ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 17 No. 1
March 2017
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2017; **17(1):** 11603-11615 **DOI: 10.18697/ajfand.77.15440**

TACKLING COMMUNITY UNDERNUTRITION AT LAKE BOGORIA, KENYA: THE POTENTIAL OF SPIRULINA (*ARTHROSPIRA FUSIFORMIS*) AS A FOOD SUPPLEMENT

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ABSTRACT

Undernutrition remains a major public health concern for many developing nations, particularly in sub-Saharan Africa. In Kenya, undernutrition affects a substantial portion of the Kenyan population, especially children and those living in rural areas. Local and sustainable means of addressing undernutrition is still lacking in many communities in urban, but more so in rural areas of Kenya. Spirulina (*Arthrospira fusiformis*), a cyanobacterium from alkaline inland waters, high in nutrient content, is a potential means of treating undernutrition in the developing world, where it can be easily grown. This paper presents a feasibility study on the harvest of Spirulina from Lake Bogoria in the Kenyan Rift Valley for use as a food supplement for undernutrition mitigation in the surrounding rural communities. A nutrition survey revealed the local population to be deficient in a number of micronutrients, specifically vitamins E and B12 that could be provided through dietary supplementation with Spirulina. A sample of Spirulina was collected from Lake Bogoria and analyzed for nutrient content and the presence of toxins. It was found that Lake Bogoria Spirulina had a dry protein content of 14.6% and is a rich source of dietary iron, with an iron content of 1.86%. A toxicity analysis revealed that Lake Bogoria Spirulina contained 1.15ng/g of microcystins (a group of hepatotoxic small polypeptides produced by several strains of cyanobacteria), which is within levels safe for human consumption according to World Health Organization standards. It was concluded that Lake Bogoria Spirulina is an easily accessible source of food and has the potential to be a sustainable means for the Lake Bogoria community to tackle undernutrition. Finally, using the data gathered, consultation sessions were arranged with key community groups and members to discuss the feasibility and potential for incorporation of Spirulina into the local diet – an ongoing collaborative process between researchers and the community that has already been met with some success.

Key words: Spirulina, food, diet, undernutrition, Bogoria, toxicity, microcystins, community, Landsat

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INTRODUCTION

Undernutrition remains a serious public health challenge in developing nations, especially in sub-Saharan African nations such as Kenya [1-3]. Undernutrition, defined as an insufficient intake of either macronutrients or as a deficiency in essential micronutrients [1], manifests clinically in various ways including stunted growth and wasting (defined as an abnormally low weight-to-height ratio) [4]. Undernutrition disproportionately affects children, especially those under 23 months of age [1, 4]. In malnourished populations, individuals typically suffer from both macronutrient and micronutrient undernutrition [5]. Worldwide, undernutrition is directly responsible for approximately 300,000 deaths per year and has been estimated to contribute to half of all deaths amongst young children [1]. Although the global incidence of undernutrition has decreased in the past two decades, it has become more prevalent in certain regions of the developing world [1].

In Kenya, undernutrition affects approximately one third of children under 72 months of age. A substantial portion of the Kenyan population, young children in particular, is deficient in dietary iron. A 1999 survey of all eight Kenyan provinces found that the prevalence of stunting, wasting and a low BMI amongst children under 72 months of age to be 37%, 6% and 27%, respectively [4]. It is difficult to estimate the exact contribution of undernutrition to mortality rates in Kenya since patients with undernutrition are also more susceptible to infectious diseases such as malaria or pneumonia, which carry significant mortality rates in young, rural children who are hospitalized due to infection [1, 6].

The implementation of measures to improve food access and security in poor, rural communities in Kenya and other developing nations has been a slow and sporadic process [7]. Measures such as fortifying cooking pots with iron and the development of kitchen and community gardens have met some success in meeting micronutrient deficiencies in rural communities [1], but many communities remain dependent on external aid to meet basic nutritional needs or are unable to meet these needs at all [8]. Consequently, there is a pressing need for a means of addressing undernutrition that can be implemented at the community level.

