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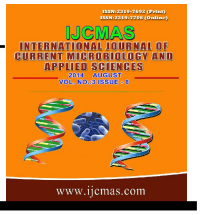

Original Research Article

Macro - and micronutrients status in *Arthrospira platensis* grown in Fresh water and brackish water medium

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A B S T R A C T

Malnutrition is considered as the most significant risk factor for illness and death. Supplementation with Spirulina *(Arthrospira)* is a solution for malnutrition resulting from inadequate nutrient in food. Spirulina is widely used as food source due to its high protein content, easier digestibility and presence of high-value nutritional components. The aim of this study is to analyze the macro- and micronutrients content in *Arthrospira platensis* cultured in freshwater with clewat (FC), freshwater without clewat (FWC), brackish water with clewat (BC) and brackish water without clewat (BWC). Rice was selected as a control. Macro- and micronutrients content in digested *Arthrospira* samples were analysed using Inductive Couple Plasma-Mass Spectometry (ICP-MS). *Arthrospira platensis* cultured in BC contains significantly higher macronutrients (360.90 ± 2.86 µg/g dry weight) while, rice contains significantly lower macronutrient content (18.42 ± 0.11 µg/g dry weight). *A. platensis* cultured in FWC has significantly higher (p <0.05) amount of micronutrients (88.88 ± 0.36 µg/g dry weight) and it is followed by FC (53.90 ± 1.10 µg/g dry weight). The superiority of macro- and micronutrients in Spirulina are evidently higher compared to rice, hence the potential of using Spirulina in alleviating malnutrition is promising.

K e y w o r d s

Arthrospira platensis; freshwater; brackish water; clewat, Macro - and micronutrients

Introduction

In the 20 and 21st century, world is experiencing rapid advancement in various fields. This is accompanied with numerous problems. One of the problems is soil depletion which is more precisely described as deterioration in soil physical, chemical and biological properties. This phenomenon occurs when the soil fertility is not replaced adequately and not maintained after been used (Dalal and Probert, 1997). Based on FAO (1994), the combined effects of growing population densities, large-scale industrial logging, agriculture practices (intense cultivation and inadequate soil management), and other factors depleted soils through rapid and almost total nutrient removal. The inadequate nutrient replacement of nutrient depleted soils as well as nutrient losses are not only intensifying soil degradation, but also threatening agricultural sustainability (Ayoub, 1999; Sheldrick *et al*., 2002).

Therefore, soil nutrient depletion is an important concern directly linked to food insecurity. This results in the continuing diminishing in crop yields under circumstances of low-input and unstable fertilization in many parts of Africa, Asia, and Latin America (FAO/UNDP /UNEP/World Bank, 1997).

Accordingly, soil depletion affects food production and nutritional composition (Tan *et al*., 2005) and hence it also affects food chain (Trussell *et al*., 2006). This is due to the direct connection between minerals in our bodies and fertility of our soil, since soil is the key factor in nutrition because our primary food comes from the earth. As depleted soils do not produce healthy and nutrient-rich crops (Harry and Graham, 1989), malnutrition became a true challenge more than hunger in developing and least developed countries (Henao and Baanante, 1999). Hence, malnutrition is considered as the most significant risk factor for illness and death, affecting mainly hundreds of millions of pregnant women and young children (Muller and Krawinkel, 2005).

Malnutrition is a state of nutrition in which insufficient, excessive or imbalanced of protein, energy or micronutrients (vitamins and minerals) that causes measurable adverse effects on our body function and clinical outcome (Puntis, 2010). Malnutrition leads to mortality, increases risk of infection, and is a major cause of sickness and death related with various syndromes (Stephen *et al*., 2002; Black, 2003). Black *et al*. (2003) stated that, malnutrition directly leads to 300,000 deaths per year and indirectly causes half of all deaths in young children. A higher degree of malnutrition is directly correlated with increasing risk of death with severely malnourished children facing considerably higher mortality risk (Chen *et al*., 1980).

