Journal of Animal & Plant Sciences, 2012. Vol. 13, Issue 3: 1811-1822
Publication date: 30/4/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

Changes in the physico-chemical properties of *Spirulina platensis* from three production sites in Chad

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Key words: *Spirulina platensis*, physico-chemical properties, macro and microelements, Chad

1 SUMMARY

The cyanobacterium *Spirulina platensis* is an attractive source of valuable protein for both human and animal consumption. Investigations were carried out in Chad to evaluate the influence of the production sites on the physico-chemical properties of *S. platensis*. Samples from Artomissi, Brandi and Sugar Producing Company of Chad (CST) were analyzed and compared. The pH (H_2O) in the production sites was basic and ranged from 9.98 to 10.01. The temperature also varied from 36.15°C for Artomissi to 31.3°C for Brandi. The protein content in samples significantly differed ($p < 0.0001$) from 50.21% for CST to 58.61% for Artomissi and Brandi. Similarly, there was a highly significant difference ($p < 0.0001$) between the saponin contents in samples originated respectively from Artomissi (319), Brandi (286.6) and CST (136.6 mg/100g DM). Brandi was the production site with the greatest total chlorophyll content (973.51 mg/100g DM) ($p < 0.0001$)), while the smallest accounted for Artomissi samples (725.62 mg/100g DM). *S. platensis* from Brandi (35.14%) and Artomissi (46.43%) consistently contained more phycocyanin than that of CST (10%). Apart from calcium that did not show significant difference ($p = 0.224$) between the production sites, the studied micro and macroelements in *S. platensis* significantly differed ($p < 0.0001$) from one production site to another. No toxic heavy metals such as nickel, chromium, sulphates, and cyanides were detected in *S. platensis* samples. The results reveal that macro and microelements essential to animal and human nutrition are present in *S. platensis* which can be in use as food supplement.

2 INTRODUCTION

Malnutrition is one of the major problems facing by developing countries which are characterized by increased population growth rate compared to agricultural production, a situation that has led to high food demand and children mortality (World Bank, 2002; Geo4, 2009). Averagely, 12 million children worldwide from zero to five years old are dieing every year from malnutrition (Fox & Manin, 2000).The most devastating disease is kwashiorkor, caused by lack of proteins, vitamins and oligo-elements (Yacoub, 2005). At a stage where natural resources are disappearing, it is necessary and urgent to summarize their previous and present

utilizations, in order to better elaborate efficient strategies for their protection and sustainable management (Nkongmeneck, 2002). Local measures include water management; agro-sylviculture and other strategies such as pastures that cannot only increase the yield, but also, healthy reconstitute the soils, maintain the diversity of cultures, and avoid the expensive chemical fertilizers and pollutants (Geo4, 2009). Techniques such as the rational management of the environment by introduction of new production systems in growing areas have enabled the yield improvement, while preserving the natural ecosystems (Mapongmetsem & Ibrahima, 1999). Among these techniques, the growth of spirulina seems to be one of the major solutions, since it necessitates little means or space, and is economically important as far as yield improvement is concerned.
In spirulina growing areas, yields are in fact the equivalent of 9 tones of protein per ha, compared to 1 tone/ha for barley (Fox, 1999). Nowadays, spirulina represents a hope for the future. Like other edible microalgae, it produces a healthy food, and in enough quantity as single cells proteins. In its composition, spirulina contains 70% (w/w) of proteins, all the other essential nutrients needed for child growth such as ion, magnesium, zinc, vitamin A, and has been reported to better contribute to solving the malnutrition problem in humans and other animals (Jourdan, 2006). Despite these attributes, sanitary constraints are often linked to the production due to impurities that impede the quality of this resource in the international markets (Yacoub, 2000; 2005). The improvement of its quality may require adequate analysis in laboratories. *S. platensis* originating from Hawaii and California has been advised for consumption compared to unspecified quality ones from developing countries that has not yet been appropriately analysed (Jourdan, 2006). Spirulina grown in Chad is known for its various utilizations, although it has not been subjected to complete analysis. Apart from recent works on the drying technique, nutritional and organoleptic qualities (Abdulader *et al.*, 2000, Sorto, 2003; Sorto *et al.,* 2006, Yacoub, 2005), the only physico-chemical and biological results on spirulina of Chad origin concern the total sugar, ashes, lipids, proteins and microbiology (Gongnet, 1984; Mbaïguinam *et al.*, 2006, ITRAD, 2009). Nowadays, spirulina production worldwide is more and more oriented towards the health food market, although it has been reported to be influenced by environmental (Vonshak *et al.*, 1996), chemical (Richmond & Grobbelaar, 1986; Ortega-Calvo *et al.*, 1993) and biological (Venkataraman, 1997; Habib *et al.*, 2008) factors. It is assumed that these factors vary from one production area to another. In Lake Chad, only specific sites are considered as growing area of spirulina. Hence, we hypothesized that these production areas are heterogeneous, and could impact the physico-chemical quality of the harvested product.
Therefore, investigations were undertaken to complete the physico-chemical properties profile of the microalgae *S. platensis* from Chad by comparing data between three location sites, one of which was artificial (CST) and the others natural (Artomisti and Brandi). The expected results will give more information on macro and microelements, as well as carbonate compounds found in spirulina grown in this region.