An increasing use of Spirulina (*Arthrospira fusiformis*) as a cheap and easily accessible food in rural communities of developing nations has been witnessed in recent years [9]. Spirulina is a cyanobacterium that occurs naturally in central Africa and Southeast Asia, and is commercially grown and marketed as a health supplement in the West [10]. Spirulina is thus an attractive candidate for use in combatting undernutrition: it has high protein content, abundance of essential micronutrients and it can be cultivated with limited technical knowledge and resources [9, 10]. Spirulina has already been used as a nutritional supplement in malnourished populations in a few African communities, notably in the Lake Kossorom region of Chad [11] and in Burkina Faso [7]. One study has also examined the feasibility of using Spirulina to treat undernutrition in Kisumu, Kenya [8].

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This study investigates the feasibility of employing Spirulina as a means of combating undernutrition in communities surrounding Lake Bogoria in the Kenyan Rift Valley. Lake Bogoria differs from Kisumu in that it is a primarily rural area, with the population consisting of mainly subsistence farmers, whereas Kisumu is a major urban center [8]. Lake Bogoria is also unique as it is one of a few regions in the world that has an alkaline-soda lake supporting natural Spirulina growth. The lake's unusually high pH and salt concentration results in its biomass being dominated by Spirulina, making the direct harvest of Spirulina possible [12]. Despite this, there is no historical precedent of the people surrounding Lake Bogoria having used Spirulina as a food source. There have been conflicting reports, however, of whether Lake Bogoria Spirulina produces microcystins, a group of hepatotoxins that is highly hazardous to human health [13]. While certain studies conclude that Lake Bogoria Spirulina might be a source of microcystins, other studies could not find significant levels of them [13, 14]. Should Lake Bogoria Spirulina be toxin-free, then it has the potential to serve as a local, sustainable means of addressing undernutrition in the Lake Bogoria region.

MATERIALS AND METHODS

Dietary Survey

Community members in the Lake Bogoria region were interviewed regarding their normal dietary habits. The Lake Bogoria region was defined for the purposes of this study as composed of the communities of Emsos, Loboi and Kapkuikui. These communities combined have a population of 5,805, of which 2,896 are male and 2,909 are female according to the 2009 Kenyan national census [15]. A total of 28 males and 25 females were interviewed, ranging in age from 12 to 68. Each participant was asked to recount their dietary intake over the past four days and provide a description of how each meal was prepared. Estimates of the intake of key nutrients were generated, based on the description provided of how each meal was prepared, then using information on the nutrient content of common foodstuffs from the US National Agricultural Library, the average daily intake of nine essential nutrients (vitamins B6, B12, C, E, thiamin, riboflavin, calcium, iron and zinc) were calculated for each participant.

Spirulina Sample Collection

Spirulina was collected through filtration of Lake Bogoria water using a simple hand-made apparatus (**Figure 1**). All materials required for construction were acquired locally with four pieces of 50µm mesh purchased in the United Kingdom. Lake water was allowed to filter through the apparatus and retained biomass was collected and allowed to dry under direct sunlight for a period of 3-5 hours. Dried biomass was ground into a fine powder and stored dry at room temperature, and then taken to McMaster University in Hamilton, Ontario, Canada for analysis.

