INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 8, No. 4, p. 125-134, 2016

RESEARCH PAPER OPEN ACCESS

Growth and chemical composition of hydroponically cultivated *Lactuca sativa* using phytoplankton extract

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Article published on April 30, 2016

Key words: *Lactuca sativa*, Hydroponic system, Phytoplankton extract, *Gephyrocapsa oceanica*, Growth and chemical composition.

Abstract

The implementation of sustainable agricultural practices, which allow for more efficient utilization of natural resources, as well as reduced pollutant emissions, has become an imperative. Given this context, different cultivation solutions, such as hydroponic methods and alternative fertilizer sources, should be considered. This study evaluated the potential of phytoplankton, *Gephyrocapsa oceanica*, as a substitute for secondary macronutrients and micronutrients.. Lettuce plants (*Lactuca sativa* L. var. *capitata*) were grown in a non-circulating hydroponic system in order to test different nutrient solutions. We assessed four different growing media containing: distilled water without added nutrients, distilled water enriched exclusively with nitrogen, phosphorus, and potassium, distilled water enriched with nitrogen, phosphorus, and potassium, and *Gephyrocapsa oceanica* extract, and distilled water enriched with traditional inorganic fertilizers. Growth parameters of the treated lettuce, such as the fresh and dry weight of the shoot and roots, head diameter, root length, leaf area, and specific leaf area were determined. Additionally, we evaluated plant composition in terms of micronutrient profile (Ca, Mg, Fe, Mn, Zn) and crude protein and fiber production (neutral detergent fiber NDF, acid detergent fiber ADF, acid detergent lignin ADL, cellulose, hemicellulose). Lettuce plants grown with *Gephyrocapsa oceanica* extract presented complete development and agronomic parameters, comparable to those of plants cultivated using the conventional nutrient solution. With emphasis to all the parameters, phytoplankton extract result to be suitable for use in hydroponic cultivation and may serve as a promising tool in sustainable agriculture.

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Introduction

Economic growth and the increase in world population have contributed to a drastic rise in the demand for food and natural resources. Consequently, the agricultural sector has grown over the past 50 years; cultivable areas have been expanded and the use of irrigation and fertilizer has become more widespread, especially since the end of the 1960s. According to continuous agricultural expansion scenarios, there will be an 18% increase in natural ecosystems converted into land to be used for cultivation by 2050 (Tilman *et al.,* 1999). This projected change will be accompanied by an increase in the use of chemical fertilizers and pesticides as well as water demand. This can lead to environmental change on both the local and global scale (with effects such as water eutrophication and soil acidification) and have a drastic impact on the natural ecosystem. Accordingly, developing new strategies for more competitive and sustainable agriculture through cultivation solutions has become an imperative.

Despite these constraints, Azoreans may overcome these issues through different cultivation solutions and taking advantage of the islands' abundant natural ocean resources. In this context, hydroponics, a technique in which plants are grown in a nutrient solution without use of soil may present an alternative to traditional cultivation methods. Moreover, the use of ocean phytoplankton, which are rich in elements that, in small quantities, are essential for plant development, could serve as a substitute for traditional fertilizers. The production of inorganic fertilizer is an energy-intensive process that uses raw materials, requires electricity, petroleum, and natural gas for manufacturing and transportation, and entails pollutant emission. Thus, the development and implementation of alternative solutions could reduce these negative impacts on the environment and reduce the economic losses associated with inorganic fertilizers.

Hydroponics is currently used in laboratory experiments and in extensive crop production due to its numerous advantages: it reduces the need to control soil-borne diseases, pests, and weeds through the use of herbicides and pesticides (Chinta *et al.,* 2014). It also allows for more efficient water use as well as for the precise control of plant nutrition (Fallovo *et al.,* 2009) and development (Zekki *et al.*, 1996), which decreases soil degradation and the need for fertilizers. Moreover, it is safe for the environment (Nhut *et al.*, 2006) and can be applied in regions with soil-related problems and limited cultivable land.

Traditional hydroponic methods feature circulating closed systems, where the nutrient solution is re-circulated which allows for increased water and nutrient efficiency and reduces its environmental impact. However, the utilization of the circulating systems is limited by the availability of electricity associated with operating the pump. An alternative is the non-circulating or passive hydroponic method, which avoids the use of electrical pumps for mechanical aeration and circulation, saving energy. The most common non-circulating hydroponic system consists of a covered tank that is filled with 4 to 8 liters of nutrient solution per plant prior to planting. Plants are grown in suspended net pots and supported by an expanding polystyrene cover held in a fixed position on the top of the growing tank. The lower portions of the pots are initially immersed in the nutrient solution and the plants are automatically watered by capillary wetting of the growing medium in the net pots. While the plants are growing, the nutrient solution level drops below the net pots, which creates and enlarges the moist air space. In this way, the portion of the expanding root system occupying the moist air space supplies aeration to the plant while the portion that extends through the solution is capable of absorbing nutrients.