3 MATERIAL AND METHODS

3.1. Study sites and Microorganisms: Experiments were carried out in the sahelian zone of Chad, where spirulina is reported to be consumed as early as in 1960s (Fox, 1999). Lake Chad and Kanem are two naturally producing regions of *Spirulina platensis* in Chad. They are located at 300 km in the North West of N'Djamena (13°, 17° latitude North and 13°, 18° longitude

East), and where the rainfall oscillates between 400 and 800 mm/year (Yacoub, 2005). *S. platensis* was sampled from Artomissi and Brandi (the two natural producing sites), and Mani (the artificial site) at 90 km in the south of N'Djamena within the Sugar Producing Company of Chad (CST) (Figure 1). In spite of difficulties encountered to access the study sites, *S. platensis* was sampled in each site in triplicate for statistical analysis purpose. The experiment was conducted partly directly in the field, and partly in the Research laboratories of Natural Substances (LRSN), Water and Environment (LABEEN), or in the Laboratory of Soil and Plant Analysis (LASEP) of ITRAD (Research Institute for Agronomic Development), all in N'Djamena, Chad.

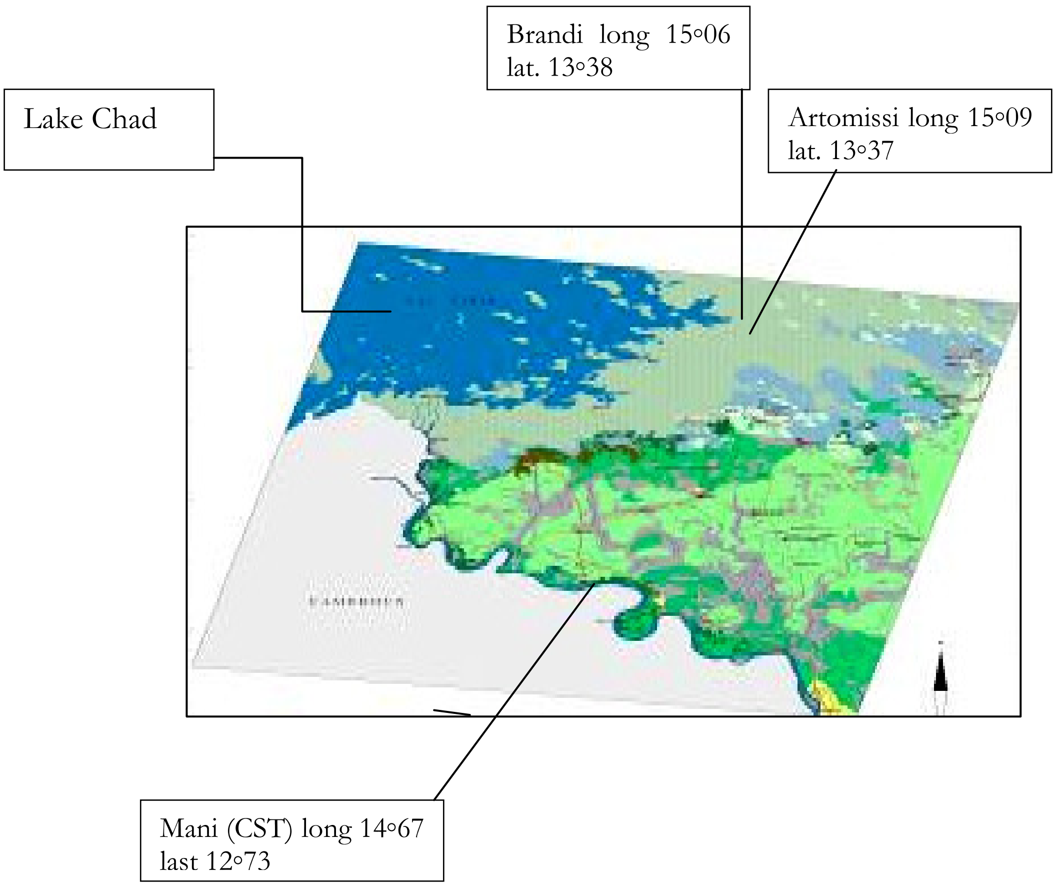

Figure 1: Study sites of spirulina in Chad **(**Adapted from CNAR: National Centre for Research Allowance)

3.2. Physical analysis

3.2.1. Temperature and conductivity: Water temperature in the production sites was measured with an electronic thermometer (Eutech and Cyberscan) in three replicates. The water quality was assessed by measuring the conductivity in the production sites using an electronic conductivimeter HACH (USA), (Jourdan, 2006).

3.3. Chemical Analysis

3.3.1. Measurement of pH: An electronic pH-metre of Cyberscan type was used to measure the water pH of the production sites.

