-Carig Campus, Tuguegarao City, Cagayan. The seagrass samples collected (dried during collection and considered wastes in the coastal areas of Sta. Ana) were air-dried for two weeks in a room temperature. The collected samples were identified and verified in the National Museum. It was identified belonging to *Cymodecea* genera and *serrulata* species.

Experimental design and treatments

The experiment was arranged in a completely randomized design (CRD) with three replications. The four treatments include (1) control, (2) enzyme (cellulase), (3) acid, and (4) enzyme and acid pre-treatments combined. In treatment 1, the samples had undergone yeast fermentation. In treatment 2, the samples were pre-treated with cellulase enzyme with a ratio of 4ml cellulase to 996ml of prepared sample (Sander and Murthy 2009). For treatment 3, the samples were pre-treated with 75% sulfuric acid and neutralized using 25% NaOH (Zhang *et al.*, 2006). For treatment 4, the samples were first pre-treated with 75% sulfuric acid and neutralized using 25% NaOH and then added with 4ml cellulase (Frantz *et al.*, 2008).

Pasteurization of the fermented samples

Each fermented samples was pasteurized at 60°C for 30 minutes. The samples were subjected to laboratory analyses for the determination of macro and micronutrient contents.

Laboratory analysis

Laboratory analysis (macro- and micronutrients) was done at DA RFO2 (Department of Agriculture – Regional Field Office 02), San Gabriel, Tuguegarao City, Cagayan. Silica (SiO_2) content was determined by Intertek Testing Services Philippines, Inc. in Makati City. Standard procedures for analyzing total macro- and micronutrients were employed. Moreover, silica content of the samples was determined using UV/Vis Spectrophotometry.

Statistical analysis

The data were analyzed using STAR (Statistical Tool for Agricultural Research) to determine the differences between treatment means at 5% level of significance by least significant difference (LSD).

Results and discussion

Macronutrient content of seagrass extracts

Kg 1 shows the total N, P and K of the extracted samples with the different pre-treatments. Macronutrients are absorbed by plants in large amounts. Based on the standards (PNS-BAFPS, 2013) for the total percentage of NPK in liquid fertilizers it should have 2 to 5% to consider it as a fertilizer.

In this study, it was noted that total N, P and K extracted from seagrass using the different pre-treatments were relatively low but it reached the minimum standard for total macronutrient of 2%. Each macronutrient contents of plant extracts or plant juices is recorded not to exceed 2% except in acid pre-treatment (total P). No significant difference was observed in total N and total P, however, in acid pre-treatment, the highest total P was extracted (2.14%).

Moreover, significant effect was observed in enzyme pre-treatment having lower total K content (1.15%) which is comparable to total K from acid pre-treatment (1.48%). Moreover, combination of enzyme and acid pre-treatment was also comparable to control treatment.

Kg 1. Total macronutrient (N, P and K) content of the seagrass extracts.

Treatment	Total N,%	Total P,%	Total K,%
Control (yeast fermentation)	0.12 a	1.68 a	1.77 a
Enzyme (cellulase) pre-treatment	0.12 a	1.15 a	1.15 b
Acid pre-treatment	0.14 a	2.14 a	1.48 ab
Enzyme and acid pre-treatment	0.12 a	1.86 a	1.71 a

Means with the same letter are not significantly different at 5% level.

Micronutrient content of seagrass extracts

The total micronutrient (Zn, Cu, Mn and Fe) contents of the seagrass extracts are shown in Kg 2. Plants differ in their requirements for certain micronutrient. Foliar sprays are widely used to apply micronutrients, especially iron and manganese, for many crops (Bacchus, 2010). Soluble inorganic salts generally are as effective as synthetic chelates in foliar sprays and they are also cheaper, so the inorganic salts usually are being applied.

Kg 2. Total micronutrient (Zn, Cu, Mn and Fe) content of the seagrass extracts.

Treatment	Total Zn, ppm	Total Cu, ppm	Total Mn, ppm	Total Fe, ppm
Control (yeast fermentation)	0.43	0.04	0.08	7.97
Enzyme (cellulase) pre-treatment	0.32	0.05	0.33	10.99
Acid pre-treatment	0.05	0.04	0.15	7.21
Enzyme and acid pre-treatment	0.05	0.03	0.20	4.88

*Means with the same letter are not significantly different at 5% level

Very low Zn content was observed in all treatments, although no significant difference was observed. Zinc (Zn) is taken up by plants as the divalent Zn^{2+} cation. It is one of the first micronutrients recognized as essential for plants and the one most common yield limiting element. Although Zn is required only in small amounts, high yields are impossible without it. As with copper, residual effects of applied zinc are substantial, with responses found at least 5 years after application. Because of these residual effects, soil test levels of available zinc generally increase after several applications (Fernandez *et al.,* 2013). In this extraction study, it can be concluded that seagrass (*Cymodecea* sp.) is not a good source of Zn.