Micronutrient deficiency is a type of malnutrition which leads to human physiological disorder due to consumption of crops grown in a nutrient depleted soil. Micronutrient insufficiencies have been associated with significantly high reproductive risks, ranging from sterility to fetal structural defects and long-term illnesses (Cetin *et al*., 2010). Previous studies recognized the role of trace elements in the activity of virtually all enzymes, whether within the active site of the enzyme or as co-factors, and of the vitamins as co-enzymes (Shenkin, 2006). Micronutrients play vital role in metabolism and the maintenance of tissue functions (Shenkin, 2006).

One way to alleviate malnutrition resulting from inadequate nutrient in food is through supplementation with Spirulina (*Arthrospira)*. Spirulina is widely used as food source due to its high protein content, easier digestibility and presence of high-value nutritional components like Vitamin A, chlorophyll, essential fatty acids and so on (Piorreck *et al*., 1984). Besides that, Spirulina, the blue-green algae is considered as an ideal food for astronauts by NASA and has been addressed as the 'food of the future' (Raoof *et al*., 2006). Furthermore, Spirulina is the second most vital commercial microalgae in nutritious food production and livestock feed (Vonshak and Tomaselli, 2000).

Basically, unlike terrestrial crop which grown on soil, Spirulina is growing in water containing various minerals and free from substances inhibiting nutrient uptake. Hence, water with proper nutrient supplementation enhances nutrient uptake, promoting microalgal growth and intracellular substance accumulation (Yoon *et al*., 2008). Therefore, the objective of this study is to assess the macro- and micronutrients status

in Spirulina (*Arthrospira platensis)* grown in freshwater and brackish water compared to rice which is a major food crop especially in Asia.

Materials and Methods

Culture Maintenance

The cyanobacterium *Spirulina platensis* UTEX 1926 which has been reclassified as *Arthrospira platensis* (Tomaselli *et al*., 1996) was obtained from The Culture Collection of Algae at The University of Texas, Austin (UTEX) and maintained in 10 mL test tubes at 28°C under continuous Philips TLD fluorescent light with intensity ranging 40-50 $\mu molm^{-2}s^{-1}$ for 3-4 weeks. A continuous observation was made to identify the successful bloom and was re-inoculated on stab slate agar and pour plate for stock culture maintenance. The whole process was done in pure culture room of Plant Physiology Lab, Biology Department, Universiti Putra Malaysia.

A. platensis Cultivation

There were four treatments in this study; freshwater with clewat (FC), freshwater without clewat (FWC), brackish water with clewat (BC) and brackish water without clewat (BWC). Each treatment were prepared using Kosaric medium following Tompkins *et al*. (1995). Besides that, 1 mg/L of clewat 32 (commercial mixture of various trace metals produced by Teikoku Sangyo, Inc.) consisting of Fe, Zn, Mn, Co, Cu and Bo with EDTA (Kungvankij, 1988) was added to treatments with clewat. The environmental factors in the pure culture room were controlled and monitored throughout the growth period. 12:12 h light:dark cycle photoperiods was employed. The salinity for freshwater medium and brackish water medium was maintained at 5 ppt and 15 ppt respectively.

A. platensis Harvesting

The growth of *A. platensis* was determined by optical density at 620 nm (Puganeswary *et al*., 2014) using Perkin Elmer's Lamba 25 UV/VIS spectrophotometer. Upon reaching stationary phase, *A. platensis* was harvested through centrifugation at 5000 rpm for 10 minutes. The harvested sample was freeze-dried at -40°C for 24 hours and kept in freezer at -80°C before analysed. Rice sample (control) was grinded using mortar to fine powdered form and kept in freezer at -80°C.