3.4. Analytical methods: The dry weight of biomass was determined by filtration of sample (25 ml) through Whatman filter paper No.4 after washing with 3N acetic acid to eliminate salt precipitate. Crude protein was determined by micro Kjeldahl method through nitrogen content determination followed by its conversion by a factor of 6.25 (Dubois, 1956). Total lipid was measured by Soxhlet solvent extraction (UICPA, 1979), and then the lipid content was expressed by the formula:

Total lipid content (%) = $[(P_1 - P_0) \times 100]/M$

Where,
P_0 is the weight of the empty container; P_1 is the weight of container with lipid; and M the mass of sample.
Sugar was determined based on method of Kochart (1978) using glucose as a standard. Fiber content in *S. platensis* was determined as described by Wolff (1968), consisting hydrolyzing sample with H_2SO_4 followed by alkaline treatment with KOH, and calcination of the residue. The fiber content of the sample was given by the relation:

Fiber content (%) = $[(P_2 - P_3)/P_1]\ [100/100\text{-}TE] \times 100$

Where,
P_1 is the mass expressed in g of the sample without lipid; P_2 the mass expressed in g of the residue after treatment with H_2SO_4 and KOH; P_3 the ash mass expressed in g; and TE the water content of the sample.
The different chlorophyll contents were determined after absolute methanol extraction utilizing the absorption coefficient factor reported by Vonshak (1997), by optical density measurement at 645, 652 and 663 nm according to the following Kinney (1941) equations:
- Total chlorophyll = 20.2 OD 645 nm + 8.02 OD DO 663 nm (mg/l of solution);
-Chlorophyll a = 12. 7 OD 663 nm - 2.8 OD 645 nm (mg/ml of solution);
- Chlorophyll b = 22.9 OD 645 nm- 4.68 OD 663 nm (mg/ml of solution).

