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Seasonal variation in the nutrient profile of Arthrospira fusiformis biomass harvested from an Ethiopian soda lake, Lake Chitu

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Abstract:	Abstract The extent of seasonal variation in the nutrient profile of Arthrospira biomass harvested from Lake Chitu was investigated to evaluate the variability of the quality of the product over a period of a year. Protein content varied from 47.9 to 55.7% for wet season biomass samples and from 39.2 to 40.8% for dry season samples. Dry season samples were characterized by relatively higher carbohydrate values (38.0 - 41.3%). Higher proportion of amino acids and unsaturated fatty acids were recorded for biomass harvested in wet season. Similarly, higher contents of phytonutrients (pigments) were recorded for wet season biomass samples: chlorophyll a (8.2-10.3mg g-1), phycobiliproteins (104.1-120.7mg g-1), total carotenoids (3.17-4.31mg g-1) and β-carotene (1.24-1.61mg g-1). The contents of Na and K were higher for a dry season biomass whereas other major (Ca, P, Mg) and trace (Mn, Fe, Cu, Zn, Se) minerals were found relatively in higher quantities in a wet season biomass. The nutritional composition of Arthrospira from Lake Chitu was found to be relatively comparable to that found in commercial Arthrospira products in the market. The significance of the findings is discussed in relation to potential sustainable production of Arthrospira biomass from this lake.
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	Sincerely,

Seasonal variation in the nutrient profile of *Arthrospira fusiformis* biomass harvested from an Ethiopian soda lake, Lake Chitu

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Introduction

Arthrospira is known for its excellent nutritional composition as it contains high protein, essential fatty acids, minerals (especially iron), vitamins (especially beta-carotene and vitamin B12) and various bioactive phytonutrients with potential therapeutic effects (Belay 2002). For instance, its blue pigment, C-phycocyanin is reported to have antioxidant (Riss et al. 2007; Guan et al. 2009), anti-inflammatory (Shih et al. 2009), hepatoprotective (Aissaoui et al. 2017), neuroprotective (Bermejo-Bescós et al. 2008) and anti-cancer (Subhashini et al. 2004) effects. Besides, this pigment has high economic potential ~~by being used~~ as a natural pigment for food, feed, drug and cosmetics (Cohen 1997). Carotenoids are also found in this cyanobacterium ~~in large quantities~~ (Mendiola et al. 2005). Carotenoids are strong antioxidants as they are capable of scavenging potentially harmful radicals, which are commonly associated with the induction of some types of cancers (Stahl and Sies 2005). Secondary metabolites from *Arthrospira* ~~have also got~~also have some biotechnological applications. For instance, a novel sulfated-polysaccharide isolated from *Arthrospira*, Calcium Spirulan (Ca-SP), was found to be an inhibitor in the replication of several enveloped viruses including HIV-1 virus in vitro (Hayashi et al. 1996). This isolate has also ~~showed~~ shown ~~an~~ inhibitory activity against metastasis and tumor invasion (Mishima et al.1998). Similarly, Okuyama et al. (2017) reported the ability of *Arthrospira* lipopolysacharides ~~in inhibiting~~to inhibit tumor growth. Consequently, *Arthrospira* is produced in many countries either from natural lakes or artificial pond cultures to be used as a health food or as a protein supplement (Vonshak and Richmond 1988; Belay 2013). From data obtained from websites of various companies, Belay (2013) estimated an annual production of 10,000 t~~ons~~ of *Arthrospira* worldwide.

Arthrospira grows abundantly almost as a unialgal population in an Ethiopian soda lake, Lake Chitu, throughout the year (Wood and Talling 1988; Kebede 1997). Research done so far on Lake Chitu's *Arthrospira* mainly focused on its limnological aspects such as its growth behavior and light utilization efficiency (Kebede and Alghrein 1996), ~~its~~ tolerance limits to salinity and ionic concentrations (Kebede 1997), ~~its~~ biomass concentration (Wood and Talling 1988), ~~its~~ morphological variability in relation to environmental conditions (Ogato and Kifle 2014) and absence of cyanobacterial toxins (Willen et al. 2011). However, published data on the food value of Lake Chitu's *Arthrospira* biomass are lacking. The objective of this study was to examine the nutrient profile of *Arthrospira* biomass harvested from Lake Chitu in order to assess its suitability for use as food.

Materials and Methods

Description of Lake Chitu

Lake Chitu (7°23'N 38°24'E) is a crater lake which lies at an altitude of 1600 m above sea level (Kebede et al. 1994) ~~and located~~ in the vicinity of Lake Shala about 287 km south of Addis Ababa, Ethiopia (Fig 1). The lake is within a closed basin and lacks obvious surface outlet and inflows. It receives water from hot springs located at its shores as well as from direct precipitation (Kebede 1997).

Sampling and sample preparation

Samples of *Arthrospira* biomass were collected every two months from March 2012 to January 2013. The biomass was harvested manually in plastic jars during morning hours when wind normally brings the biomass to the shallow area of the lake. Samples were taken randomly at the different sites of the lake and pooled. About 40 ~~liters~~ L of biomass samples were taken at a time. During harvesting, the algal biomass was allowed to pass through a 0.425 mm sieve to remove insects, ~~birds' hair~~feathers and other extraneous materials. The harvested biomass samples were immediately transported to laboratory, filtered under vacuum by using a ~~nylon cloth with~~ 25 µm nylon cloth~~openings~~ and washed with distilled water to remove salts. The washed biomass samples were freeze dried ~~using a freeze drier~~. The dried biomass samples were then ground~~ed~~ to powder and stored in brown bottles at -20◦C until analysis.