Sample Digestion

Digestion of *A. platensis* and rice sample was done following Huang *et al*. (2004) with modification. Prior to digestion, all the samples were dried in oven at 60°C for 24 hours to obtain dry weight. A triplicate of 1 g samples was put into digestion tube and 10 mL of nitric acid was added. The tubes were placed on digestion block and the temperature was set to 40°C for 1 hour. For next 2-3 hours the temperature was increased to 140°C. The resultant solution was filtered through Whatman GF/C (47 mm Ø) to obtain a clear solution. Finally, the solutions were left to cool to room temperature and then top up to 50 mL with double distilled water.

Elemental analysis

Elements in digested samples were analysed using Inductive Couple Plasma-Mass Spectometry (ICPMS) Perkin Elmer (Elan 6000). Concentration of each of the macro- and microelements such as Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Boron (B), Titanium (Ti), Vanadium (V), Chromium (Cr), Iron (Fe), Manganese

(Mn), Nickel (Ni), Cobalt (Co), Copper (Co), Zinc (Zn) and Selenium (Se) was analysed in µg/L and converted to µg/g of sample using following calculation:

1 g (sample)/0.05 L solution = 20 g/L (concentration of sample)

Weight of micronutrient (µg/g) =

$$\frac{\text{Concentration of micronutrient (µg/L)}}{20g/L}$$

Data analysis

The composition of micronutrients that present in control (rice) and treatments were analyzed using the SPSS software through one-way independent analysis of variance (ANOVA) and followed by Tukey HSD (Honestly Significant Difference) multiple comparison test.

Results and Discussion

Macronutrients composition

Results of macronutrients composition of *Arthrospira platensis* grown in various treatments and control (rice) is presented in Figure 1. Total macronutrients in *A. platensis* cultured in BC is significantly higher (p <0.05) among other treatments and control which is 360.90 ± 2.86 µg/g dry weight.

This is followed by *A. platensis* cultured in FWC and FC which are 305.75 ± 1.81 and 264.14 ± 6.13 µg/g dry weight respectively. *A. platensis* cultured in BWC contains 95.13 ± 2.90 µg/g dry weight which the least total macronutrient composition among other treatments. Rice contains significantly lower total macronutrient composition which is 18.42 ± 0.11 µg/g dry weight. Na and K is significantly higher (p <0.05) in *A. platensis* cultured in FWC with 143.56 ± 1.29 and 120.55 ± 0.84 µg/g dry weight respectively, while Mg and Ca is significantly higher (p <0.05) in *A. platensis* cultured in BC that is 47.47 ± 0.91 and 146.37 ± 1.19 µg/g dry weight. In overall, composition of macronutrients in *A. platensis* grown in freshwater is siginificantly higher (p <0.05) than brackish water and rice.

Micronutrients composition

Results of micronutrients composition of *Arthrospira platensis* grown in various treatments and control is presented in Table 1. *A. platensis* cultured in FWC has significantly higher (p <0.05) content of micronutrients (88.88 ± 0.36 µg/g dry weight) and it is mostly comprised of iron at 82.86 ± 0.26 µg/g dry weight. It is followed by *A. platensis* cultured in FC that contains 53.90 ± 1.10 µg/g dry weight. Both *A. platensis* cultured in BWC and rice contains significantly lower amount of micronutrients which are 2.64 ± 0.05 µg/g dry weight and 2.67 ± 0.09 µg/g dry weight respectively.

The amount of Fe and Mn are significantly higher (p <0.05) in *A. platensis* cultured in FWC that is 82.864 ± 0.25575 and 4.073 ± 0.02788 µg/g dry weight respectively. Se is significantly higher in *A. platensis* grown in FC while Cr is significantly higher in *A. platensis* grown in FC and FWC.

Roughly, 97% of the water on Earth is comprised of salt water with an average salinity of 3.5% by weight (Eakins and Sharma, 2010). Seawater contains more dissolved ions than all types of freshwater and the most abundant dissolved ions in seawater are sodium, chloride, magnesium, sulfate and calcium (Hogan, 2010). Brackish water contain more or less the similar elements composition albeit less NaCl.