The method used for estimating the total carotenoids was spectrophotometry (CECIL, Model- 7200 Spectrophotometer) after extraction in 90% acetone (Vonshak & Borowitzka, 1991). For phycocyanin determination, 3 g of sample solution was centrifuged and decanted. From the supernatant, 0.5 ml solution was sampled diluted 100 times with distilled water. The optical density (OD) of each sample was spectrophotometrically measured at 615 and 652 nm. The phycocyanin content expressed as % of dry weight matter (DW) was estimated through the relation:
Phycocyanin content (%) = [1.873 x (OD 615 – 0.473 x OD 652) x 100]/C
Where,
C is the concentration of spirulina in the solution
Carotenoid content was determined by adding 25 ml acetone to 3 g sample and maintaining it at 24 h in the refrigerator. The supernatant was centrifuged and 0.5 ml of it aliquot and diluted with acetone. Considering DIL as the dilution factor, the optical density (OD) of the solution was measured at 450 nm and the carotenoid content expressed as follows:

Carotenoid content (%) = [OD 450 x DIL x C]/2.8

Where,
C is the concentration of spirulina in the supernatant. The chemical composition of the dried biomass was determined according to AOAC methods (AOAC, 1984).
3.5. Statistical analysis: Data were statistically analyzed by ANOVA using a Statgraphic Plus, version 5.0 (SIGMA PLUS, California, USA) computer program. Means were compared between treatments using the Least Significant Difference (LSD) procedure at 5% level. Correlations between parameters were assessed using the SPSS computer program.

4 RESULTS AND DISCUSSION

4.1 Characteristics of the growing medium:

The experimental results (table 1) demonstrate that the pH of medium was alkaline and did not show significant differences ($p > 0.059$) between treatments. The pH was between 9.98 and 10.05. These findings are in line with other results reported by Papa (2005) and Charpy *et al.* (2008), who indicated the growth of *Spirulina* in natural medium is characterized by warm and alkaline water rich in carbonates/bicarbonates. According to these authors, pH ranged between 8 and 11.5. *S. platensis* was reported to grow well at 32°C at pH 10 (Al-Homaidan *et al*., 2005). According to Jourdan (2006), the optimal pH of a growing medium for a set up of a *spirulina* culture should be at least 9. In fact, cyanobacteria growing in a medium lead to a progressive pH increase, in agreement with Pelizer (2003) findings. This observation can be correlated with the carbon-source consumption.

Table 1: Physico-chemical characteristics of the production sites

Parameters	Production sites			*P-values*
	Artomissi	Brandi	CST	
pH at 25°c	9.98	10.01	10.00	0.059
Conductivity (ms/cm)	22.7a	23.20a	24.0b	0.019
Salinity (g/l)	14.35a	23.20b	14.5a	0.029
Temperature (°C)	36.15c	31.3b	28.0a	0.042

Values in a row followed by different letters differ by LSD test (p < 5%)

Bicarbonate ions for example are assimilated by the cyanobacteria and subsequently, converted into carbon dioxide and carbonate. The carbon dioxide is utilized in photosynthesis and the carbonate is excreted into the medium. The increase in pH of the system is due to the shift of the bicarbonate-carbonate equilibrium towards the carbonate (Rangel-Yagui, 2004). Temperature varied from one production site to another. This variation was close to the optimum range reported for *S. platensis* cultivation (Danesi *et al*., 2002; Sanchez-Luna *et al*., 2004). The salinity of 14.5 g /l for Artomissi and CST sites was similar to 15g/l reported by Zarrouk (1966) from an artificial medium. Increasing salinity of the medium was shown to reduce the specific growth rate of *S. platensis* (Kebede, 1997). The results of the present work indicate that Brandi site was more concentrated in salt that is known to be involved in growth retardation of spirulina (Fodorpataki & Bartha, 2004).