Rainfall and temperature data

Rainfall and temperature data of the study area during the sampling period (March 2012-January 2013) were obtained from Ethiopian Meteorological Agency. Data recorded for the nearest meteorological station to the lake, Arsi Negele station, was used ~~for the study~~. A "rainfall coefficient" for each month was calculated as the ratio between the monthly rainfall and one twelfth of the annual rainfall. A month is designated "wet"(rainy) when the monthly rainfall coefficient is greater than or equal to 0.6 (60% of the rainfall module) (Zinabu 2002).

Physical and chemical parameters

The pH of the lake was measured with a pH-meter (Oakton pH 110, Eutech Instruments ~~Pty. Ltd.~~, Singapore) and salinity was measured with a standard refractometer (0–100%, Atago ~~Co. Ltd.~~). Carbonate-bicarbonate alkalinity was determined according to Golterman et al. (1978) by titration of the water with 1N HCI to a pH of 4.5.

Water samples were also collected during wet and dry months to determine some inorganic minerals of the lake. Minerals were determined according to the standard analytical methods described in APHA/AWWA/WEF(1999): Na^+ and K^+ by flame photometric method, Ca^{2+} by direct nitrous oxide-acetylene flame method, Mg^{2+}, Fe, Zn, Mn, and Cu by direct air-acetylene flame method.

Biomass nutrient profile analysis

Proximate composition

Freeze dried *Arthrospira* samples were analyzed for their proximate composition using AOAC procedures. Moisture content was determined by drying a representative (5 g) sample for 4 h~~rs~~ at 105ºC (AOAC 2000; 925.09). Crude protein content (N x 6.25) was determined by employing the Kjeldahl method (AOAC 2000; 979.09) ~~from~~ on a 0.5g

~~*Arthrospira* powder~~sample. Lipids were quantified gravimetrically by exhaustively extracting 2 g sample in diethyl ether (boiling point, 55ºC) in a Soxhlet apparatus (AOAC 2000; 4.5.01). Ash was determined by incineration of 2.5 g sample in a muffle furnace at 550ºC until a gray ash was obtained (AOAC 2000; 923.03). Crude fiber was determined after digesting 1.5 g of sample by refluxing with 1.25% boiling sulfuric acid and 28% of boiling potassium hydroxide (AOAC 2000; 962.09). Carbohydrate content was determined as the weight difference using protein, lipid, fiber, moisture and ash content data (James 1996).

Determination of amino acids

Amino acid analysis was performed according to AOAC (2005; 994.12). Briefly, samples were oxidized with a hydrogen peroxide/formic acid/ phenol mixture. Excess oxidation reagent was decomposed with sodium metabisulfite. The oxidized samples were hydrolyzed with 6M hydrochloric acid for 24 h~~rs~~. The hydrolysate was adjusted to pH 2.2, centrifuged, and filtered. Amino acids were separated by ion exchange chromatography (Biochrom 20+ Amino Acid Analyser, Biochrom Ltd, ~~Cambridge,~~ UK) and determined after reacting with ninhydrin by using photometric detection at 570 nm. The amino acids were identified and quantified by comparing peak profiles of the proteins with amino acid profiles from external amino acid standards.

Determination of fatty acids

For fatty acids determination, lipids were extracted from 0.1 g of biomass samples with chloroform–methanol–water (2:1:0.8) according to Bligh and Dyer method (Bligh and Dyer 1959). The extracted lipid was dissolved in 2ml chloroform. 1 ~~ml~~ mL of the mixture was transferred to a new glass bottle and 100 ~~µl~~ µL of internal standard (mixture of methyl pentadecanoate glyceryl triheptadecanoate) was added ~~to it~~. Fatty acids were transmethylated by treatment with trimethylsulfonium hydroxide (Sigma). 10 ~~µl~~ µL of fatty acid methyl esters (FAMEs) was injected into the capillary column (Phenomenex Zebron, ZB-FFAP, 30 m x 0.22 mm internal diameter) using a vaporising injector (split flow of 50 mL~~/~~ min^{-1}). The oven temperature was maintained at 120°C for 1 min, and then increased to 250°C (5°C/ min^{-1}~~min~~) for 2 min~~utes~~. Hydrogen was used as a carrier gas at a flow rate of 0.5 ~~Ml~~ mL min^{-1}. Identification of fatty acids was made by comparing the relative retention times of FAME peaks of the samples with FAME standards. A standard library was also used through the "Thermo Scientific Xcalibur" software programme, to confirm identification by comparison of the mass spectrum.

Determination of mineral content

The mineral content was determined from microwave digested *Arthrospira* samples by ICP-MS (~~Thermo Fisher Scientific~~ Icap-Q; Thermo Fisher Scientific, ~~Bremen,~~ Germany). The instrument was run employing collision-cell technology with kinetic energy discrimination (CCT-KED) to remove polyatomic interferences; the collision cell gas was He. Samples were introduced from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a PEEK nebulizer (Burgener Mira Mist). Internal standards were introduced to the sample

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stream on a separate line via the ASXpress unit and included Sc (20 µg L^{-1}), Rh (10 µg L^{-1}), Ge (10 µg L^{-1}) and Ir (5 µg L^{-1}) in 2% trace analysis grade (Fisher Scientific, UK) HNO_3. External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) for Cu, Fe, Mn, , Se and Zn were prepared in the range 0 – 100 µg L^{-1} (0, 20, 40, 100 µg L^{-1}). A bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, and France) was used to create Ca, Mg, Na and K standards in the range 0-30 mgL^{-1}. Phosphorus calibration utilized an in-house KH_2PO_4 solution standard (10 mg L^{-1} P).

