International Journal of Horticultural Science and Technology
Vol. 2, No. 2; December 2015, pp 133-140

Mineral Content of Mediterranean Seaweeds, *Padina pavonica* L. *(Pheophytae)*, *Ulva lactuca* L. and *Ulva linza* L. *(Chlorophytae)* for Biofertilizing Use

Asma Chbani[*], Sandy Majed, Hiba Mawlawi
Laboratory of Applied Biotechnology for Biomolecules, Biotherapy and Bioprocess, Doctoral School for Sciences and Technology, Azm Centre for Research in Biotechnology and its Application, Lebanese University, El Miten Street, Tripoli, Lebanon.

(Received: 21 June 2014, Accepted: 27 June 2015)

Abstract

Nowadays, organic fertilizers play an important role in agriculture. They are progressively substituting chemical fertilizers to prevent their harmful impact on human health and the environment. They provide high yield, better quality products and a shorter period of harvesting crops. In this study, the mineral elements: primary macronutrient (N, PO^{3-} and K^{+}), secondary macronutrient: (Ca^{2+}, Mg^{2+} and SO^{2-}), micronutrient (Na^{+} and Cl^{-}), alkalinity (HCO_3^{-}) and other elements (NO_2^{-} and NO^{-}), of three seaweeds were determined: chlorophytae (*Ulva lactuca, Ulva linza*) and phaeophytae (*Padina pavonica*). The nitrogen content was the most abundant element in the three Mediterranean seaweeds [79.85 - 57.16 - 126.09 [$\times 10^3$ mg L^{-1}], respectively, with a maximum to the chlorophytae *U. lactuca*. This is true also for other macroelements (K and P); their values are higher in green seaweed than the brown *Padina pavonica*. Secondary elements (Ca and Mg) also show higher values in green algae, with a maximum value in *Ulva lactuca*. There is no significant difference concerning the values of microelements Na^{+} and Cl^{-} between green and brown algae. The values of nitrite and nitrate are equivalent for the two green algae, while these items are virtually nonexistent in the brown algae. In conclusion, interesting values of the green alga *Ulva lactuca* could suggest the use of aqueous extract of this alga such as biofertilizant.

Keywords: Biofertilizer, biostimulant, minerals composition.

Introduction

Since the use of chemical fertilizers is widespread, scientists study their impacts on human health. They were able to establish links between chemical fertilizers and diseases, cancers of the stomach, pancreas, kidney, as well as leukemia, and multiple myeloma (Schmidt *et al*., 2003). Unlike chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereira 2005). Any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. One such approach is the use of biostimulants, which can enhance the effectiveness of

---

[*] Corresponding author Email: asmashbani61@gmail.com

conventional mineral fertilizers (Abdalla and El-Khoshiban, 2012). Several regions of the world have to be explored and exploited for the richness of marine plants and macro-algae that they contain (El Abed *et al*., 2002). Marine bioactive substances extracted from marine algae are used in agricultural and horticultural crops, and many beneficial effects, in terms of the enhancement of yield and quality, have been reported (Erulan *et al*., 2009). The application of seaweed liquid extract as foliar spray has recently gained importance in agriculture. This extract contains growth hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids. When seaweed extracts are applied to seeds or added to the soil, they promote plant growth (Kumar and Sahoo, 2011). Liquid extracts obtained from seaweeds have recently gained importance as foliar sprays for many crops including various grasses, cereals, flowers and vegetable species for a comprehensive list (Ismail and Kardoush, 2011). Seaweed extracts contain major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances and have been reported to stimulate the growth and yield of plants (Khan *et al*., 2009), develop tolerance to environment stress (Zhang *et al*., 2003) and increase nutrient uptake from soil (Turan and Köse, 2004; Rathore *et al*., 2009). The beneficial effect of seaweed extract application is the result of many components that may work synergistically at different concentrations, although the mode of action still remains unknown (Fornes *et al*., 2002). In recent years, the use of seaweed extracts have gained in popularity due to their potential use in organic and sustainable agriculture (Khan *et al*., 2009; Kumar and Sahoo, 2011), especially in rained crops, as a means to avoid excessive fertilizer applications and to improve mineral absorption.