Phytoplankton, highly diverse unicellular autotrophs that are passively transported in the water column, can be considered a good source of nutrients. Their cellular elemental composition includes primary macronutrients (nitrogen, potassium, phosphorus), secondary macronutrients (silica, calcium, magnesium), and micronutrients (iron, manganese, zinc, copper, lead) (Ho *et al.*, 2003; Twining and

Baines, 2013). Moreover, phytoplankton takes CO_2 from the atmosphere and uses this carbon to produce biomass, being responsible for over half of the global net primary biomass production per year (Behrenfeld *et al.*, 2001). Phytoplankton is rich in bioactive compounds and is already used in various capacities, such as in human food supplements, medicinal products with antimicrobial, antibiotic, and anticancer properties (Ordog *et al.*, 2004) as well as in bioremediation, and as an energy source in biofuel production (Metting, 1996). Despite its having various applications, the use of phytoplankton in agriculture is still largely unexplored and information on the elemental composition of specific microalgae species is scarce.

In the present study, we investigated the potential use of a calcifying microalga *Gephyrocapsa oceanica*, which is naturally rich in calcium, as a substitute for secondary macronutrients and micronutrients in a conventional hydroponic nutrient solution. Hydroponic growing media were tested utilizing a low-cost, low-maintenance non circulating system (Kratky, 1993; Kratky, 2005; Kratky, 2010) which only requires initial water and nutrients, can function without electricity and pumps, and can be easily built from locally obtained materials. Lettuce plants (*Lactuca sativa* L. var. *capitata*) were chosen for the experiment since they are the plant that is most commonly cultivated through hydroponics and because they are a model crop due to their fast growth and high sensitivity to nutrient concentrations. We compared the use of phytoplankton extract (produced from cell lysis) to the use of conventional secondary macronutrients and micronutrients in hydroponic nutrient solutions. Specifically, we assessed growth parameters of the treated lettuce, such as the fresh and dry weight of the shoot and roots, head diameter, root length, leaf area, and specific leaf area. Additionally, we evaluated plant composition in terms of micronutrient profile and crude protein and fiber production.

Materials and methods

Experimental setup

The study was conducted on the Terceira Island Campus of the University of Azores between March and May 2014. The experiment was carried out using lettuce seeds (*Lactuca sativa* L. var. *capitata*) grown in a non-circulating hydroponic system. Plants were kept in greenhouse conditions, temperatures ranged between 15°C and 36°C, and light was provided through natural radiation.

Suspended pot non-circulating hydroponic systems were constructed following the method described by Kratky (1993). Systems consisted of polystyrene tanks coated with black polyethylene and filled with 6 liters of nutrient solution per plant. Plants were placed in plastic net pots supported by the tank wood cover and positioned randomly and at an equidistant distance with a plant density of 1.86 plants per square meter (Fig. 1).

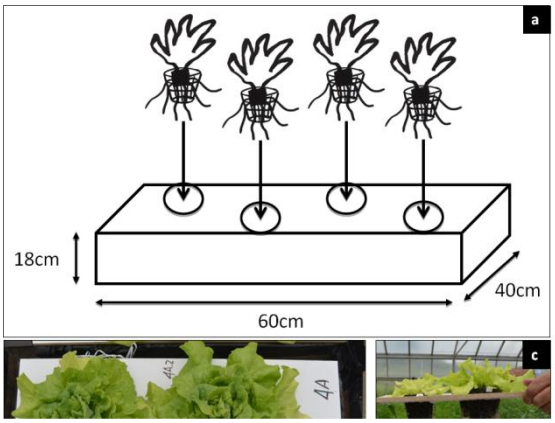

Fig. 1. a) Schematic of suspended pot non-circulating hydroponic system constructed following the method described by Kratky (1993); b) Plants of lettuce (Lactuca sativa L. var. capitata) after 5 weeks; c) Lettuce plants grown in plastic net pots supported by the tank wood cover; roots extend down through an air space below the panels and into the nutrient solution.