Determination of phytonutrients

All phytonutrients were determined spectrophotometrically using Lamda 950 UV/VIS/NIR Spectrophotometer (Perkin Elmer, USA). Chlorophyll *a* was determined according to AOAC (1995) after complete extraction (at least four times extraction) of the pigment from *Arthrospira* powder with 85% acetone. The absorbance of the extract was read at 666_nm and 642_nm against an 85% acetone blank. Chlorophyll *a* content of the samples was then calculated using the formula below.

$$\text{Chlorophyll } a\ (\%) = \frac{[(9.93 \times Abs_{666}) - (0.0777 \times Abs_{642})] \times 0.05 \text{ liter} \times 100}{\text{Sample weight (mg)} \times \%\text{dry weight}}$$

The contents of phycobilproteins were estimated following the procedure developed by Yoshikawa and Belay (2008). These pigments were extracted from *Arthrospira* powder with 0.1M Na-~~Phosphate~~ phosphate buffer (pH 6.0) and the content of each phycobiliprotein was measured at 618, 620 and 650 nm.

Total carotenoid content was estimated according to the method used by Cyanotech Corporation (2002) from the methanolic extract of the powder with subsequent extraction with diethyl ether. The maximum absorbance of the ether layer which contained the total carotenoids of the powder was measured at a wave length between 450nm and 453nm against a diethyl ether blank and the total carotenoids content was calculated using the following formula.

$$\text{Total carotenoids (\%)} = \frac{\text{Max Abs (450-453)} \times 25\text{ml} \times 1.5 \times 100}{259.2 \times \text{sample weight (mg)} \times \%\text{ dry weight}}$$

Where: Max Abs= maximum absorbance

$$\text{Percent dry weight} = \frac{\text{Weight of empty dish (g)} + \text{dried powder(g)} - \text{Weight empty dish(g)}}{\text{Powder weight (not dried) (g)}}$$

Beta-carotene was estimated from the methanolic extract of the powder with subsequent extraction with diethyl ether. The absorbance of the diethyl ether layer which contained the extracted β-carotene of the powder was measured at 436nm against a diethyl ether blank and the β-carotene content was calculated using the following formula.

$$\beta\text{-carotene (\%)} = \frac{\text{Abs 436 x 25ml X 1.25 x 100 x 0.84}}{\text{196 x sample wt (mg) X (\%) dry weight}}$$

Where: Abs =Absorbance

$$\text{Percent dry weight} = \frac{\text{Weight of empty dish (g)+ dried powder(g)-Weight empty dish(g)}}{\text{Powder weight (not dried) (g)}}$$

Statistical analysis

All data are expressed in terms of mean ± standard deviation. The effects of seasonal variation on the proximate and phytonutrients composition of the dry biomass were analyzed by one-way ANOVA followed by post-hoc analysis with Tukey HSD test using the statistical program SPSS ver. 20. The effect of seasonal variation on the mineral composition of the biomass was analyzed using student's t-test. Significant levels for all analyses were set to $p < 0.05$.

Results and discussion

Rainfall and temperature regime

Rainfall and temperature pattern of the lake area during the study period are shown in Fig 2a and b respectively. Based on the data, months from March to September were found to be wet (rainfall coefficient > 0.6) and months from November to January were found to be dry (rainfall coefficients < 0.6). The rainfall pattern was similar with that reported by Wood and Talling (1988) and Klemperer and Cash (2007) for the general climate of Ethiopian rift valley lakes where wet season ranges from March to September and dry season ranges from October to February.

The maximum air temperature of the lake area varied from 24.0 to 28.9ºC and from 26.7 to 29.4ºC for wet and dry seasons respectively. The minimum air temperature varied from 11.6 to 16.8ºC in the wet season and from 15.3 to 16.1ºC in the dry season.

Physical and chemical characteristics of Lake Chitu

Some physical and chemical characteristics of Lake Chitu are presented in Table 1. The average pH value of the lake was 10.3±0.1 and in both wet and dry seasons the pH was within the optimal pH range (9.5–10.5) that support the growth of many *Arthrospira* species (Vonshak 1997). High alkalinity and salinity values were recorded for the lake, which according to Wood and Talling (1988) is associated with the high evaporative concentration prevailing in the lake. Salinity values are usually expected to be lower in rainy seasons due to high precipitation and low evaporation. However, in this study high salinity values were recorded in wet months. Zinabu (2002) proposed the

possible reason for these phenomena as exchange with sediments within the water bodies especially during frequent mixing which mostly occurs in rainy seasons.