Numerous studies have demonstrated a broad spectrum of beneficial effects of extract of algae applications on plants, such as seed germination, improved crop yields and performance, a high resistance to biotic and better preservation post-harvesting of perishable goods (Norrie and Keathley, 2006). Currently, experiments conducted on biostimulant have mixed results with respect to growth, development and quality of plants.

The Goëmar Laboratories have forwarded the benefits observed following the application of their cream algae on the growth of corn and spinach in experiments in the laboratory of INRA Versailles. They emit other products that increase the production of fresh weight, manifesting in stems and roots, and stomata resistance increases, decreasing the percentage of perspiration (Ginestet, 1994).

15 million metric tons of seaweed products are produced each year, a considerable part is used for nutritional supplements and bio-stimulants or as organic fertilizers to increase plant growth and yield (FAO, 2006).

Macronutrients and micronutrients play a vital role in all plants. Although they are present in infinitesimal doses, they're essential for normal metabolism. The chemical composition of mineral elements of *Enteromorpha linza* or *Ulva linza* determined by (Jiang *et al*., 2013): Na^+, k^+, Ca^{2+}, Mg^{2+}, Cl^- and NO_3^- demonstrated that these green seaweeds could be considered for future applications in medicine, dietary supplements, cosmetics or food industries.

In Lebanon, seaweeds are poorly exploited. To replace chemical fertilizers and their harmful impact on health and the environment, this study aims to use aqueous extracts of marine algae as biofertilizer. Three seaweed aqueous extracts of the Mediterranean species from Lebanon, *Padina pavonica* (Phaeophytae), *Ulva lactuca* and *Ulva linza* (Chlorophytae) were studied for their mineral composition. These algae are present in abundance on the coastal area of the Mediterranean, El Mina (34 ° 26'N 35 ° 50'E), in Tripoli - Lebanon.

Materials and Methods

Seaweed materials and treatments

The seaweeds were collected in March 2014, raw and fresh from the coastal area of the Mediterranean, El Mina (34 ° 26'N 35 ° 50'E), in Tripoli – Lebanon. Fresh plants were thoroughly removed of their epiphytes, rinsed on the spot with seawater, and then placed in plastic bags. On their arrival at the laboratory, the seaweeds were thoroughly washed using tap water and ultrapure water to remove sea salts on the surface of samples.

Preparation of seaweed liquid extracts (SLE) for Physico-chemical analyses

To study and compare the composition of minerals and nutrients contained in three algae *Padina pavonica* (Pheophytae), *Ulva lactuca* and *Ulva linza* (Chlorophytae) liquid extract, a dosage of the primary nutrients (N, P, K), secondary nutrients (Ca, Mg, S), trace elements (Cl, Na), alkalinity ($HCO3^-$), nitrite and nitrate, were analyzed with the method described by Normalization French Association (AFNOR). The pH of the SLE was directly measured using a pH meter ORION type. (*P. pavonica*: 7.05, *U. linza*: 5.85, *U. lactuca*: 5.51

Preparation of aqueous liquid extracts (ELA)

200 g of each fresh alga were ground and then boiled with 200 ml of ultrapure water and then stirred for one hour on a hot plate. Then the extracts were filtered twice through a muslin cloth and then allowed to cool to room temperature (Kumar and Sahoo, 2011). 1 liter of liquid extracts concentration (1%) was prepared by diluting the extract with ultrapure water for analysis. Each assay was performed three times.

Volumetric assays

The calcium, magnesium, chlorine and alkalinity were performed according to French standard NF of the French Association for Standardization (AFNOR). The determination of total concentration of calcium and magnesium (hardness or total) in the aqueous extract was performed by the titrimetric method to EDTA. The chlorine concentration is determined by measuring with a solution of silver nitrate $AgNO_3^-$ (4.791 g L^{-1}). The alkalinity of the ELA was determined by measuring the “Full Title alkalimetric" or TAC.