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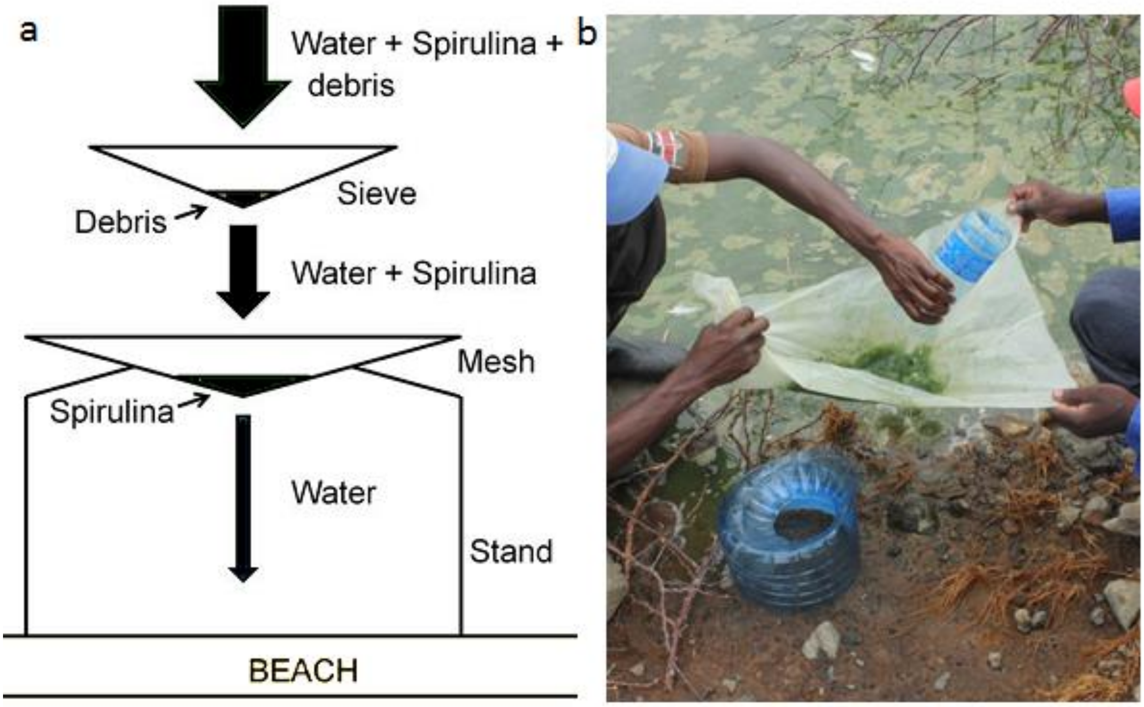

Figure 1: Mechanical filtration system employed to collect Spirulina biomass
Schematic (**a**) and photo of apparatus in use (**b**). Water from Lake Bogoria is filtered through a sieve, which removes dirt and other macroscopic particles from the water, and then passed through a 50µm mesh, which collects the Spirulina biomass.

Bicinchoninic acid (BCA) protein assay

Total protein content of a Spirulina sample collected from Lake Bogoria was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific) according to the manufacturer's instructions. Dried Spirulina was dissolved in distilled water to a concentration of 100mg/ml and extracted through two minutes of sonication. This extract was incubated for 30min at 37°C in BCA reagents as per the manufacturer's instructions. At the end of the incubation period, protein concentration was quantified by reading absorbance at 562nm. Protein content of the sample was determined by comparison to a standard curve generated from albumin standards of known concentration (see **Supplementary Figure 1**).

Mineral titration

A dried sample of Spirulina was ashed by heating at 500°C in a Thermolyne Atmosphere Controlled Ashing Furnace (Thermo Scientific) for one hour followed by cooling for one hour in a desiccator. Ashed samples were dissolved in distilled water to a concentration of 0.01mg/ml and titrated with either permanganate for iron content or EDTA for calcium content. For permanganate titration of iron, a small volume of dissolved sample was mixed with an equal volume of 1M sulfuric acid and titrated with 0.01M potassium permanganate until a purple colouration was observed, indicating the presence of excess permanganate and the exhaustion of iron in the sample. For EDTA titration of calcium, 10ml of dissolved sample was mixed with 1ml of a pH 10.0 phosphate buffer and two drops of eriochrome black T solution. This mixture was titrated with 0.001M EDTA solution until a blue-purple colouration was retained, indicating complete complexation of available calcium with EDTA.

