



Correlation between chemical characteristics and optical spectra of *Spirulina* commercially available on the Bulgarian market

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Abstract: The aggregate of various chemical substances useful for the functioning of the human body are known as nutrients. *Spirulina* has been present in human nutrition since ancient times, but in recent years the interest in it has been particularly increased due to the emergence of numerous alternative methods of nutrition. This study aimed to compare the functional and elemental composition as well as the optical properties of commercial *Spirulina* products available on the Bulgarian market. For this purpose, fluorescence spectroscopy in the ultraviolet and visible range, fourier transform infrared spectroscopy and inductively coupled plasma optical emission spectroscopy were used. The basic components of the analyzed *Spirulina* samples are proteins (1657 and 1537 cm^{-1}) and carbohydrates (1069 and 1054 cm^{-1}) and no meaningful differences between the IR spectra of the samples. Concentrations of important microelements Mg, Fe, Cu, Zn, and Mn varies with the manufacturer. The highest levels for Mg (6.69 g kg^{-1}) were measured in samples from USA, while the *Spirulina* fabricated in Bulgaria exhibits the highest contents of Zn (242 mg kg^{-1}) and Cu (25.4 mg kg^{-1}). All samples followed the tendency $\text{Mg} > \text{Fe} > \text{Mn} > \text{Zn} > \text{Cu}$. Making use of a fiber optic spectrometer the fluorescence spectra of the studied samples of *Spirulina platensis* for an excitation wavelength of 380 nm were measured. In these spectra we observe three fluorescence maxima: at 465 nm – nicotinamide dinucleotide phosphate, 640 nm chlorophyll a, and 736 nm due to similar to chlorophyll pigments. A strong positive correlation between the contents of Zn and Cu on the one side and the second fluorescence peak ($\lambda=640$ nm) for excitation wavelength at 380 nm. In contrast, a high negative correlation for Fe and the third fluorescence maximum ($\lambda=736$ nm) is observed for all excitation wavelengths. The correlation dependencies were obtained with the least squares method with a significance level of $p \leq 0.05$.

Keywords: *Spirulina*, fluorescence spectra, infrared spectra, elements.

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1. INTRODUCTION

In 1827 P.J.Turpin isolated *Spirulina* from a freshwater sample, and in 1852 Stizenberger wrote the first taxonomic report. These photosynthetic organisms were first considered algae, but in 1962 Stanier and Van Neil included blue-green algae in the prokaryotic kingdom and proposed that these microorganisms be called cyanobacteria (1).

Based on the cylindrical arrangement of the multicellular trichomes, there are two types of filamentous cyanobacteria: *Arthrospira (Spirulina) maxima* and *(Spirulina) platensis* (2). Their main photosynthetic pigment is phycocyanin, which is blue in color (3), they also contain chlorophyll a, carotenoids and various pigments that can give them a red or pink color (4). *Arthrospira (Spirulina) platensis* is a natural inhabitant of tropical lakes with alkaline waters (pH>11), these conditions restrict the growth of other microorganisms but allow its cultivation (5). The distribution of *Arthrospira platensis* in nature is not limited only from lakes in Africa to Lake Texcoco in Mexico, it can be found in soil, marine and fresh water, swamps and thermal springs (6). *Arthrospira platensis* is the most widely cultivated alga because it is rich in protein (between 50 % and 70%) with a high biological value due to the content of all essential fatty acids (7, 8). Long-chain fatty acids are dominant, especially palmitic and gamma linoleic acids (9). The latter is of particular importance in the treatment of chronic diseases. *Spirulina* contains polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (10). The consumption of microalgae by vegetarians is extremely valuable due to the high content of vitamins of group B as well as of type A, E, D, K (11). It is a source of the minerals calcium, iron, selenium, and fluorine (11, 12) as well as large amounts of carotenoids such as astaxanthin and zeaxanthin, beta carotene, polyphenols, and chlorophyll (4).

The huge number of bioactive compounds in *Arthrospira platensis* makes it suitable for its inclusion in foods and nutritional supplements (13). There are data that microalgae lower LDL cholesterol and blood pressure (14). It can be prescribed to patients with diabetes because it reduces blood sugar levels (15), relieves mental and physical fatigue of the body (16), increases immunity, and can be used as a probiotic (17). There are already many commercially available brands of health foods made from cultured algae and *spirulina*. Phycocyanins, which are now extracted from *Spirulina*, are used as industrial and food colorings (17).

As fluorescence measurements are far simpler and less costly than chemical analysis of substances it is of practical importance to establish the correlations between fluorescence spectra and the chemical contents of commercially available *Spirulina* samples from different parts in the world. The objective of this study is to investigate and compare the functional and elemental composition and optical characteristics of commercial *Spirulina* products available on the Bulgarian market.