Very low insignificant Cu copper content was also observed in all treatments. Copper (Cu) activates enzymes and catalyzes reactions in several plant-growth processes (Fernandez, *et al*., 2013). The presence of copper is closely linked to Vitamin A production, and it helps ensure successful protein synthesis. It can also be concluded that seagrass (*Cymodecea* sp.) could not be a Cu source for plant production. Copper toxicity will not be a problem even if seagrass is a silicon accumulator (Frantz *et al.*, 2011). No significant difference was also noted in Mn content in all extracts. Manganese (Mn) functions primarily as part of enzyme systems in plants. It activates several important metabolic reactions and plays a direct role in photosynthesis (Zhang, *et al.*, 2006). Manganese accelerates germination and maturity while increasing the availability of phosphorus (P) and calcium (Ca). Application rates of MnO would be similar if applied as a fine powder or in NPK fertilizers. It can be inferred from this study that Mn content of seagrass (*Cymodecea* genera) is relatively low. The highest Iron (Fe) content was noted in cellulase pre-treatment, but it was not significantly different from the other treatments. Iron (Fe) is generally considered to be an intermediately mobile nutrient in higher plants and can be re-translocated in small amounts from the old leaves to the younger ones (as cited by Fernandez *et. al*., 2013). A typical concentration of Fe in the phloem is 9.4µgmL^{-1} which is too low to supply plant demand and the pH of the phloem is about 7.8-8.0 which favors Fe^{3+} insolubility (Hocking, 1994). The degree of mobility of Fe clearly varies with species, stage of plant growth and Fe supply amongst other factors.

Kg 3. Silica (SiO_2) content of the seagrass extracts.

Treatment	Silica (mg L^{-1})
Control (yeast fermentation)	65.3 c
Enzyme (cellulase) pre-treatment	82.3 b
Acid pre-treatment	103.7 a
Enzyme and acid pre-treatment	73.0 c

Means with the same letter(s) are not significantly different at 5% level.

Silica (SiO_2) content of seagrass extracts

Highest significant silica content (103.7mg L^{-1}) was noted in acid pre-treatment. It was followed by cellulase pre-treatment extract with 82.3mg L^{-1}. However, combination of enzyme (cellulase) and acid pre-treatments and control (yeast fermentation) were comparable in terms of silica content. Based on standard percentage required by plants, total silica content optimum level should at least be 5% (Fernandez *et al*., 2013). In this study, it was found out that seagrass could really be a good source of silica even with yeast fermentation alone.

Conclusion

To assist in ameliorating biotic and abiotic stresses, reliable detection, extraction and quantification methods of Si in other plant materials will be increasingly important. The use of acid pre-treatment in Si extraction was found to be effective for seagrass specifically *Cymodecea serrulata*; however, the liquid extracts should be tested in field crops to determine its effect. Seagrass, although considered under threat due to the increasing utilization of the coastal environment for economic purposes such as eco-tourism, fish caging, docking areas and recreation areas, is now being realized as one of the plant sources of silica. As mentioned, this study used the dried ones and considered wastes in coastal areas of Sta. Ana, Cagayan, Philippines.

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References

Acchus GL. 2010. An Evaluation of the influence of biodynamic practices including foliar-applied silica spray on nutrient quality of organic and conventionally fertilized lettuce (*Lactuca sativa* L.). Journal of Organic Systems **5(1),** 2010. pp. 4-13.

Fernandez V, Sotiropoulos T, Brown P. 2013. Foliar Fertilization: Scientific Principles and Field Practices. 1st ed. International Fertilizer Industry Association (IFA), Paris, France.

Fortes MD. 1989. Seagrasses: A resource unknown in the ASEAN Region. ICLARM Ed. Ser. 5, 46 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Frantz JM, Khandahar S, Leisner S. 2011. Silicon differentially influences copper toxicity response in silicon-accumulator and non-accumulator species. Journal of the American Society for Horticultural Science **136,** 329-338.

Frantz JM, Locke JC, Datnoff L, Omer M, Widrig A, Sturts D, Horst L, Krause CR. 2008. Detection, distribution and quantification of silicon in floricultural crops utilizing three distinct analytical methods. Communications in Soil Science and Plant Analysis. 39:17, 2734-2751.

Hocking PJ. 1994. Dry-matter production, mineral nutrient concentrations, and nutrient distribution and redistribution in irrigated spring wheat. Journal of Plant Nutrition **17,** 1289-1308.

Jana S, Jeong BR. 2014. Silicon: The most under-appreciated element in horticultural crops. Academic Journals. Trends in Horticultural Research **4(1),** 1-19, 2014.

Sander K, Murthy GS. 2009. Enzymatic degradation of microalgal cell walls. American Society of Agricultural and Biological Engineers Paper No. 1035636.

Yoshida S, Navasero SA, Ramirez EA. 1969. Effects of silica and nitrogen supply on some leaf characters of the rice plant. Plant and Soil Journal **31(1),** August 1969, pp.48-56.

Zhang B, Wang L, Shahbazi A, Diallo O, Whitmore A. 2006. Dilute-sulfuric acid pretreatment of cattails for cellulose conversion. Bioresource Technology **102,** (2011) 9308-9312.