Microcystin estimation using Enzyme-linked immunosorbent assay (ELISA)
The concentration of microcystins in the Spirulina sample was assessed using the Microcystins-ADDA ELISA Kit (Abraxis) according to the manufacturer's instructions. Briefly, standards, controls or a Spirulina sample extracted into distilled water was treated for 90min with an anti-microcystin antibody and a further one hour with an enzyme-conjugated secondary antibody. A colorimetric substrate was applied and absorbance at 450nm was measured. Concentration was determined by comparing absorbance values with those obtained from measurement of microcystin solutions of known concentration supplied by the manufacturer (see **Supplementary Figure 2** for the standard curve).

Satellite image analysis
Images of Lake Bogoria taken by the NASA Landsat 7 Enanced Thematic Mapper (ETM+) sensor over a period of 14 years were analysed as previously described [16]. Briefly, the ratio of red to near infrared (NIR) reflectances for the lake's water were measured from 1999 to 2012. *In situ* chlorophyll-α measurements for Lake Bogoria were taken from 2003 to 2005 and correlated to the red:NIR reflectance ratio to determine the average chlorophyll content of Lake Bogoria over the entire 13-year period. Chlorophyll concentration in the lake water was estimated as a proxy for Spirulina concentration and segregated by season to determine annual variation. Chlorophyll concentration can be used as a proxy for Spirulina density because Spirulina is the dominant phytoplankton species supported by Lake Bogoria due to its extreme salinity [17].

Statistical analysis
All statistical analysis was carried out using Student's two-tailed t-test on GraphPad Prism 6 (GraphPad Software). Results were considered significant if $p < 0.05$.

RESULTS

Dietary imbalances exist in the Lake Bogoria community
Community members in the Lake Bogoria region were consuming significantly higher levels of vitamin C (180% of RDI) out of the nine nutrients examined, but significantly lower levels of vitamins E (5% of RDI) and B12 (50% of RDI) (**Figure 2**). The average daily intakes of the other nutrients did not vary significantly from recommended values according to data obtained from the research surveys.

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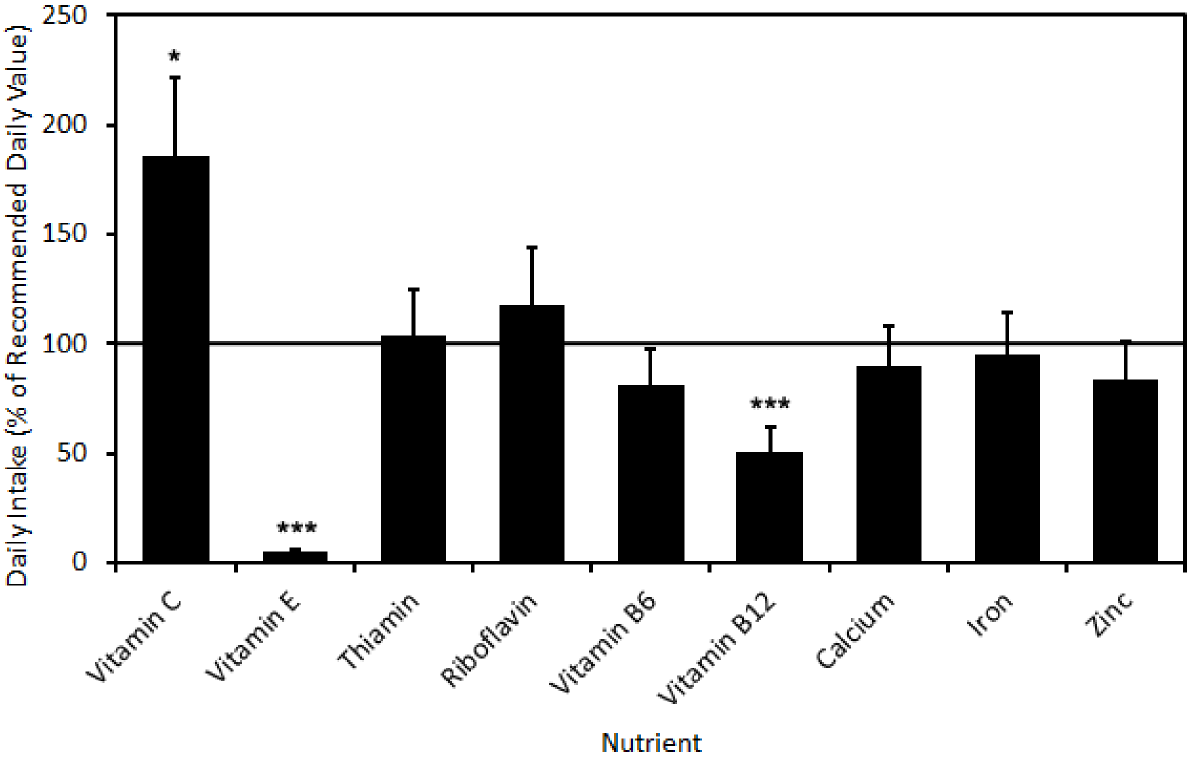