Spectrophotometric assays

The nitrate, nitrite, phosphate, sulfate, sodium and potassium were performed according to French standard NF of the French Association for Standardization (AFNOR). The concentration of the reagent of ascorbic acid and of a reagent (reagent molybdic) giving a colored derivative with phosphate (blue phosphomolybdic complex) were determined. Principle of determination of sulphate: precipitating sulphate ions in the presence of barium chloride in hydrochloric acid medium in the form of barium sulphate. The dosage potassium and sodium ions Na^+ and K^+ was performed by measurement of the test solution using a flame spectrophotometer Type PFP JENWAY 7 to a wavelength of 589 nm and 766nm, respectively. The principle of nitrite assay is to use a calibration curve to be able to deduce the concentration from the absorbance at a wavelength of 543 nm. For the determination of nitrate, the method of sodium salicylate was used and it was measured at the wave length of 415 nm.

Determination of total nitrogen

Kjeldahl nitrogen (NK) was analyzed using a method based on Kjeldahl mineralization (AFNOR T 90–110) (Martin 1987) that includes Org–N and NH_4^-N, in which 25 mL of sample was decomposed and oxidized by an excess of concentrated sulfuric acid (10 mL) in the presence of a catalyst mixture digestion ($CuSO_4$ + K_2SO_4) (5g) and heated to a temperature of 350°C for three hours. At the end of this mineralization step, nitrogen was obtained in the same mineral form (NH_4^+). This Kjeldahl digestion solution was then passed through a programmable type distiller Vapodest Gerhardt 30s allowing the distillation of the mineral deposit by steam stripping.

Results and Discussion

In this study, different mineral elements: nitrogen (N), phosphate (PO_3^-), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfate (SO^{2-}), sodium (Na^+), chlorine (Cl^-), carbonate (HCO_3^-), nitrite (NO_2^-) and nitrate (NO^-), were determined in chlorophytae and phaeophytae. The average mineral compositions of the liquid extract of *Padina pavonica*, *Ulva linza* and *Ulva lactuca* seaweed samples are shown in Table 1.

Table 1. Concentration of macronutrients (primary and secondary nutrients), micronutrients, alkalinity and other minerals of the aqueous liquid extract of the chlorophytae *Ulva lactuca* and *Ulva linza* and of the pheophycae, *Padina pavonica*

Concentration (mg L^{-1})		*Padina pavonica*	*Ulva linza*	*Ulva lactuca*
Macronutrients				
Primary macronutrients	Azotes (N) x10³	79.85	57.16	126.09
	Phosphates (PO^3)×10^2	0.69	1.2	3
	Potassium (K^+)×10²	2.78	12.65	16.34
Secondary macronutrients	Calcium (Ca^{2+})×10²	4.8	6.8	13.6
	Magnesium (Mg^{2+})×10²	1.94	23.8	36.9
	Sulphate (SO^{2-})×10³	1.52	0.108	0.262
Micronutrients	Chloride (Cl^-)×10³	7.207	4.45	8.35
	Sodium (Na^+)×10²	12.62	10.4	13.02
alkalinity	Carbonate (HCO^-) ×10²	1.95	7.8	10
Other minerals	Nitrite (NO^-)×10	0.41	3.84	5.66
	Nitrate (NO^-)×10²	0	7.58	7.24

The Nitrogen (N) content was the most abundant element in the three species of seaweed extract 79.85 - 57.16 - 126.09 [x10³ mg L^{-1}], respectively, with a maximum in the chlorophyta *U. lactuca* (Table1). The fact that *Ulva lactuca* presents a higher affinity for nitrogen might be related to the less energy which is required to assimilate this nutrient (Abreu *et al.*, 2011). Several field studies on bloom-forming species found their effective nitrogen uptake and tissue accumulation capability of green algae in ambient water with higher nutrient availability (Pérez-Mayorga *et al.*, 2011; Runcie *et al.*, 2003).