4.2. Chemical composition: The water content was significantly greater (p < 0.0001) in spirulina samples harvested from the natural sites (Artomissi and Brandi) than from the artificial site (CST) as indicated in table 2. In all the cases, the water content of our sample was higher than 5% reported by Flamant vert (1988), and was close to 10 % requested by French Norms (1979). The dry matter content was greater than 90% in all the studied sites and was not significantly different from one site to another. These results are higher than those reported by ITRAD (2009) who obtained 69.44 and 69.52 % dry matter from spirulina harvested from Artomissi and Brandi respectively.

Table 2: Chemicals composition of spirulina samples from various production sites in Chad (%)

Production sites	**Chemical composition**						
	Water content	**Ash**	**Dry matter**	**Sugar**	**Lipids**	**Proteins**	**Fibers**
Artomissi	9.36b	7.96	90.64	13.8	3.4 a	58.61b	7.58a
Brandi	9.08b	7.8	90.92	14.2	9.86b	50.24a	7.55 a
CST	7.11a	7.91	92.8	14.06	4.6a	58.61b	7.78b
p-value	< 0.0001	ns	ns	ns	< 0.0001	< 0.0001	= 0.0053

Values in a column followed by different letters differ by LSD test (p < 5%)

The lipid content was significantly (p < 0.0001) influenced by different sites and varied from 3.4% to 9.86% for Artomissi and Brandi samples. Several authors have reported values ranging from 5.6 to 7% (Earthrise, 1986; Fox, 1996) or from 6 to 13% (Cohen, 1997; Xue, 2002). ITRAD (2009) claimed total lipid content in spirulina from 4.00 to 4.44 %, which is comparably lower than that of the present work. The lipid content in spirulina might be influenced by the extraction method or the type of solvent used. The CST lipid content was 4.6 % which is close to the value of 5.46% obtained from Kanem samples in Chad (Mbaïguinam *et al*., 2006). According to Piorreck *et al.* (1984), Danesi et al. (2002) blue-green algae do not show any significant change in the percentage and composition of their lipids and fatty acids when grown at different concentrations of nitrogen. In this study, although the protein content (which is closely related to nitrogen content) of Artomissi and CST samples (58.61%) was significantly higher ($p < 0.0001$) than Brandi (50.24 %), the lipid content in Brandi sample was in contrast was higher than CST and Artomissi sites. The protein content fails between the range of 50-70% (AFAA, 1982), 53-62% (Alaniesse, 2009), 55- 65% (Banks, 2007) that were previously reported in spirulina. According to AFAA (1982), the protein content in spirulina may vary with the sampling period in relation to the photoperiod, the highest contents being obtained at the beginning of the sunlight period. Indeed, all of experimental samples were collected in the sites located within the sahelian zone where the photoperiod is high. Moreover, these results are in accordance with 58.20% reported by Alvarenga *et al.* (2011), but lower than 69.2 % obtained by Mbaïguinam *et al*., (2006) from *in vitro* samples culture or the range of 60-71% reported by Fox (1996). The increase in protein content was probably due to the nitrogen level in the medium. Piorreck *et al.* (1984) reported that increasing the nitrogen level in the nutrient medium leads to an increase in the biomass and protein content of spirulina. Indeed, Nitrogen is required for the synthesis of amino acids which make up proteins and other cellular components (Colla, 2007). A lower protein content value of 42.3 % was obtained in spirulina from the same lake, but this was justified by contamination with sand (Gongnet, 1984). As far as the total sugar content in spirulina is concerned, there was no difference between the production sites. These results are similar to those of Fox (1996) with values of between 13 and 16.5 %, but are lower than 17.07 % reported in spirulina from Kanem-Lake Chad (Mbaïguinam *et al*., 2006). It appears that the sugar content in spirulina may vary with the culture conditions and the production site. According to Walach *et al.* (1984), higher quantities of carbohydrates are synthesised when nitrogen availability is decreased, while carbon availability is constant. Nitrogen deficiency has been found to stimulate the synthesis of all carbohydrate fractions (intracellular, capsular and soluble) in a cyanobacterium (Fresnedo & Serra, 1992).