Table 2 shows the inorganic mineral content of Lake Chitu as compared to the synthetic Zarrouk's medium (Zarrouk 1966). Sodium and potassium levels of Lake Chitu were very high compared to other minerals of the lake and levels found in the synthetic medium which can be attributed to their large concentration in the trachytic and rhyolitic rocks of the Ethiopian rift (Klemper and Cash 2007). The Ca and Mg contents of the lake were low and this can be explained by the high alkalinity of the lake which causes the removal of these ions from the solution as carbonate precipitates (Wood and Talling 1988). This phenomenon also occurs in some commercial production facilities where the raw water used to cultivate *Arthrospira* contains high content of calcium or magnesium (Belay 1997).

Biomass nutrient profile

Seasonal variation in the proximate composition of *Arthrospira* biomass harvested from Lake Chitu is presented in Table 3. The moisture content of the samples varied from 5.1 to 6.7% for wet season and from 5.8 to 6.1 % for dry season samples with a mean value of 5.9±0.5%. The moisture content of all biomass samples were within the recommended value of such types of products (<7%) (Belay 2013) which is necessary to reduce water activity and inhibit microbial growth in the product.

The protein content of the biomass varied from 43.0 to 55.7% for wet and from 39.2 to 40.8% for dry season samples (Table 3). Wet season biomass samples had higher protein contents which may be related to nutrient availability in the lake during this season (Zinabu and Taylor 1989) as nutrients are released due to mixing caused by prevailing winds and possibly from runoff. The protein contents of wet season samples were within ranges (45–71%) reported for commercial *Arthrospira* (~~Belay 2008;~~ Ortega et al. 1993; Fox 1996; ~~Ortega et al. 1993~~Belay 2008). They are, however on the low side of values reported for most commercial products that are grown under more controlled conditions.

Changes in the carbohydrate content of the biomass were observed throughout the study period and varied from 30.9 to 36.4% for wet season and from 38.0 to 41.3% for dry season samples. Relatively higher carbohydrate contents were recorded in dry season biomass samples. Dry season is characterized by high salinity, high light intensity and nutrient deficiency (Kebede et al. 1994) which influence the synthesis of high amounts of carbohydrates (Zeng and Vonshak 1998; Olguin et al. 2001). *Arthrospira* is known to synthesize high amounts of trehalose at times of osmotic stress (Belay 2013).

In this study, the crude fat content of the biomass oscillated from 1.4 to 3.2% for wet season and from 1.9 to 2.5% for dry season samples. The fat content of the biomass was comparable to results obtained by others: 3.0% (Becker and Venkataraman 1984); 2-3 % (De la Noiie and De Pauw 1988) and 2.5% (Cafiizares-Villanueva et al. 1995).

The crude fiber content of the biomass varied from 0.7 to 0.8% for wet season and from 0.6 to 0.9% for dry season biomass samples. These values were within the lower ranges reported for commercial *Arthrospira (*0.1 to 7.7%) (Ortega et al. 1993; Fox 1996; Belay 2008~~Belay 2008; Fox 1996; Ortega et al. 1993~~).

The ash content of the biomass varied from 10.1 to 13.9% for wet season and from 16.1 to 18.7% for dry season biomass samples. All biomass samples had high ash contents compared to commercial *Arthrospira* (6.4 to 13%) (Ortega et al. 1993; Fox 1996; Belay 2008~~Belay 2008; Fox 1996; Ortega et al. 1993~~) which might be due to the high amount of carbonate salts present in the lake. Additionally, it is possible that the washings with distilled water done before the samples were freeze dried may not have removed these salts completely. Washing with dilute acid may be necessary to reduce the ash content (Richmond 1998). High ash content may also result in high heavy metal content (unpublished data).

Amino acid composition

The amino acid profile of the biomass is presented in Table 4. Higher content of amino acids was recorded for a wet season biomass which could be associated with the difference in the protein and ash content of the samples. The essential amino acids isoleucine (Ile), leucine (Leu), methionine (Met), threonine (Thr), valine (Val), phenylalanine (Phe) and histidine (His) comprised 39.7% of the total amino acids for the wet season sample and 39.5% for the dry season sample . In both wet and dry season samples, leucine (Leu) was the highest essential amino acid. Misurcova et al. (2014) also reported the dominance of leucine among essential amino acids in their study on the amino acid composition of commercial algal products including *Arthrospira*.

The non-essential amino acid content (NEAs) of wet and dry season *Arthrospira* samples comprised 60.4% and 60.5% of the total amino acids respectively. In both samples, glutamic acid (Glu) was the highest amino acid taking a share of about 18% of the total amino acids followed by aspartic acid (Asp). The glutamic acid content of the biomass (especially the wet season sample) was higher compared to that reported for commercial *Arthrospira* (Richmond 1998; Belay 2008). The EA/NEA ratio of the biomass was 0.66% for the wet season and 0.65% for the dry season sample and these values were comparable with other *Arthrospira* strains: 0.50 – 0.65% (Ortega et al. 1993) and 0.60 –0.70% (Fatma et al. 1994).

The amino acid composition of the biomass was compared with amino acid composition of artificially grown *Arthrospira* (Table 4). *Arthrospira* from Lake Chitu was inferior in the levels of most amino acids when compared to artificially grown *Arthrospira*. However, it is important to note that the quantities of sulphur containing amino acids (methionine and cysteine) were higher in Lake Chitu's *Arthrospira* compared to the artificially grown *Arthrospira* as well as values reported for commercial *Arthrospira* (Richmond 1998; Belay 2008). Ciferri (1983) indicated the existence of considerable variation in the amino acid profiles of *Arthrospira* from different sources especially in the concentration of the essential sulphur amino acids. There is currently a big interest by consumers

for plant-based proteins. Due to problems of allergy associated with soy proteins, alternative proteins are being investigated, *Arthrospira* protein being one. However, *Arthrospira* is generally deficient in sulfur-containing amino acids compared to other conventional sources like soy protein and any improvement in the profile of these amino acids will be of great advantage.