Lettuce plants were grown with four treatments: no nutrients, distilled water without added nutrients; primary macronutrients, which consist of distilled water containing exclusively nitrogen, phosphorus, and potassium at the following concentration: N, 93

mg L^{-1}, P, 33 mg L^{-1}, and K, 108 mg L^{-1}; primary macronutrients + phytoplankton extract, composed of distilled water enriched with nitrogen, phosphorus, and potassium at the following concentration: N, 93 mg L^{-1}, P, 33 mg L^{-1}, and K, 108 mg L^{-1} and phytoplankton extract containing Ca, 101 mg L^{-1}, Mg, 8 mg L^{-1}, Fe, 3 mg L^{-1}, Mn, 0.2 mg L^{-1}, Zn, 0.2 mg L^{-1}, Cu, 0.2 mg L^{-1}; inorganic fertilizer, which consist of distilled water enriched with N, 93 mg L^{-1}, P, 33 mg L^{-1}, and K, 108 mg L^{-1}, Ca, 110 mg L^{-1}, Mg, 18 mg L^{-1}, S, 23 mg L^{-1}, Fe, 2 mg L^{-1}, Mn, 1 mg L^{-1}, Zn, 0.3 mg L^{-1}, Cu, 0.3 mg L^{-1} and Mo, 0.05 mg L^{-1} as described by Kratky (1993). As the proportion of nutrients varies between phytoplankton extract and the inorganic fertilizer, we chose to follow a proportion based on the concentration of calcium, which is the secondary macronutrient that lettuce plants require the most. The pH of the nutrient solutions were measured potentiometrically with an electrode cell (WTW 340i pH meter) and adjusted to a value of 6.3-6.5 with HCl 3M and NaOH 0.1M. The electrical conductivity was determined conductively through a conductivity cell (WTW 340i cond) and adjusted to a value ranging between 1.5 and 2.0 dS m^{-1}. Eight plants were subjected to each treatment, for a total of 32 pots.

Phytoplankton extract

Monospecific cultures of the coccolithophore *Gephyrocapsa oceanica* were grown in sterile, filtered North Atlantic seawater (0.2 μm) enriched with nitrate, phosphate, trace metals and vitamins according to the f/40 medium (Guillard, 1975). When the cultures reached the stationary phase of growth, the cells were let to sediment and the sea water was removed. In order to break cellular membranes and obtain cell lysis, cells were dried at 90°C for 72 hours and subsequently re-suspended in ultrapure water; afterwards, the solution obtained was centrifuged (at 4000 rpm) and frozen at -20°C for 24 hours. The extract was then stored at -80°C until the experiment was conducted to preserve nutrient composition and guarantee cellular lysis. For micronutrient content quantification, sub-samples of *Gephyrocapsa oceanica* extract were reduced to ash at 500°C for 8 hours. Mineral content (magnesium, calcium, iron, manganese, zinc) was determined by re-suspending the ash in 3M HCl (3:25w/v), according to Duque (1971), and assaying the obtained solution using atomic absorption spectroscopy (Varian, Spectra 240FS). The phytoplankton extract was then defrosted and added to ultrapure water already enriched with nitrogen, phosphorus, and potassium in the previously described concentrations.

Plant material and growth conditions

In order to reduce contamination, seeds were surface sterilized in 7% sodium hypochlorite for 3 minutes and rinsed 5 times with ultrapure water. Afterwards, seeds were pre-germinated for 24 hours at 20°C in 9 cm petri dishes between two layers of Whatman No.181 paper filters soaked with sterilized ultrapure water. The seeds were then transferred to rockwool cubes (2.5 cm × 2.5 cm × 2.5 cm) and germinated until the four-leaf stage in ultrapure water at greenhouse light and temperature conditions. During the seedling period, no nutrient solution was applied. Uniformly-sized lettuce seedlings at the four-leaf stage were transplanted into plastic net pots and placed in the non-circulating hydroponic systems.

Harvest and plant growth measurement

Plants were harvested 5 weeks after transferring them to the net pots in the non-circulating hydroponic system. Immediately upon harvesting, shoot and root parts were separated by hand-cutting and fresh parameters of all lettuce plants from each of the treatment groups were evaluated. Fresh measurements included shoot weight, root weight, head diameter, root length, leaf area, and specific leaf area. In order to determine the fresh weight of shoots and roots, both parts of each plant were weighed using a scale. Root lengths were measured with a meter ruler from the bottom of the shoot to the tip of the longest root in centimeters to one decimal of precision. The leaf area of each plant was measured with a LA meter Delta-T Device; specific leaf area (mm^2 mg^{-1}) was determined by dividing the measured leaf area by the dry weight.