Chapman and Craigie (1977) found that nitrate (NO_3^-) represented a nitrogen reserve, and discovered that the green algae present a higher affinity for nitrogen (Abreu *et al.*, 2011) which can be elucidated as a consequence of the high rate of nitrate and nitrite. In addition, as algal NO_3^- reductase (NR) converts NO_3^- to NO_2^- in light (Albert *et al.*, 2013), it involves more enzymes activation and significant transformation in green algae which are more exposed to light than brown algae.

The alkalinity (HCO_3^-) values are close to the two green seaweeds (7.8 – 10 [×10² mg L^{-1}]), whereas this value is very low in the brown seaweed (1.95 ×10² mg L^{-1}). Carbonate alkalinity (CA) and pH are considered to be two important stress factors (Lin *et al.*, 2013). *U. lactuca* was highly efficient in removing the inorganic nutrients from the culture systems (Van Khoi and Fotedar, 2011). Therefore, maintaining a certain level of inorganic carbon is necessary in order to prolong ammonia removal (Park *et al.*, 2010). In general, green algae were able to reach the highest pH (10.8), and thus to achieve the highest level of inorganic carbon via a simple HCO3 −/OH− ion exchange process. For brown algae, pH increases due to carbon uptake never exceeded pH 9.7. Within each algal class, differences in pH and dissolved inorganic carbon compensation points could be related to differences in the depths at which the algal species occurred (Axelsson and Uusitalo, 1988). It is well known from higher plants that nitrate assimilation increases alkalinity. This has also been shown for algae by (Wolf-Gladrow *et al.*, 2007). We can then explain the fact that chlorophyta present a more important *Phaeophyta* alkalinity.

According to the results, *U. lactuca* contained significant amounts of all the essentials minerals except the sulfate compound (Table 1). Magnesium (36.9×10^2 mg L^{-1}) was the most abundant element in the *U. lactuca* seaweed after the nitrogen elements, followed by potassium (16.34×10^2 mg L^{-1}). The other elements were calcium (13.6×10^2 mg L^{-1}), sodium (13.02×10^2 mg L^{-1}), chlorine (8.35×10^3 mg L^{-1}) and phosphate (3×10^2 mg L^{-1}), and this is correlated with other studies such as the study conducted by (Yaich *et al.*, 2011). The high content of minerals for this *U. lactuca* seaweed was explained by the consumption of the alga of nutritive elements in the medium, where it survives, and their accumulation (Bartoli *et al.*, 2005; Tsagkamilis *et al.*, 2010). *Ulva (Chlorophyta)* is one genus of opportunistic green macroalgae that owing to its foliose morphology has efficient nutrient uptake and high growth rates, enabling these organisms to proliferate fast in favorable conditions (Pedersen and Borum, 1997; Sode *et al.*, 2013)

This characteristic was attributed to sulphated polysaccharides present in the cellular wall of the alga. Indeed, the groups hydroxyl, sulphate and carboxyl of polysaccharide were ion exchangers; consequently, they were important sites of complexation of the metallic cation (Vasconcelos and Leal, 2001).

In this study, we were also interested in the large difference which exists between Chlorophyta and *Phaeophyta* in the concentration of magnesium (1.94 - 36.9 [$\times 10^2$ mg L^{-1}], respectively) (Table 1). The brown algae have low concentration of magnesium, whereas the green algae are well provided with magnesium, especially *Ulva* sp. and *Enteromorpha* sp., and this is correlated with other studies (Costa *et al.*, 1997; Marfaing and Lerat, 2007).