Figure.1 Macronutrient composition (µg/g dry weight) in *A. platensis* cultured in freshwater medium with clewat (FC), freshwater medium without clewat (FWC), brackish water medium with clewat (BC) and brackish water medium without clewat (BWC) and rice. Sodium; ( ), Magnesium; ( ), Potassium; ( ), and Calcium; ( ). Values are presented as means of triplicates ± SE and Means within each similar patterned bar with different letters (a-d) differ significantly (p <0.05).

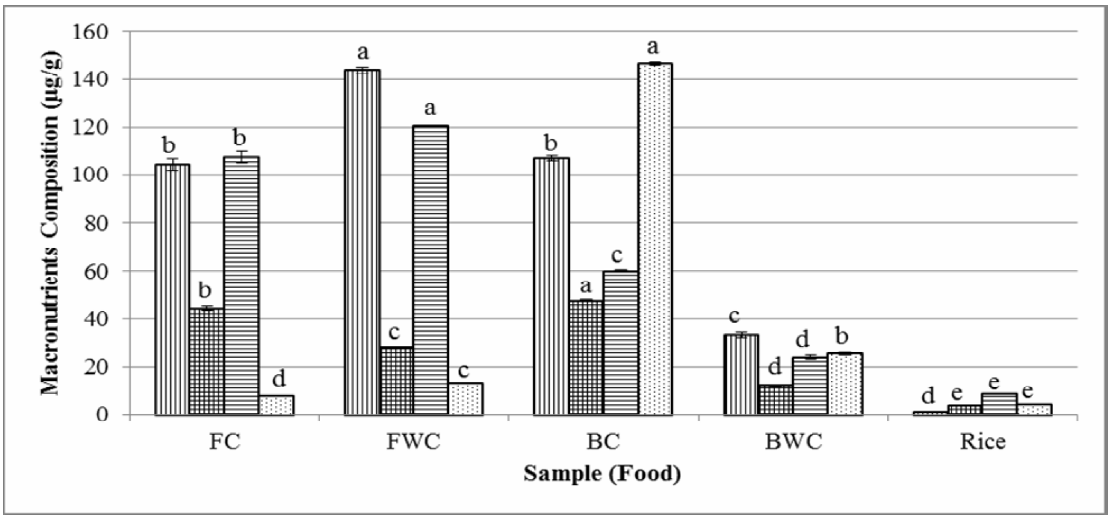

Table.1 Results of micronutrient composition in (µg/g dry weight) in *Arthrospira platensis* cultured in freshwater medium with clewat (FC), freshwater medium without clewat (FWC), brackish water medium with clewat (BC) and brackish water medium without clewat (BWC) and rice.

Elements	FC (µg/g dry weight)	FWC (µg/g dry weight)	BC (µg/g dry weight)	BWC (µg/g dry weight)	Rice (µg/g dry weight)
B	0.540 ± 0.01370^{b}	0.323 ±0.00411^{c}	0.615 ±0.00354^{a}	0.299 ±0.01395^{c}	0.248 ± 0.00358^{d}
Ti	0.113 ± 0.00361^{b}	0.091 ±0.00013^{c}	0.183 ±0.00707^{a}	0.047 ±0.00226^{d}	0.052 ±0.00009^{d}
V	0.003 ± 0.00011^{a}	0.002 ±0.00009^{b}	0.002 ±0.00007^{b}	0.001 ±0.00006^{c}	0.002 ±0.00014^{b}
Cr	0.033 ± 0.00050^{a}	0.034 ±0.00071^{a}	0.010 ±0.00132^{c}	0.003 ±0.00018^{d}	0.016 ±0.00026^{b}
Fe	48.895 ±0.95730^{b}	82.864 ±0.25575^{a}	5.516 ±0.01239^{c}	1.429 ±0.02319^{d}	1.382 ±0.06389^{d}
Mn	2.916 ±0.05884^{b}	4.073 ±0.02788^{a}	0.494 ±0.00267^{c}	0.122 ±0.00307^{d}	0.157 ±0.00053^{d}
Ni	0.099 ±0.00108^{a}	0.100 ±0.00584^{a}	0.014 ±0.00113^{b}	0.002 ±0.00064^{c}	0.005 ±0.00085^{b}
Co	0.014 ±0.00036^{a}	0.002 ±0.00013^{c}	0.006 ±0.00009^{b}	0.002 ±0.00012^{c}	0.002 ±0.00003^{c}
Cu	0.050 ±0.00096^{b}	0.052 ±0.00027^{ab}	0.050 ±0.00092^{b}	0.028 ±0.00065^{c}	0.054 ±0.00104^{a}
Zn	0.875 ±0.05048^{ab}	1.208 ±0.06161^{a}	0.744± .05543^{bc}	0.531 ±0.07023^{c}	0.651 ±0.01947^{bc}
Se	7.147 ±0.28415^{a}	2.311 ±0.06620^{c}	0.871 ±0.20648^{d}	3.597 ±0.24743^{b}	1.973 ±0.11208^{c}
Total	53.897 ±1.09707^{b}	88.876 ±0.35520^{a}	7.679 ±0.08693^{c}	2.644 ±0.05085^{d}	2.668 ±0.09482^{d}