Figure 2: Daily intake of vitamins and minerals in the Lake Bogoria region relative to recommended daily intake values
The average daily intake of nine vitamins and minerals of members of the Lake Bogoria community was found through a four-day survey of dietary habits. The average daily intakes of the survey participants were compared to the recommended daily intake values of each nutrient obtained from the literature and plotted as a percentage of the recommended values. The average daily intake of vitamin C was found to be significantly above the recommended value (*, $p = 0.0209$) while the intake of vitamins E and B12 were found to be significantly lower (***, $p < 0.001$ for both). Data are shown as the mean of $n = 53$ participants ± SEM.

Lake Bogoria Spirulina protein, vitamin and mineral content
Protein concentration and vitamin and mineral content of the sample were measured in order to determine the nutritional value of Lake Bogoria Spirulina versus the commercially manufactured equivalent. The sample analyzed contained high levels of both vitamin E and B12 (**Table 1**). Its protein content was 14.6mg in a 100mg sample. Moisture content of the sample was 68%, as determined by comparing wet and ashed masses.

The contents of iron and calcium were 1.86 ± 0.76% and 0.11 ± 0.01%, respectively (**Table 2**). Compared to commercial Spirulina strains [18, 19], the calcium content of Lake Bogoria Spirulina was significantly lower ($p < 0.0001$) and its iron content higher, though not significantly so ($p = 0.632$).

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Microcystin content of Lake Bogoria Spirulina

A test was performed to determine whether microcystins were present in Lake Bogoria Spirulina in order to ensure that it would be safe for human consumption. From the same dried Spirulina sample a measure of total microcystin content was obtained by ELISA. Total microcystin content of the sample was found to be 1.15 ± 0.05 ng/g.

Optimal season of Spirulina harvest from Lake Bogoria

Landsat images of Lake Bogoria over 13 years, from 1999 to 2012, showed that the mean chlorophyll-α content peaked between March to June (corresponding to the wet season in Kenya) and declined from July to November. The interval from December to March was a period of especially rapid Spirulina growth, with a significant increase in chlorophyll content ($p = 0.0164$) (**Figure 3**).