The magnesium is easily consumed in green seaweed compared to other metallic components (Park *et al.*, 2010). The chlorophyll molecule comprises a magnesium ion (Mg^{2+}), and forms a chelate with the 4 nitrogen atoms in the middle of cycle. Magnesium chelatase is a heterotrimeric enzyme complex that catalyzes a key regulatory and enzymatic reaction in chlorophyll biosynthesis, the insertion of Mg^{2+} into protoporphyrin IX (Rissler *et al.*, 2002). Therefore, chlorophyteas have the highest levels in magnesium compared to Phaeophyceae.

The results show that the green seaweed is an important source for minerals including potassium (rang between 12.65-16.34 [$x10^2$ mg/L]). There is no significant difference in both green algae, but this difference is high between brown algae and green seaweed (2.78 – 16.34 [$\times 10^2$ mg L^{-1}], respectively), and this is correlated with other studies (Lill *et al.*, 2012).

Concentration calcium in chlorophytea is significantly higher than that among in pheotophytae. This result is attributed to the Photosynthetically saturating irradiances of continuous white light that enhance the active and passive fluxes of Ca^{2+} across the plasmalemma by a factor of two (Wagner and Bellini, 1976; Bauer *et al.*, 1997), and normally green algae are more exposed to light than brown algae, which is translated by the high concentration of calcium in green algae.

The mean levels of tissue phosphorus in green seaweed were important, specially *U. lactuca* , which were related to the high efficiency of *U. lactuca* in removing the PO_4^{3-} nutrients from the culture systems. In consequence, the potential use of Ulva as an indicator species for eutrophication is discussed (Ho, 1981; Sode *et al*., 2013; Van Khoi and Fotedar, 2011). (Aratani *et al*., 2007) show that the higher the PO_4^- concentration, the higher the biomass of periphytic algae and the more dominant the Chlorophyceae.

For microelements, Na^+ and Cl^-, the values are roughly in the range of the brown and green algae ($[Cl^-]$:7.207 - 8.35 [$\times 10^3$ mg L^{-1}]/$[Na^+]$:12.62 - 13.02[$\times 10^2$ mg L^{-1}] respectively). On the other hand, the sulfate concentration is most important in the brown alga *Padina pavonica* (1.52[$\times 10^3$ mg L^{-1}]). These results are correlated with other results showing that the extract of brown algae presented higher percentage of sulphate (6.6±1.42%) (Vijayabaskar and Vaseela, 2012). Furthermore, studies on chemical compositions from brown algae showed their relatively high sulfate content in comparison with green algae (Haroun-Bouhedja *et al*., 2000; Al-Amoudi *et al*., 2009).

In photosynthetic organisms, sulfur is taken up by the cells mostly in the form of sulfate anions (Melis and Chen, 2005). In plants and algae, primary assimilation of sulfate takes place in the chloroplast. Seawater sulfate concentration is high and very little is known about the sulfur metabolism of marine organisms. It is the consequence of 1718 genes that they retain the ability to re-program their gene expression in response to reduced sulfate availability (Anastyuk *et al*., 2011; Bochenek *et al*., 2013; Bilan *et al*., 2014).

Conclusion

After comparing the composition of minerals and nutrients from the aqueous extract of the chlorophyta (*Ulva lactuca, Ulva Linza)* and Phaeophyta (*Padina pavonica),* we can conclude that the mineral compounds present a significant difference between the green and brown algae, except for the concentration of chlorine and sodium. In general, Chlorophyta, especially *Ulva lactuca,* contained significantly larger amounts of essential mineral elements than Phaeophyta. Finally, considering the fact that substantial values of mineral compounds were found in *Ulva lactuca*, and that these algae are abundant on the Lebanese coast throughout the year, it can be suggested that the use of these algae as biofertilizers replace the consumption of chemical fertilizers and, therefore, eliminate their harmful impact on people's health and the environment.

References

Abdalla, M. and N. El-Khoshiban. 2012. The Palliative Effect of Bio-Organic Fertilizer on Lead Pollution. J. Basic Appl. Sci. 8:399-410.