Each value is presented as mean ± SE (n = 3). Means within each row with different letters (a-e) differ significantly (p < 0.05).

Previous studies shows that, seawater has the potential to grow *Arthrospira platensis* (Devanathan and Ramanathan, 2012; Wu *et al*., 1993). But, there is no study showing the effect of high salinity and complexing capacity of seawater on elemental accumulation in Spirulina.

Spirulina cultured in different media able to bioaccumulates minerals at different temperatures, pH, and salinity (Sharma and Azeez, 1988). In brackish water medium, salinity stress (Gabbay-Azaria and Tel-Or, 1993) and complexing capacity (Mackey, 1983) limits accumulation of macro- and micronutrients in *A. platensis*. During acclimatization process in saline medium, salt ions will be effluxed (Gabbay-Azaria and Tel-Or, 1993) and carbohydrate compound been accumulated by *A. platensis* as osmoprotector (Mary Leema *et al*., 2010). Meanwhile, the complexed trace metals that formed by presence of ligands in certain organic compounds in brackish water (Mackey, 1983), are not available to be absorbed by *A. platensis.* This has been proved in this study where by the total macro- and micronutrients were siginificantly lower ($p > 0.05$) in *A. platensis* grown BWC.

On the other hand, addition of new chelated micronutrients such as clewat in the brackish water medium, may become synergestic or antagonistic with certain elements. Presence of chelates such as EDTA in clewat (James, 1984) enables Spirulina to uptake and accumulates macro- and micronutrients. This has been proved in this study where the total macro- and micronutrients were siginificantly higher ($p < 0.05$) in *A. platensis* grown BC compared to BWC. Ronquilo *et al*. (1997) stated that addition of clewat in media showed a positive effect on cell growth of *Tetraselmis tetrathele*.

Since the affinity of complexation by ligands for most trace metals is much greater than those of the corresponding calcium or magnesium complexes (Mackey, 1983), the role of clewat is more advantageous in term of total macronutrients content in *A. platensis*. Due to hardness of brackish water, content of Ca and Mg is significantly higher ($p < 0.05$) in *A. platensis* cultured in BC (with 2:1 ratio) compared to *A. platensis* in freshwater. The role of clewat in freshwater is beneficial in boosting the macro- and micronutrients content in a balanced proportion. Eventhough *A. platensis* in FWC contains higher macro- and micronutrients content compared to FC, the proportion of each elements are not in balance. In this study, level of clewat is too low in comparison with other studies (Ronquilo *et al*., 1997; Abu-Rezq *et al*., 2010). Perhaps, higher concentration will give different value.

As mentioned in introduction, the superiority of macro- and micronutrients in Spirulina (Henrikson, 2009; Ortega-Calvo *et al*., 1993) are evidently higher compared to rice, hence the potential of using Spirulina in alleviating malnutrition is promising.

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