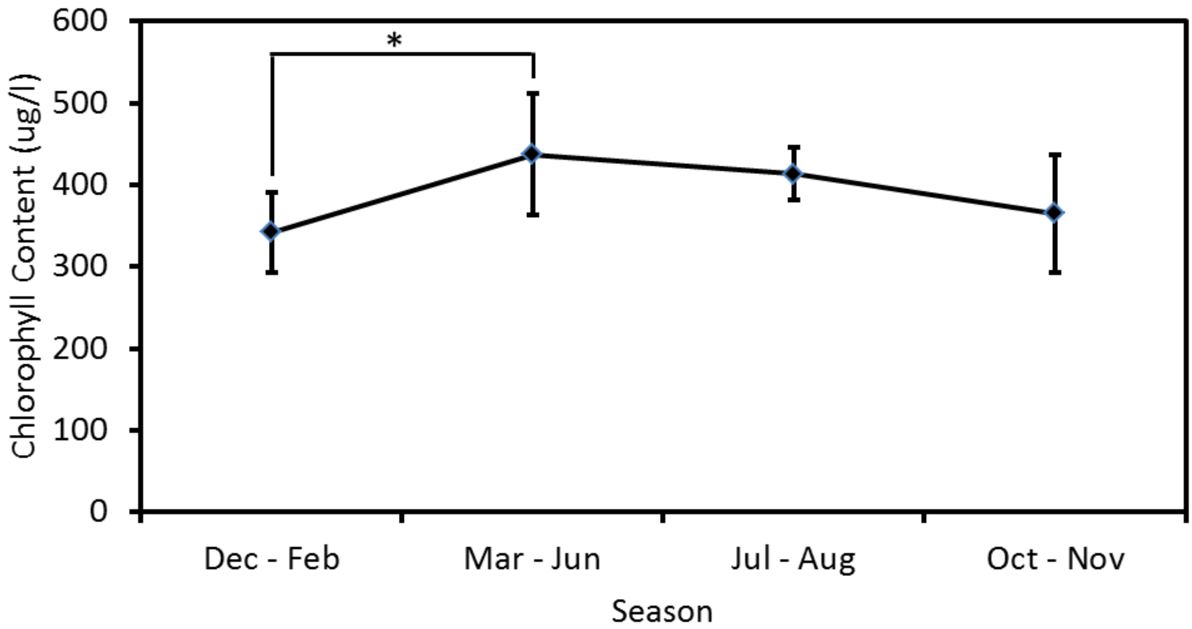

Figure 3: Variation of Spirulina biomass in Lake Bogoria over an average seasonal cycle

Landsat 7 ETM+ data measuring chlorophyll content of Lake Bogoria water over a period of 13 years were categorized by season with $n \geq 12$ measurements per category. Spirulina biomass showed significant variation between December to February and March to June (*, $p = 0.0164$) but little difference between the other seasons, with biomass being highest in March to June. Data are displayed as mean ± SD

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DISCUSSION

This study examined nutritional deficiencies amongst a rural, subsistence agricultural community at Lake Bogoria, Kenya and the possibility of using Spirulina, as a means of addressing these nutritional deficits.

Deficiencies in micronutrient intake have serious health consequences. For example, vitamin E deficiency can have serious consequences, especially in developing children who are at increased risk for anemia, retinopathy and abetalipoproteinemia [20]. Vitamin B12 deficiency is another risk factor for developing anemia, and the combined deficiencies of both E and B12 indicates that the population at Lake Bogoria may be especially vulnerable to anemia [21]. In this study, the researchers did not examine the intake of lipids and cholesterol, something that will need to be measured in future studies to gain a complete understanding of the nutritional profile of the Lake Bogoria community.

It is often challenging for poor, rural communities to make significant alterations in diet. When supplements are available to address nutritional deficits, these are often provided temporarily by outside sources and cannot be considered a sustainable solution. Spirulina, which grows in abundance in Lake Bogoria, contains many of the nutrients found to be lacking amongst Lake Bogoria community members. Lake Bogoria Spirulina has similar iron content but drastically lower protein levels when compared to commercially-grown strains of Spirulina.

Although iron was not found to be deficient in the diet of the community at Lake Bogoria, additional iron intake may help offset the risk of anemia in young women undergoing menses, a population in which anemia is known to be more prevalent in Kenya than in developed nations [22]. Since Lake Bogoria Spirulina is especially high in iron, it would likely make an ideal dietary supplement to address this issue. The protein content of Lake Bogoria Spirulina compared unfavourably to commercially-produced or lab-grown strains of Spirulina, which typically have protein contents of approximately 50-60% [18]. The observation may be a result of only the strains with the highest protein contents being selected for commercial development. It would be worthwhile to examine the nutrient content of Spirulina found growing in the wild, in both central Chad and Vietnam to examine the protein content of these wild strains.