2. MATERIALS AND METHODS

2.1. Samples Under Study

Three commercially available *Spirulina* samples were purchased, cultivated in bioreactors in USA, China (CHN) and Belgium (BEL) as well as *Spirulina/Arthrospira platensis* cultivated in a bioreactor close to Varvara, Bulgaria (BGR).

Three packages from each tested product were purchased. Measurements have been performed on each of them. Average results from three replicates of the experiment were presented.

The data for dietary and energy value expressed in g/100g for *Spirulina* products purchased from the Bulgarian dietary store and taken from the producer's label are given in Table 1.

Table 1: A main dietary and energy value of *Spirulina**.

	CHN	BEL	USA
Energy value, kcal	336	358	333
Fats , g/100g	1	6	5
Proteins, g/100g	65	65	56
Carbohydrates, g/100g	13.1	9.5	15

*The indicated table does not present data on *Spirulina* from a bioreactor in Bulgaria, since the latter is not available in organic stores and it is in the process of certification and research of the indicated parameters.

2.2. Methods

2.2.1. Fluorescence measurements

The fluorescence characteristics of organic matter from seaweeds were measured using an Ocean Optics QE65000 fiber optic spectrometer, an Ocean optics MonoScan 2000 fiber optic monochromator and broadband Energetiq Laser driven light source (190 nm to 2500 nm). The samples were excited at wavelengths from 220 nm to 720 nm at a 10 nm increment and a spectral bandwidth of around 15 nm. The sample is illuminated by a 1 mm core fiber and fluorescence is captured by a receiving 1mm core fiber oriented at 45° with respect to the excitation fiber to minimize reflected and diffused light. Integration time was 5 s. The experimental set-up is shown in Figure 1.

2.2.2. Excitation emission matrices (EEM)

At each excitation wavelength in the 220 nm to 720 nm the emission spectrum from 200 nm to 1000 nm of each *Spirulina/Arthrospira* sample was captured which permits 3D excitation emission plots to be presented. These color coded 3D spectra for each sample are presented in side and in topographic view in Figure 2. In these figures the emission spectrum is presented in the range of 400 - 800 nm.

2.2.3. Determination of the elements' content

A sample of about 0.3 g is weighed on an analytical balance in Teflon vessels for a microwave digestion system and 10 mL of 67% HNO₃ (Suprapur®) was added. Microwave digestion was carried out according to the following procedure: 10 minutes to reach 180 °C and maintain this temperature for 10

minutes. After cooling solution was transferred into a 25 mL volumetric flask and dilute to the mark with deionized water. The blank sample was run through the entire analytical procedure. The samples were finally filtered through 0.45 µm cellulose membrane filters (Millipore) and kept at 4 °C.

The content of Mg, Fe, Mn, Cu, and Zn was measured by ICP-OES system ULTIMA 2, Jobin Yvon, (Longjumeau, France). Multi-element standard solution IV for ICP (TraceCERT®, Merck) was used to prepare diluted working standard solutions for instrument calibration.

2.2.4. FT-IR spectra

The FT-IR spectra were recorded on a Thermo Fisher Nicolet iS50 spectrometer equipped with a diamond ATR Accessory and are presented after standard ATR correction performed on the OMNIC software. The IR spectra were recorded from 4000 cm⁻¹ to 400 cm⁻¹ with an average of 64 scans at a resolution of 4 cm⁻¹. The measurements were carried out directly on the *Spirulina* powder samples.

2.2.5. Statistical analysis

Analysis of variance was used to compare means with a significance level of $p \leq 0.05$ by using SPSS Statistic 22. One-way analysis of variance and Duncan's post hoc test for multiple comparisons based on the parameters studied were performed for all samples studied. The correlation dependences were obtained with the least squares method with a significance level of $p \leq 0.05$.

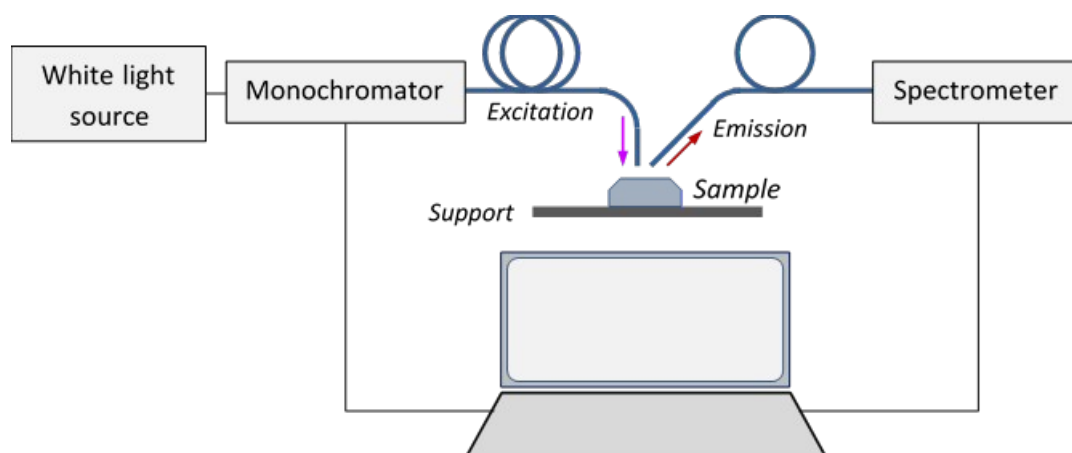


Figure 1: Experimental set-up for measurement of 3D emission excitation matrices.