Fatty acid composition

Five fatty acids corresponding to the most abundant fatty acids reported for various *Arthrospira* strains (Ortega et al. 1993; Campanella et al. 1999; Muhling et al. 2005) were identified including another medium-chain fatty acid (lauric acid) and expressed as weight percentage of total fatty acids (Table 5). Palmitic acid (16:0) was the dominant fatty acid in both wet and dry season samples followed by linoleic acid (18:2n6), γ- linoleic acid (18:3n6) and oleic acid (18:1n9). While lauric acid (12:0) was the least abundant fatty acid for a wet season sample, it was found in relatively higher proportions in the dry season sample. The dominance of palmitic acid in the biomass is in line with results reported for artificially grown *Arthrospira* sp. (Cohen et al. 1987) which is due to the fact that the synthesis of fatty acids generally begins with saturated fatty acids in photosynthetic microorganisms (Morais et al. 2009). The gamma linoleic acid (GLA) content of the samples was found in higher quantities than that reported for some commercial *Arthrospira* (Campanella et al. 1999). GLA is a rare essential fatty acid which is found only in breast milk, the oil of primrose and *Arthrospira* and is reported to have several health benefits (Cohen et al. 1987; Dubacq and Pham-Quoc 1993).

Table 5 also shows the percentage contribution of saturated, monounsaturated and polyunsaturated fatty acids of the total fatty acids of the biomass. Relatively higher contents of saturated fatty acids were recorded for a dry season sample. Cohen et al. (1987) reported that the levels of saturated fatty acids increased in various *Arthrospira* strains when cultivation temperature increased. Although the biomass had low fat content, its fatty acid profile had some interesting characteristics. PUFAs (linoleic and GLA) contributed a high percentage of the total fatty acids in both wet and dry season samples. A higher content of polyunsaturated fatty acids (PUFA) increases the nutritional value of foods.

Mineral composition

The mineral content of the biomass is shown in Table 6. Significant differences were observed in the mineral contents of wet and dry season samples ($p<0.05$). Sodium was the most abundant mineral in both wet and dry season samples followed by potassium. The contents of these minerals were very high which might be due to the high concentration of these minerals in the lake. The mineral content of *Arthrospira* biomass from Lake Chitu is somewhat comparable to commercial *Arthrospira* with the exception of iron which is much lower in the lake's *Arthrospira* (28 ~~mg /100 g~~ vs 70 mg/ (100 g^{-1})(Belay, unpublished data). *Arthrospira* is known to reduce iron-deficiency anemia and has been studied for such in India and Africa. It is not known why iron content of the product is low even though the ash content of the biomass from the lake is significantly higher than that of commercial *Arthrospira*.

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Phytonutrients composition

Figure 3 shows seasonal variation in the phytonutrients composition of the biomass. Chlorophyll *a* contents of the biomass varied from 6.7 to 10.3 mg g^{-1} for wet season samples and from 6.5 to 7.13 mg g^{-1} for dry season samples. The presence of high concentration of chlorophyll *a* in wet season biomass might be associated with light limitation due to clouds during this season (Zinabu 2002) as cyanobacteria produce higher amounts of chlorophyll *a* to optimize the light harvesting process in lower light intensities (Ravelonandro et al. 2008; Danesi et al. 2012).

In this study, the total carotenoids content of the biomass varied from 1.97 to 4.31 mg g^{-1} for wet season and from 1.86 to 2.63mg g^{-1} for dry season samples. Similarly, the β-carotene content of the biomass varied from 0.7 to 1.61mg g^{-1} and from 0.88 to 1.0 mg g^{-1} during wet and dry season respectively. For both phytonutrients, higher values were recorded for wet season samples which may be due to synthesis of accessory pigments for light capture under the low light conditions of the wet and cloudy season (Kebede 1997). It could also be due to the higher availability of nutrients in the wet season.

The crude phycocyanin content of the biomass varied from 61.5 to 120.7mg g^{-1} for wet and from 58.3 to 98.7mg g^{-1} for dry season samples. Higher contents of these chromoproteins in wet season samples might be attributed to the low light conditions and high nutrient availability that prevail in the lake during this season. Seasonal and diurnal changes in phycocyanin content due to varying irradiation have been reported by Hidasi and Belay recently (in press).

Conclusions

The present study provided the first information on the seasonal profile of the nutritional composition of *Arthrospira* from a natural lake with the exception of that from a semi-natural Lake Texcoco in Mexico. The nutritional profile of *Arthrospira* biomass from the lake was found to be relatively comparable to commercially grown *Arthrospira*. The data presented provide baseline information for future studies on the sustainable exploitation of these natural resources. While direct harvest and utilization is not sustainable, it is possible to utilize the water and biomass from the lake to grow *Arthrospira* in ponds alongside the lake, with supplemental nutrients and recycling as is practiced in some commercial facilities in Inner Mongolia, China. Seasonal studies on biomass productivity and a thorough analysis of the safety of the product from this lake are essential in order to evaluate the economic feasibility of production of *Arthrospira.* The sustainable production and use of *Arthrospira* from this lake would no doubt have significant health and economic benefit to the local community and beyond.