Abreu, M.H., R. Pereira , A.H. Buschmann, I. Sousa-Pinto and C. Yarish. 2011. Nitrogen Uptake Responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under Combined and Single Addition of Nitrate and Ammonium. J. Exp. Mar. Biol. Ecol. 407:190-199.

Al-Amoudi, O.A., H.H. Mutawie, A.V. Patel and G. Blunden. 2009. Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia. Saudi J. Biol. Sci. 16:23-29.

Albert, K.R., A. Bruhn and P. Ambus. 2013. Nitrous Oxide Emission from *Ulva lactuca* Incubated in Batch Cultures is Stimulated by Nitrite, Nitrate and Light. J. Exp. Mar. Biol. Ecol. 448:37-45.

Anastyuk, S.D., N.M. Shevchenko, P.S. Dmitrenok, and T.N. Zvyagintseva. 2011. Investigation of a Sulfate Transfer during Autohydrolysis of a Fucoidan from the Brown Alga Fucus Evanescens by Tandem ESIMS. Carbohydr. Res. 346:2975–2977.

Aratani, Y., A. Tajima, and M. Minamiyama. 2007. Relationship of Nutrient and Residual

Chlorine Concentration in Treated Wastewater with Periphytic Algae Grown in a Stream Receiving Treated Wastewater. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 55:375–386.

Axelsson, L. and J. Uusitalo. 1988. Carbon Acquisition Strategies for Marine Macroalgae. Mar. Biol. 97:295-300.

Bartoli, M., D. Nizzoli, M. Naldi, L. Vezzulli, S. Porrello , M. Lenzi and P. Viaroli. 2005. Inorganic Nitrogen Control in Wastewater Treatment Ponds from a Fish Farm (Orbetello, Italy): Denitrification Versus Ulva uptake. Mar. Pollut. Bull. 50:1386-1397.

Bauer, C.S., C. Plieth, U.-P. Hansen, B. Sattelmacher, W. Simonis and G. Schönknecht. 1997. Repetitive Ca^{2+} Spikes in a Unicellular Green Alga. FEBS Lett. 405:390-393.

Bilan, M.I., A.S. Shashkov and A.I. Usov. 2014. Structure of a Sulfated Xylofucan from the Brown Alga Punctaria Plantaginea. Carbohydr. Res. 393:1-8

Bochenek, M., G.J. Etherington, A. Koprivova, S.T. Mugford, T.G. Bell, G. Malin and S. Kopriva. 2013. Transcriptome Analysis of the Sulfate Deficiency Response in the Marine Microalga Emiliania Huxleyi. New Phytol. 199:650–662.

Chapman, A.R.O. and J.S. Craigie. 1977. Seasonal growth in Laminaria Longicruris: Relations with Dissolved Inorganic Nutrients and internal Reserves of Nitrogen. Mar. Biol. 40:197-205.

Costa, M., J.-M. Fontaine, S.L., Goër and F. Michel,. 1997. A Group II self-splicing Intron from the Brown Alga Pylaiella Littoralis is Active at Unusually Low Magnesium Concentrations and forms populations of molecules with a uniform conformation. J. Mol. Biol. 274:353–364.

Dhargalkar V.K. and N. Pereira. 2005. Seaweed: Promising Plant of the millennium, Sci. Cult . 71:60-66.

El Abed, A., R. Ben Said and M.S. Romdhane. 2002. Etude d’une Population de l’algue Brune *Padina pavonica* (L) Lamouroux a Cap Zebib (nord de la Tunisie). A Study of the Brown Alga *Padina pavonica* (L.) Lamouroux in Cap Zebib (North of Tunisia).

Erulan V., G. Thirumaran, P. Soundarapandian and G. Ananthan. 2009. Studies on the Effect of Sargassum Polycystum (C. Agardh, 1824). Extract on the Growth and Biochemical Composition of *Cajanus cajan* (L.) Mill sp, American-Eurasian J. Agr. Environ. Sci. 6(4):392-399

FAO. 2006. Yearbook of Fishery Statistics, vol. 98, pp. 1-2. Food and Agricultural Organization of the United Nations, Rome.