Like dry matter and sugar content, the ash content in spirulina did not differ ($p > 0.05$) from one production site to another, with values of 7.96, 7.8 and 7.91% respectively for Brandi, Artomissi and CST. These results line are within the range of 6.4 - 9 % revealed by Fox (1996). These values were similar to 7 % (Henrickson, 1997, Flamant vert, 1988), and 8.41 % (Mbaïguinam *et al.* 2006) reported in other samples. In contrast, the fiber content of *S. platensis* differed ($p < 0.005$) in Artomissi (7.58%), Brandi (7.55%) and CST (7.78 %) samples. This finding is slightly higher than 6.15 % revealed in spirulina sample from Brazil (Alvarenga *et al.* 2011). According to Alaniesse (2009), the raw content of fiber in spirulina varies from 4 to 7 %, which is in accordance with results of the present investigation.

4.3 Inorganic components

4.3.1 Calcium: The calcium content did not differ ($p = 0.244$) in samples varying between 3.1 mg/100g and 3.2 mg/100g DM for Artomissi and Brandi (Table 3). These values are higher than 13 to 140 mg Ca/g spirulina obtained by Johnson and Schubert (1986), but were lower than 1000 mg Ca/100g DM of spirulina reported (Montasell, 2009).

4.3.2 Iron: The iron content differed ($p < 0.0001$) in spirulina samples from one production site to another. Iron content from between 600 and 6000 mg/kg was revealed in spirulina grown in artificial medium (Falquet & Hurni, 2006).

4.3.3 Cobalt: The analysis of variance also indicated significant differences between spirulina from different production sites. However, the cobalt content in samples from the artificial site CST (49.10 mg/g DM) was lower than those of the natural production sites which are Artomissi (68.100 mg/100g DM) and Brandi (76.5 mg/100g DM).

Journal of Animal & Plant Sciences, 2012. Vol. 13, Issue 3: 1811-1822
Publication date: 30/4/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

4.3.4 Copper: Spirulina from Artomissi and Brandi was consistently rich in copper compared to samples from CST as indicated in table 3. Other published results have reported copper contents of between 0.8 and 1 mg/100g DM (Johnson & Schubert, 1986), or between 0.7 to 1.2 mg/100g DM (Montasell, 2009).

4.3.5 Magnesium: The magnesium content in our sample ranged between 1.8 mg/100g DM for Artomissi and Brandi and 7.8 mg/100g DM for CST. Montasell (2009) reported higher magnesium content (4000 mg/100g DM) in other spirulina samples. Spirulina is naturally rich in Mg due to its high chlorophyll content.

Table 3: Inorganic components in *Spirulina* from various production sites in Chad (mg/100g DM)

Inorganic components	Treatments			
	Artomissi	**Brandi**	**CST**	**p-value**
Calcium	3.1a	3.2a	3.15a	P = 0.244
Iron	788.88b	683.33a	1666.6c	p < 0.0001
Magnesium	1.8a	1.8a	7.85a	p < 0.0001
Total phosphorus	357.5b	353b	20a	p < 0.0001
Cobalt	68.62b	78.65c	49.12a	p < 0.0001
Copper	250b	250b	128.12a	p < 0.0001
Potassium	188.87c	140.62b	128.12a	p < 0.0001
Manganese	2925b	3220c	975a	p < 0.0001
Sodium	762.5c	275b	143.75b	p < 0.0001
Zinc	298c	242b	216a	p < 0.0001
Chlorures	62.75c	50.27b	42.28a	p < 0.0001

Values in a row followed by different letters differ by LSD test (p < 5%)

4.3.6 Manganese: The manganese content from CST (975 mg/100g DM) was significantly lower than those of Artomissi (2925 mg/100g DM) and Brandi (3220 mg/100g DM). Theses values were consistently high compare to 55.4 and 37.5 mg/100g reported from a related study (Avino *et al.*, 2000).