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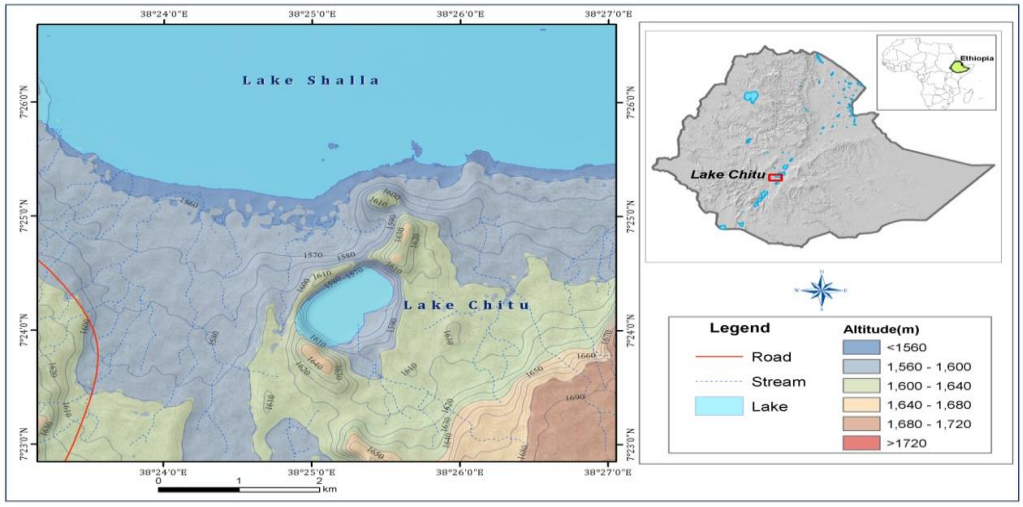
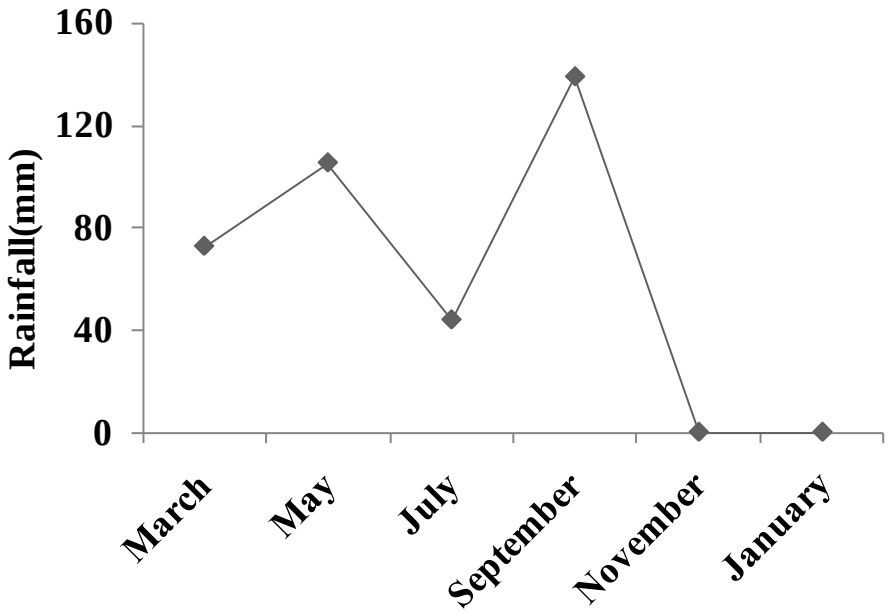
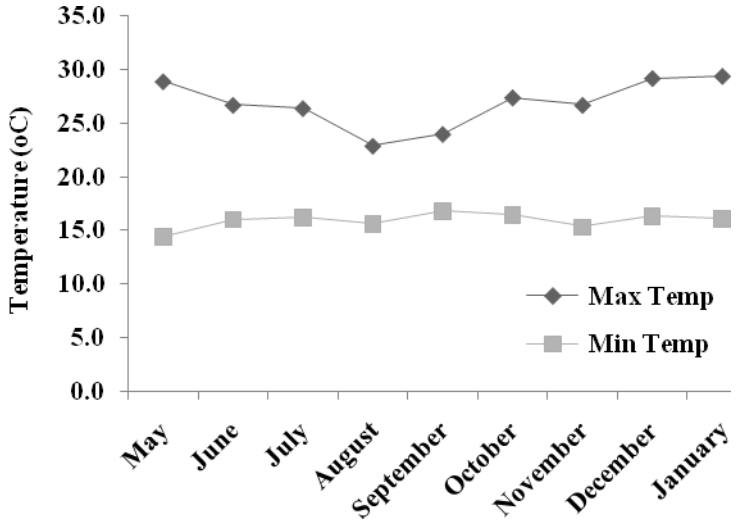


Fig 1. Location of the study site (Lake Chitu)