Fornes J.F., M.Sànchez-Perales and J.L.Guadiola. 2002. Effect of a Seaweed Exrtact on the Productivity of 'de Nules' Clementine Mandarin and Navelina Orange. Bot. Mar. 45:486-489.

Ginestet, F., Laboratoires Goëmar. 1994. Recherches et Mise en Oeuvre des Biostimulants. Lien horticole. 14:22.

Haroun- Bouhedja F., M. Ellouali, C. Sinquin and C. Boisson-Vidal. 2000. Relationship between Sulfate Groups and Biological Activities of Fucans. Thromb. Res. 100:453–459.

Ho, Y.B., 1981. Mineral Element Content in *Ulva lactuca* L. with Reference to eutrophication in Hong Kong Coastal Waters. Hydrobiologia 77:43–47.

Ismail O.M and M. Kardoush. 2011. The Impact of Some Nutrients Substances on Germination and Growth Seedling of *Pistacia vera* L. Aust. J. Basic Appl. Sci. 5(5):115-120.

Jiang, H., B. Gao, W. Li, M. Zhu, C. Zheng, Q. Zheng and C. Wang. 2013. Physiological and Biochemical Responses of *Ulva prolifera* and *Ulva linza* to Cadmium Stress. Sci. World J.

Khan, W., U.P. Rayirath, S. Subramanian, M.N. Jithesh, P. Rayorath, D.M. Hodges, A.T. Critchley, J.S. Craigie, J. Norrie and B. Prithiviraj. 2009. Seaweed Extracts as Biostimulants of Plant Growth and Development. J. Plant Growth Regul. 28:386–399.

Kumar, G. and D. Sahoo. 2011. Effect of Seaweed Liquid Extract on Growth and Yield of *Triticum aestivum* var. Pusa Gold. J. Appl. Phycol. 23:251–255.

Lill, J.-O., S. Salovius-Laurén, L. Harju, J. Rajander, K.-E. Saarela, A. Lindroos, and S.-J. Heselius. 2012. Temporal Changes in Elemental Composition in Decomposing Filamentous Algae (*Cladophora glomerata* and *Pilayella littoralis*) Determined with PIXE and PIGE. Sci. Total Environ. 414:646–652.

Lin, T., Q. Lai, Z. Yao, J. Lu, K. Zhou and H. Wang. 2013. Combined Effects of Carbonate Alkalinity and pH on Survival, Growth and Haemocyte Parameters of the Venus clam Cyclina sinensis. Fish Shellfish Immunol. 35:525–531.

Marfaing, H. and Y. Lerat. 2007. Les Algues Ont-Elles Une Place en Nutrition? Phytothérapie 5:2–5.

Martin, G. 1987. Point Sur l'épuration et le Traitement des ef f Luents (Air, Eau) 3:298. Lavoisier: Phosphore.

Melis, A. and H.-C. Chen. 2005. Chloroplast Sulfate Transport in Green Algae – Genes, Proteins and Effects. Photosynth. Res. 86:299–307.

Norrie J. and J.P. Keathley. 2006. Benefits of *Ascophyllum nodosum* Marine-Plant Extract Applications to 'Thompson Seedless' Grape Production. (Proceedings of the Xth International Symposium on Plant Bioregulators in Fruit Production, 2005). Acta Hort. 727:243–247.

Park, J., H.-F. Jin, B.-R. Lim, K.-Y. Park and K. Lee. 2010. Ammonia Removal from Anaerobic Digestion Effluent of Livestock Waste using Green Alga Scenedesmus sp. Bioresour. Technol. 101:8649–8657.

Pérez- Mayorga, D.M., L.B. Ladah, J.A. Zertuche-González, J.J. Leichter, A.E. Filonov and M.F. Lavín. 2011. Nitrogen Uptake and Growth by the Opportunistic Macroalga Ulva lactuca (Linnaeus) during the Internal Tide. J. Exp. Mar. Biol. Ecol. 406:108–115.