Chemical analysis

Plant tissue samples, both root and shoot tissues, were dried at 65°C in a forced drying oven to constant weight, in order to obtain the dry shoot weight and dry root weight. Furthermore, the total dry matter was determined according to the Weende analytic scheme (A.O.A.C., 1975). Fiber fractions (NDF [neutral detergent fiber], ADF [acid detergent fiber], and ADL [acid detergent lignin]) were evaluated as per the procedure developed by Goering and Van Soest (1970). Crude protein was determined according to the Kjeldhahl method and following Weende analytic scheme (A.O.A.C., 1975). Neutral detergent fiber (NDF) includes the total insoluble fiber in the plant cell wall, primarily cellulose, hemicellulose and lignin. Acid detergent fiber (ADF) is primarily cellulose and lignin, while acid detergent lignin (ADL) is primarily lignin. The quantity of cellulose was calculated as the difference between acid detergent fiber (ADF) and acid detergent lignin (ADL). The quantity of hemicellulose was calculated as the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF). Subsequently, 0.5 g portions of both dried root and shoot plant tissues were reduced to ash at 500°C for at least 8 hours. Calcium, magnesium, iron, manganese, and zinc concentrations were determined by re-suspending the ash in 3M HCl (3:25w/v) according to Duque (1971) and assaying the obtained solution through atomic absorption spectroscopy (Varian, Spectra 240FS Detection limits: Ca < 15 μg / 100 g dry mater, Mg<1.5 μg / 100 g dry mater, Fe < 0.25 μg / 100 g dry mater, Mn < 0.075 μg / 100 g dry mater, Zn < 0.075 μg / 100 g dry mater).

Statistical analysis

Results were expressed as the mean and standard errors (SE) and their differences were tested for significance. All measurements were evaluated using SPSS (Statistical Package for the Social Sciences) by one way analysis of variance (ANOVA) and differences of $p < 0.05$ by LSD post hoc test were considered to be significant.

Results and discussion

The biomass measurements of lettuce treated with the nutrient solution are shown in Table 1.A. Plants cultivated in distilled water with no added nutrients did not achieve mature growth, while lettuce plants grown in nutrient solution containing exclusively primary macronutrients showed reduced development. Treatments containing phytoplankton extract and traditional inorganic fertilizer had no statistically significant effect ($P<0.05$) on the shoot biomass of the plants. The biomass production of lettuce leaves, estimated as the means of fresh weight, dry weight, leaf area and head diameter per treatment, were similar in the treatment containing phytoplankton extract and in the treatment containing inorganic fertilizers, but significantly greater than the plants grown in the nutrient solution containing exclusively nitrogen, phosphorus, and potassium.

The fact that the increase of lettuce shoot biomass was greater in primary macronutrients + phytoplankton extract and in inorganic fertilizer treated plants compared to primary macronutrients treatment could be related to the enlarged leaf area and head diameter. Plants with larger leaves have been observed to have better light interception, leading to a significant increase in shoot biomass (Lin *et al.*, 2013); nevertheless, nutrient solutions with high or low nutrient concentrations cause a reduction in shoot growth rate and, consequently, a reduction in leaf size (Fallovo *et al.*, 2009). Furthermore, leaf area was used in the calculation of specific leaf area, which is a good indicator of the fundamental compromise between rapid biomass production and efficient conservation of the acquired resources that are necessary in proper plant functioning (Poorter and De Jong, 1999; Garnier *et al.*, 2001). Lettuce plants grown in the nutrient solution enriched with primary macronutrients + phytoplankton extract showed a greater specific leaf area, indicative of puffiness and a loose shoot structure, than plants treated with primary macronutrients and inorganic fertilizer. The higher specific leaf area of lettuce plants treated with primary macronutrients + phytoplankton extract can

be considered a good indicator of higher photosynthetic surface area per unit investment in leaf tissue (Lin *et al.*, 2013). This loose shoot structure allows for greater light interception and, consequently, faster production of biomass, although it may cause less efficient nutrient retention. Plants with low specific leaf area values generally have high dry matter content, exhibit longer leaf and root longevity, greater nutrient retention, and superior resistance to desiccation (Ackerly *et al.*, 2002; Lin *et al.*, 2013). None of the treatments resulted in significant differences in root development, however lettuce exposed to primary macronutrients + phytoplankton extract and inorganic fertilizer treatments exhibited a significantly greater dry shoot / root ratio than plants grown in nutrient solution containing only primary macronutrients. Primary macronutrients + phyto-plankton extract - treated plants and inorganic fertilizer - treated plants exhibited dry shoot / root ratio mean values that ranged from 6.85 to 8.43, indicating that an imbalance between shoot and root growth did not occur. Meanwhile, the primary macronutrient - treated plants showed a ratio of 4.28, which was significantly lower, indicating that the microelement concentration in the nutrient solution affected shoot and root development. Both low and high dry shoot / root ratios indicate an imbalance between shoot and root growth; low values indicate that a higher portion of dry weight accumulation occurred in the developing root system than in the shoot system (Soundy, 2005).