4.3.7 Potassium: The potassium contents were 188, 140 and 128 mg/100g DM, respectively in spirulina from Artomissi, Brandi and CST. These results are low compared to those reported by NRC (1980) with between 640-1540 mg potassium/100g DM of spirulina. There was a significant and positive correlation between the potassium and water ($p = 0.001$; $r = 0.98$), saponins ($p = 0.023$; $r = 0.73$), and phycocyanin ($p = 0.003$; $r = 0.85$) contents.

4.3.8 Sodium: Brandi as one of the natural production site had the highest sodium content in spirulina (275 mg/100g DM), compared to that of the artificial site CST (143 mg/100g DM). These values were lower than 450 mg/100g DM (Banks, 2007), or 900 mg/100g DM (Jourdan, 2006) reported. Positive and significant correlations were noticed between the sodium and water content ($p = 0.021$; $r = 0.73$), saponins ($p = 0.022$; $r = 0.74$) and phycocyanin contents ($p = 0.003$; $r = 0.85$).

4.3.9 Chlorides: Like potassium and sodium, chloride content in spirulina was significantly greater in samples from natural sites (Artomissi and Brandi) than the artificial one (CST). Chlorides may form with alkaline salts (NaCl, KCl) or acids (HCl) involved in the digestion of nutrients in human. A positive and significant difference was found between the chlorides content and that of manganese ($p = 0.03$; $r = 0.71$), and water ($p < 0.001$; $r = 0.98$).

4.3.10 Zinc: The zinc contents in spirulina were 298, 242 and 216 mg/100g DM, respectively in Artomissi, Brandi and CST samples. Banks (2007), Cornet (1992) respectively revealed zinc contents of 3 mg/100g DM and 2.1- 4 mg/100g DM in spirulina. These elevated values may be attributed to environmental pollution as early reported by Campanella *et al.* (1999). There was a significant and positive correlation ($p = 0.008; r = 0.81$) between the zinc water contents on one hand, that of phycocyanin ($p < 0.001; r = 0.91$), and saponin ($p = 0.009; r = 0.80$) on the other.

4.3.11 Chrome, Sulphates, Nickel and Cyanide : Contrary to our expectations, no presence of chrome, sulphates nickel and cyanides was found in our spirulina samples, whereas 300 µg/100g DM of chrome was reported by Montasell (2009) in spirulina, in agreement with 3 mg of chrome per kg of spirulina detected by Jourdan (2006). Apart from report on 6.1 g of sulphurs/kg obtained by Algue.sante.com (2009), no other author has revealed the presence of sulphur in spirulina.

4.3.12 Total phosphorus: The phosphorus content was significantly influenced by the production sites with value ranged from 357.5 for Artomissi, 353 for Brandi to 207 mg/100g DM for CST. These results are different from those of Cornet (1992) who reported 670- 900 mg of total phosphorus/100g DM in spirulina. A positive and significant correlation was noticed between the total phosphorus content and chlorophyll ($p = 0.033; r = 0.70$) on one hand, and phycocyanine ($p < 0.001; r = 0.95$) on the other.

4.4 Pigments and saponin: Spirulina is rich in pigments responsible to its colour. The main pigments are phycocyanin and chlorophyll. Data obtained in the present research indicate a significantly higher ($p < 0.0001$) concentration of phycocyanin in spirulina harvested from natural location sites (Artomissi and Brandi) than the one harvested from the artificial site (CST) (table 4). Results are greater than 22% value reported in other samples (Equitude, 2009). According to this author, the phycocyanin concentration in spirulina may vary with the strain and the growth conditions. In fact, phycocyanin which is the most abundant pigment in spirulina is a phycobiliprotein, the only natural and nutritional blue dye that represents more than 15 % in fresh weight and more than 20 % dry weight of these algae (Romay *et al.*, 1998).