Pedersen M.F. and J. Borum. 1997. Nutrient Control of Estuarine Macroalgae: Growth Strategy and the Balance between Nitrogen Requirements and Uptake. Mar. Ecol. Prog. Ser. 161:155–163

Rathore, S.S., D.R. Chaudhary, G.N. Boricha, A. Ghosh, B.P. Bhatt, S.T. Zodape and J.S. Patolia . 2009. Effect of Seaweed Extract on the Growth, Yield and Nutrient Uptake of Soybean (*Glycine max*) under Rainfed Conditions. South Afr. J. Bot. 75:351–355.

Rissler, H.M., E. Collakova, D. DellaPenna, J. Whelan and B.J Pogson. 2002. Chlorophyll Biosynthesis. Expression of a Second Chl I Gene of Magnesium Chelatase in *Arabidopsis* Supports Only Limited Chlorophyll Synthesis. Plant Physiol. 128:770–779.

Runcie, J.W., R.J. Ritchie and A.W.D. Larkum. 2003. Uptake Kinetics and Assimilation of Inorganic Nitrogen by *Catenella nipae* and *Ulva lactuca*. Aquat. Bot. 76, 155–174.

Schmidt R.E., E.H. Ervin and X. Zhang. 2003. Questions and Answers About Biostimulants. Golf Course Manage 71:91-94

Sode, S., A. Bruhn, T.J.S. Balsby, M.M. Larsen, A. Gotfredsen and M.B. Rasmussen. 2013. Bioremediation of Reject Water from Anaerobically Digested Waste Water Sludge with Macroalgae (*Ulva lactuca*, *Chlorophyta*). Bioresour. Technol. 146:426–435.

Tsagkamilis, P., D. Danielidis, M.J. Dring and C. Katsaros. 2010. Removal of Phosphate by the Green Seaweed *Ulva lactuca* in a Small-Scale Sewage Treatment Plant (Ios Island, Aegean Sea, Greece). J. Appl. Phycol. 22:331–339.

Turan, M. and C. Köse. 2004. Seaweed Extracts Improve Copper Uptake of Grapevine. Acta Agr. Scand. 54:213–220.

Van Khoi, L. and R. Fotedar. 2011. Integration of Western King Prawn (Penaeus latisulcatus Kishinouye, 1896) and Green Seaweed (*Ulva lactuca* Linnaeus, 1753) in a Closed Recirculating Aquaculture System. Aquaculture 322–323, 201–209.

Vasconcelos, M.T.S.D. and M.F.C. Leal. 2001. Seasonal Variability in the Kinetics of Cu, Pb, Cd and Hg Accumulation by Macroalgae. Mar. Chem. 74, 65–85.

Vijayabaskar, P. and N. Vaseela. 2012. *In Vitro* Antioxidant Properties of Sulfated Polysaccharide from Brown Marine Algae *Sargassum tenerrimum*. Asian Pac. J. Trop. Dis. 2:890–896.

Wagner, G. and E. Bellini. 1976. Light-dependent Fluxes and Compartmentation of Calcium in the Green Alga Mougeotia. Z. Für Pflanzenphysiol. 79:283–291.

Wolf-Gladrow, D.A., R.E. Zeebe, C. Klaas, A. Körtzinger, A.G. Dickson. 2007. Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. Mar. Chem. 106:287–300.

Yaich, H., H. Garna, S. Besbes, M. Paquot, C. Blecker and H. Attia 2011. Chemical Composition and Functional Properties of *Ulva lactuca* seaweed Collected in Tunisia. Food Chem. 128:895–901.

Zhang, X., E.H. Ervin and R.E. Schmidt. 2003. Plant Growth Regulators Can Enhance the Recovery of Kentucky Bluegrass Sod from Heat Injury. Crop Sci. 43:952.