To address conflicting reports of whether Lake Bogoria Spirulina produces dangerous levels of microcystins, the total microcystin levels in a Spirulina sample isolated directly from Lake Bogoria was analyzed and it was found that microcystins were present at a level of 1.15ng/g. The maximum tolerable daily intake (TDI) of microcystin is 40ng/kg body mass [23]. Given that the average adult Kenyan body weight is 56.3kg [24], their maximum TDI of microcystins should be approximately 2.2μg. The RDI of Spriulina is approximately 10g, so the daily intake of microcystins from consuming Lake Bogoria Spirulina would be 11.5ng, far below the maximum microcystin TDI. Thus, within the limits of the methods used in this study, levels of microcystins at a concentration hazardous to human health in a dried sample of Lake Bogoria Spirulina was not identified. This is in agreement with the findings of Straubinger-Gansberger *et al*. [25], who found no evidence of microcystins in planktonic biomass from Lake Bogoria over a two-year period from 2008-2009. However, it is possible that levels of microcystins present in Lake Bogoria Spirulina vary over time in response to changing environmental conditions, so further studies are needed to investigate this. Hence, the authors of this study would recommend that further research on the variability in microcystin concentrations in Lake Bogoria Spirulina is carried out before Spirulina from the lake is adopted as a nutritional supplement for local populations.

Since the Spirulina biomass in Lake Bogoria shows seasonal fluctuation [16], satellite imagery data was utilized to determine the seasonal abundance of Spirulina over the past decade and a half. This allowed the researchers to determine certain peak dates when a relatively large amount of Spirulina biomass may be easily harvested.

CONCLUSION

The community at Lake Bogoria was in an overall nutritionally deficient state, with a number of vitamins lacking in their diet. These findings all indicate that a nutritional imbalance exists amongst the Lake Bogoria community. Spirulina harvested from Lake Bogoria offers one possible means of beginning to address these nutritional deficiencies. This study has demonstrated that Spirulina contains a number of micronutrients needed in the diet of the community and is the first to report that Lake Bogoria Spirulina contains levels of microcystins within World Health Organization safe levels. Spirulina has the potential to offer a local and sustainable means of addressing nutritional deficits in the Lake Bogoria community. These results indicate that a more in-depth study of the feasibility of harvesting Spirulina from Lake Bogoria and the safety of using it as a dietary supplement is warranted and desirable.

ACKNOWLEDGEMENTS
The authors would like to thank Dr. Dawn Bowdish (Department of Pathology and Molecular Medicine, McMaster University) for generously providing the equipment and lab space to carry out the analytical work. We also thank Ezekiel Chebii for his excellent work as a translator and field guide at Lake Bogoria.

This work was carried out under research permission from the Kenyan Government’s National Commission for Science, Technology & Innovation NCST/RCD/12B/013/21 and the permission of Baringo County Council’s Chief Game Warden and the Lake Bogoria National Reserve’s Senior Warden.

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Table 1: Nutrient values of Spirulina. Nutrients listed here reflect the nutrients examined in the Bogoria community dietary survey. Data adapted from Abdulqader *et al*. [11]

Nutrient	Composition in *A. fusiformis* (mg per 10g dry mass)
Vitamin C	2
Vitamin E	0.4
Thiamin	0.37
Riboflavin	0.46
Vitamin B6	0.07
Vitamin B12	0.02
Calcium	150
Iron	18
Zinc	N/A

Table 2: Percent mineral content of Lake Bogoria Spirulina as compared to mineral content of commercial strains

Mineral	Sample Value (%)	Literature Value (%)	P-Value
Iron	1.86%	0.2%	0.0632
Calcium	0.11%	1.5%	<0.0001

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