Unlike, chlorophyll a is a primary photosynthetic pigment in algae and cyanobacteria, and is an indicator of the biomass production in the culture. Spirulina is one of the largest sources of chlorophyll in nature and contains more than 1 mg/g of chlorophyll (Rafiqul Islam *et al.*, 2003). The total chlorophyll content in spirulina was significantly different among the production sites. Results obtained show a high ($p < 0.0001$) cellular concentration of chlorophyll content in *S. platensis* from Brandi (9.73 g/kg DM) compared to those of CST (7.46 g/kg DM) and Artomissi (7.25 g/kg DM). These results are closer to those of Alaniesse (2009) with 7.9 g/kg DM, but are lower than 11 g/kg DM revealed by Fox (1999). Piorreck *et al.* (1984) also reported lower concentration of chlorophyll obtained from cultivation carried out with limited nitrogen concentration. Whereas chlorophyll a was the highest ($p < 0.0001$) in sample from Artomissi (5.34 g/kg DM), the lowest concentration of chlorophyll b (1.91 g/kg DM) was obtained in the same sample. The shading was revealed to contribute to a higher concentration of chlorophyll a observed in the biomass obtained from cultivation *Spirulina* in a medium with higher added nitrogen at a fixed light intensity (Rangel-Yagui, 2004). Higher cellular concentration in spirulina has been reported to be able to generate higher chlorophyll a biosynthesis rate in order to increase the efficiency of photon capture, and thus, compensate for the reduction of light intensity for the cells not located at the surface (Hendry, 1996). The carotenoid pigments including β-carotene are increasingly used in foods; particularly important as the main source of vitamin A. Although Brandi showed a greater salt concentration, the concentration of carotenoid from this production site did not differ ($p = 0.356$) to the two others. These results are in disagreement with significant increase in cellular carotenoids content in the highly salt tolerant algae *Dunaliella salina* (Borowitzka *et al.*, 1990).

Table 4: Differences in pigments saponin and of *Spirulina platensis* from various production sites

Production sites	Pigments					Saponin
	Total chlorophyll mg/100gMS	Chlorophyll a mg/100gMS	Chlorophyll b mg/100gMS	Carotenoïds mg/100gMS	Phycocyanin (%)	
Artomissi	725.62a	534.03c	191.59a	6.63a	35.145b	319c
Brandi	973.51c	358.51b	615c	5.68a	46.435c	286.6b
CST	746.65b	286.32a	460.33b	6.4a	10a	135.4a
p-value	< 0.0001	< 0.0001	< 0.0001	= 0.356	< 0.0001	< 0.0001

Values in a column followed by different letters differ by LSD test ($p < 5\%$)

The concentration of saponin in *S. platensis* expressed as mg/100 g of DM, and was higher ($p < 0.0001$) in Artomissi and Brandi than in CST. In the present study, Artomissi and Brandi are natural growing environments for *S. platensis*, unlike CST which is an artificial growing location.

5 CONCLUSION

The present work shows that spirulina from various production sites of Lake Chad exhibits different physico-chemical traits, which are closely dependent on the chemical composition of these sites. Although natural production sites provides better physico-chemical traits than the artificial one, findings from the present work have revealed that spirulina from all study sites in Chad are safe from heavy metal, and thus could be consumed by human and other animals. The information developed in this study should further be applied to other production sites within the lake to enable selection of those appropriate for health food market.

6 ACKNOWLEDGEMENT

The authors are very grateful to Mrs Namba Fabienne, Head of the Laboratory of Soil, water and Plant analysis (LASEP), Chad Institute for Agronomic Research and Development (ITRAD), who provided facilities for samples analysis.

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