Table 1. A) Influence of nutrient solution composition on key growth parameters: shoot fresh weight (shoot FW), root fresh weight (root FW), shoot dry weight (shoot DW), root dry weight (root DW), shoot/root dry weight ratio (S/R DW Ratio), leaf area (LA) and specific leaf area ratio (SLA Ratio). B) Influence of nutrient solution composition on lettuce composition in terms of dry matter (DM), crude protein, neutral detergent fibre (NDF), acid detergent fibre production (ADF), acid detergent lignin (ADL), cellulose and hemicellulose.

Parameters		Treatments		
		Primary Macronutrients	Primary Macronutrients + Phytoplankton Extract	Inorganic Fertilizer
A) Growth Measurement	Shoot FW (g/plant)	13.67 (2.41)^{a}	63.02 (15.89)^{b}	72.85 (5.16)^{b}
	Root FW (g/plant)	7.11 (1.37)^{a}	11.2 (2.51)^{ab}	14.06 (1.22)^{b}
	Shoot DW (g/plant)	1.29 (0.79)^{a}	5.42 (0.52)^{b}	8.75 (0.55)^{b}
	Root DW (g/plant)	0.41 (0.12)^{a}	0.82 (0.52)^{a}	1.07 (0.10)^{a}
	Shoot/Root DW Ratio	4.28 (0.72)^{a}	6.84 (0.61)^{b}	8.42 (0.46)^{b}
	Head diameter (cm/plant)	16,6875 (0.95)^{a}	23.93 (2.69)^{b}	28.62 (0.82)^{b}
	Root length (cm/plant)	36.37 (3.46)^{a}	38.75 (7.14)^{a}	37.87 (2.14)^{a}
	LA (cm^2/plant)	186.25 (32.77)^{a}	1204.75 (303.89)^{b}	1203.75 (80.96)^{b}
	SLA (mm^2 mg^{-1}DW)	160.70 (21.92)^{ab}	199.75 (16.58)^{a}	137.69 (3.03)^{b}
B) Lettuce Composition	Crude protein (g/g plant)	0.29 (0.01)^{a}	0.71 (0.07)^{b}	0.69 (0.03)^{b}
	NDF (g/g plant)	0.19 (0.01)^{a}	1.06 (0.07)^{b}	1.80 (0.14)^{c}
	ADF (g/g plant)	0.14 (0.01)^{a}	0.77 (0.08)^{b}	0.99 (0.04)^{c}
	ADL (g/g plant)	0.08 (0.01)^{a}	0.44 (0.06)^{b}	0.52 (0.10)^{b}
	Cellulose (g/g plant)	0.07 (0.01)^{a}	0.33 (0.05)^{b}	0.47 (0. 10)^{b}
	Hemicellulose (g/g plant)	0.05 (0)^{a}	0.28 (0.09)^{ab}	0.80 (0.14)^{b}

Data are expressed as means and S.E. (n=8). Means and S.E. followed by the same letter are not significantly different at $P<0.05$ by LSD test.

In contrast, high dry shoot / root ratios are indicative of poor roots that cannot supply sufficient water to large shoots. Plants with good shoot / root ratios have vigorous roots that support shoot growth by supplying water and mineral solution requirements. In lettuce plants grown in hydroponic systems, where nutrients are provided in the solution, it is not necessary for plants to develop extensive roots, and the shoot / root ratio should be of about 6.5 (Anver *et al.*, 2005). Fallovo *et al.* (2009) found that high or low nutrient solution concentrations negatively affect the growth rate of the plants, leading to a reduction of the leaf area and, consequently, to a decrease in the shoot weight. Moreover, Kursanov (1960) found that minerals and water are the limiting factors that are most capable of changing the shoot / root ratio. When the intake of minerals and water is fostered, the growth of the shoots is stimulated (Penka, 1965).