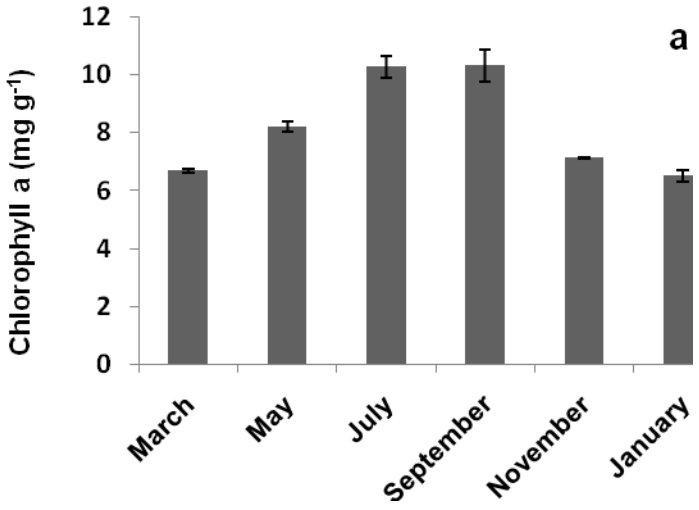


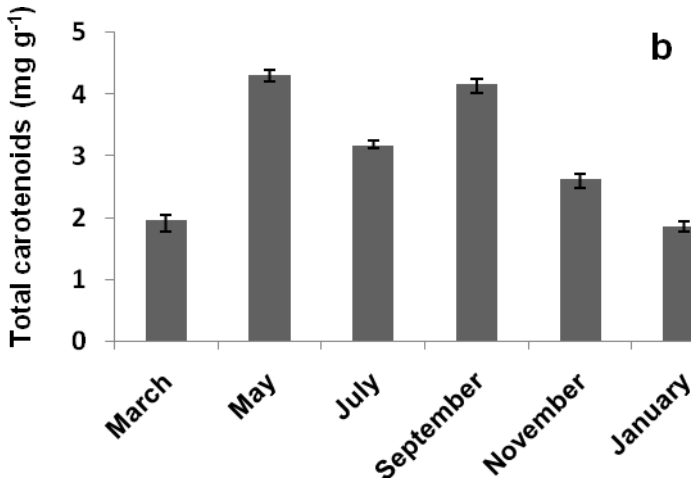
a)

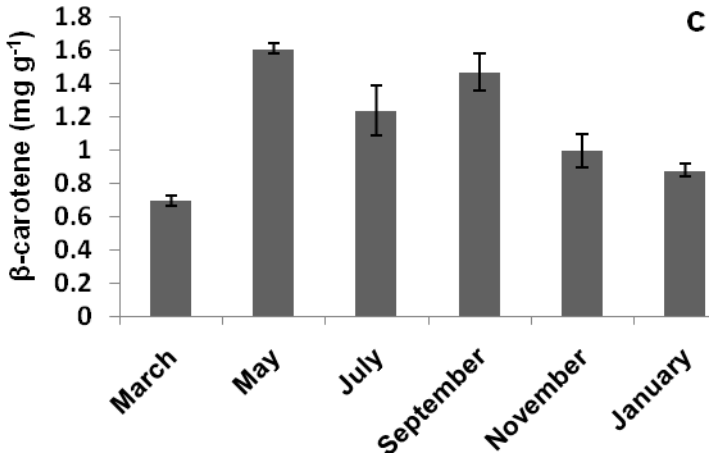


b)

Fig 2. Rainfall (a) and Temperature (b) pattern of Lake Chitu area during the sampling period (March 2012-January 2013)




b
Total carotenoids (mg g-1)
5
4
3
2
1
0
March
May
July
September
November
January


c
β-carotene (mg g-1)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
March
May
July
September
November
January

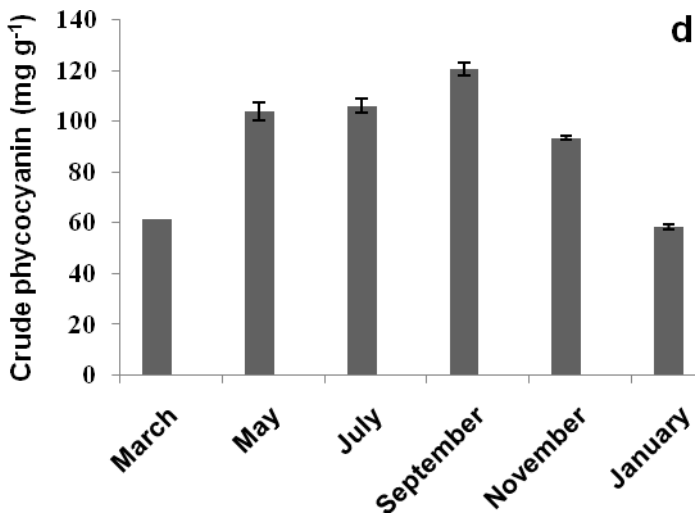


Fig 3. Seasonal variation in the phytonutrients composition of Lake Chitu's *Arthrospira (*Mean± SD; n=3). Chlorophyll *a* (a) Total carotenoids (b) β-carotene (c) and Crude phycocyanin (d). Error bars represent the standard deviation of means, n=3)

Table 1. Physical and chemical characteristics of Lake Chitu during the study period (March 2012-January 2013)

Physicochemical parameters	Mean ±SD	Range	
	(n=6)	Wet season	Dry season
pH	10.3±0.1	10.3 – 10.4	10.2 –10.3
Salinity(gL^{-1})	55±3.34	53 – 60.1	51 –55
Alkalinity($meqL^{-1}$)	681.4±44.5	637 –740	680–710