Dry matter production was greater in the plants grown in the primary macronutrients + phytoplankton extract nutrient solution and in the inorganic fertilizer nutrient solution, whereas the plants treated exclusively with primary macronutrients exhibited significantly lower dry matter production. The lower dry matter production in lettuce sampled from the primary macronutrient treatment indicates that higher levels of tissue hydration and water content.

Crude protein production was slightly higher in plants treated with primary macronutrients + phytoplankton extract than in plants grown in the inorganic fertilizer treatment, although no significant difference between the two treatments was observed. Crude protein production was significantly lower in the lettuce plants grown in the nutrient solution that contained exclusively nitrogen, phosphorus and potassium (Table 1.B).

The key chemical parameters used to evaluate the fibrosity and the energy values (neutral detergent fiber production, acid detergent fiber production, and acid detergent lignin production) of the lettuce plants are shown in Table 1.B. Neutral detergent fiber production (NDF production) and acid detergent fiber production (ADF production) were significantly different between the three treatments and greater for the plants treated with inorganic fertilizer. Moreover, the production of acid detergent lignin (ADL production) and cellulose was similar for both primary macronutrients + phytoplankton extract treated plants and all inorganic fertilizer treated plants, which also exhibited higher production than plants grown in the nutrient solution containing only primary macronutrients. Meanwhile, hemicellulose production was greater in the plants grown in the inorganic fertilizer solution and rather low in the primary macronutrients - treated plants.

Table 2. Mineral composition of leaves lettuce grown under treatments primary macronutrients + phytoplankton extract and all inorganic fertilizer expressed as mg on dry matter (DM) basis. Mineral composition of the plants grown in nutrient solution containing exclusively nitrogen, phosphorus and potassium are not shown in the table since the concentration of calcium, magnesium, iron, zinc and manganese were below the detection limit of the atomic absorption spectrometry (Mg<1.5 μg/100 g DM, Ca<15 μg/100 g DM,. Fe<0.25 μg/100 g DM, Mn<0.075 μg/100 g DM, Zn<0.075 μg/100 g DM).

Mineral	Leaf Mineral Content (mg/100 g dry matter)	
	Primary Macronutrients + Phytoplankton Extract	Inorganic Fertilizer
Mg	195.04 (11.89)[a]	100.04 (3.86)[b]
Ca	623.75 (45.6)[a]	696.13 (31.56)[a]
Fe	10.91 (2.86)[a]	15.35 (4.68)[a]
Zn	8.53 (0.80)[a]	12.11 (1.05)[b]
Mn	1.2 (0.23)[a]	12.1 (0.74)[b]

Data are expressed as means and S.E. (n=8). Means and S.E. followed by the same letter are not significantly different at P<0.05 by LSD test.

A comparison of the mineral composition of the leaves of the plants grown in the primary macronutrients + phytoplankton extract nutrient solution and in the inorganic fertilizer nutrient

solution is shown in Table 2B. The mineral composition of the plants grown in the nutrient solution containing exclusively nitrogen, phosphorus and potassium is not shown in the table since the concentration of calcium, magnesium, iron, manganese and zinc were below the detection limit of atomic absorption spectrometry. Plants treated with primary macronutrients + phytoplankton extract, compared to plants grown with inorganic fertilizer, exhibited greater values of magnesium and similar concentrations of calcium and iron; meanwhile, the concentration of zinc and manganese was slightly higher.

Conclusion

This study showed that phytoplankton extract is suitable for use in hydroponic cultivation as a complement to water and primary macronutrients (nitrogen, phosphorus, and potassium). As expected, the lettuce grown with *Gephyrocapsa oceanica* extract presented agronomic parameters comparable to those of plants cultivated with conventional nutrient solution. Therefore, our findings are relevant to regions such as the Azores, in which individuals have to contend with factors that impede conventional agriculture practices, such as limited land and resource availability and other difficulties. Additionally, the results of this study suggest that phytoplankton grown in naturally mineral - rich hydrothermal water, along with carbon dioxide sequestration, may be utilized to produce extract. In future studies, researchers could evaluate the economic and environmental advantages of producing phytoplankton extract using these methods.

Acknowledgement

This research was supported by the Azores Regional Science Fund (M3.1.7/F/028/2011). A special thanks to Meredith Cannella, Jehan Elmolla, Jorge Tiago Martins, Emanuel Silveira, Goretti Bettencourt, Lurdes Matos, Monica Ferreira, Cecilia Amaral, Tiago Maduro Dias, Francisco Reis, and João Borba for contributing to the development of this project.

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