Table 2. Inorganic mineral contents of Lake Chitu as compared to Zarrouk's medium

Inorganic minerals	Lake Chitu		*Zarrouk's medium
(mgL^{-1})	Wet season	Dry season	
Na	18065	18689	3 800
K	1981	1200	125
Fe	0.22	0.18	0.4
Cu	0.034	0.022	
Zn	0.01	0.01	
Ca	n.d	n.d	13.3
Mg	1.00	0.18	7.0

* Zarrouk 1966
n.d: not detected

Table 3. Seasonal variation in proximate composition (%, dry matter) and energy value (Kcal) *Arthrospira* biomass harvested from Lake Chitu (mean ± SD, n=3)

	Lake Chitu's *Arthrospira*					
	Wet season harvest				Dry season harvest	
	March	May	July	September	November	January
Moisture	6.7±0.07^{d}	6.4±0.34^{a}	6.2±0.09^{a}	5.1±0.05^{c}	6.1±0.06^{ab}	5.8±0.12^{b}
Protein	43.0±0.8^{d}	55.7±0.58^{a}	48.2±0.2^{b}	47.9±0.11^{b}	39.2±0.36^{c}	40.8±1.62^{c}
CHO	34.8b±1.4^{b}	30.9±0.5^{a}	36.4±0.54^{bd}	34.5±0.1^{b}	41.3±0.44^{c}	38.0±1.69^{d}
Fat	1.4±0.3^{d}	2.5±0.11^{a}	2.6±0.19^{ab}	3.20±0.07^{c}	2.5±0.18^{b}	1.9±0.06^{d}
Fiber	0.70±0.1^{a}	0.7±0.04^{a}	0.8±0.08^{ab}	0.6±0.09^{a}	0.9±0.15^{b}	0.6±0.1^{a}
Ash	13.1±0.08^{c}	10.1±0.1^{a}	12.0±0.61^{b}	14.0±0.15^{c}	16.1±0.06^{d}	18.7±0.05^{e}
Energy	323.8±2.6^{a}	369.1±0.6^{b}	361.8±1.66^{c}	358.1±0.64^{d}	344.6±1.28^{e}	332.4±0.76^{f}

In each raw different letters indicate significant differences ($p < 0.05$)

Table 4. Amino acid composition of *Arthrospira* biomass (g/100g protein) harvested from Lake Chitu

Amino acids	Wet season harvest	Dry season harvest	**Arthrospira* (Artificially grown)
Essential amino acids(EA)			
Ile	3.21	2.38	6.7
Leu	6.18	4.65	9.8
Lys	5.23	4.35	4.8
Met	2.85	2.10	2.5
Phe	3.66	2.70	5.3
Thr	4.27	3.24	6.2
Val	4.21	3.16	7.1
His	2.50	1.90	2.2
Non essential amino acids(NEAs)			
Cys	6.17	4.97	0.9
Tyr	2.72	2.11	5.3
Asp	6.92	5.20	10.3
Glu	15.30	11.80	11.8
Ser	4.46	3.35	5.1
Gly	4.22	3.17	5.7
Ala	4.01	3.13	9.5
Arg	5.08	3.85	7.3
Amm	5.44	4.06	
Total amino acids	86.43	66.12	
Essential amino acids(EA)	39.7	39.5	
Non essential amino acids(NEA)	60.4	60.5	
EA/NEA	0.66	0.65	

* Richmond 1998

Table 5. Fatty acid composition of *Arthrospira* biomass harvested from Lake Chitu during wet and dry seasons (g/100g dry weight) (mean±SD, n=3)

Fatty acid (%)	Lake Chitu's *Arthrospira*	
	Wet season harvest	Dry season harvest
Palmitic (16:0)	31.7±2.88[a]	29.2±1.65[a]
Linoleic (18:2n6)	23.7±2.61[a]	25.6±4.45[a]
Γ-linolenic (18:3n6)	20.3±5.19[a]	16.0±0.51[a]
Oleic (18:1n9)	11.9±0.35[a]	10.3±4.06[a]
Palmitoleic (16:1n9)	5.9±0.87[a]	6.3±0.15[a]
Lauric (12:0)	4.8±3.56[a]	12.7±1.33[b]
Saturated (SFA)	37.1	41.9
Monounsaturated (MUFA)	18.1	16.6
Polyunsaturated (PUFA)	44.8	41.6
PUFA/SFA	1.21	0.99

In each raw different letters indicate significant differences ($p < 0.05$)

Table 6. Major and trace minerals content (mg/100g dry matter) of *Arthrospira* biomass harvested from Lake Chitu (mean±SD, n=3)

Minerals	Lake Chitu's *Arthrospira*	
	Wet season harvest	Dry season harvest
Major		
Na	3387±330[a]	5986±507[b]
K	1564±234[a]	2044±197[b]
P	639±121[a]	514.5±64[b]
Ca	55.0±7[a]	45.7±15[b]
Mg	112.1±18[a]	76.6±7[b]
Trace		

Mn	1.81±0.31[a]	1.30±0.03[b]
Fe	27.9±5.61[a]	18.5±1.91[b]
Cu	0.06±0.04[a]	0.04±0.05[b]
Zn	0.05±0.21[a]	0.04±0.29[b]
Se	0.03±0.01[a]	0.01±0.01[b]

In each raw different letters indicate significant differences ($p < 0.05$)