3. RESULTS AND DISCUSSION

The concentration of Mg, Fe, Mn, Zn, and Cu was determined as elements performing specific functions in the human body. Since the human body cannot produce them on its own, it is necessary to take them through food.

The concentrations of the elements in the samples vary depending on the producers. The obtained values for all five analyzed elements are in the concentration range of other *Spirulina* products described in the literature (3, 18-21). The following trend is observed: Mg > Fe > Mn > Zn > Cu. As expected, the content of Mg in the four products is

the highest compared to the other elements, as it is a macronutrient. It is noteworthy that the concentration of Fe significantly exceeds the concentrations of the other micronutrients. Table 2 lists the quantity of the elements as measured in the samples. The sample from the Bulgarian reactor is the richest in Zn and Cu while in the other samples these elements are found in significantly lower concentrations. The highest concentration of Mg was observed in the USA sample.

The optical properties of *Spirulina* were investigated by fluorescence spectroscopy in the range of 220-720 nm at 10 nm increments. The excitation-emission matrices (EEM) of the studied samples in side and topographic view are presented in Figure 2. It follows from the analysis of the *Spirulina* samples that their optical properties strongly depend on the excitation wavelength. A change was observed if the individual fluorescence spectra around 410, 530, and 660 nm excitation wavelengths. At lower excitation wavelengths, the typical emission peaks around 650 nm and 672 nm associated in the literature with Phycobilisomes were not observed. However, a peak around 700 nm associated with the same substances was seen. This means that their molecules were not directly excited but had instead obtained energy from the excited molecules of carotenoids via resonance transfer or re-absorption.

For excitation in the red part of the visible spectrum from 640 nm to 660 nm range an emission peak in

the 710 nm and 715 nm due to Chlorophyll a was observed. Similar excitation wavelengths for substances as Chlorophyll a, Chlorophyll b, Phycocyanin, Phycoerythrin have been reported by Cadondon et al. (22), Li et al. (23), Gobets et al. (24), Karapetyan et al. (25), Akimoto et al. (26), Uebel et al. (27).

Some relations were established between the observed spectra and the content of the metals. A closer inspection of the 3D EEM plots reveals that the fluorescence maxima were observed at an emission wavelength around 735 nm – 736 nm for excitation from 250 nm to 720nm.

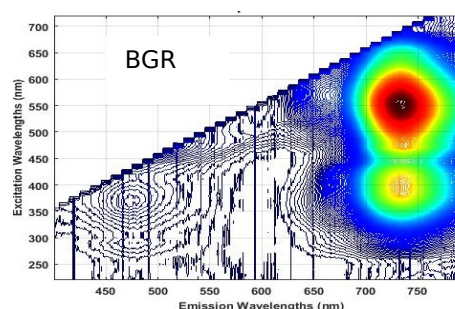
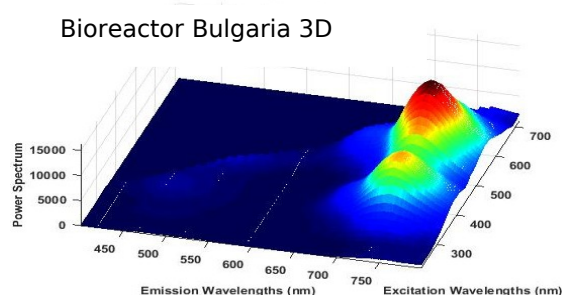
However, the maxima and their relative height were different. Fig. 3a shows how the fluorescence intensity at 736 nm changes for each sample as excitation wavelength varied from 220 nm to 720 nm.

There were some peaks for excitation wavelengths of 380, 430, and 560 nm. The emission spectra of the samples for each of these three excitation wavelengths were shown in Fig. 3b) to 3d). The emission spectra exhibited maxima at three wavelengths: 465, 640, and 736 nm were labeled as I, II, and III. Due to the Stokes' shift, fluorescence emission is observed at wavelengths higher than the excitation wavelength. Therefore, for 560 nm fluorescence is observed above 588 nm (Fig. 3d).

Table 2: Content of Mg, Mn, Fe, Zn and Cu in the four *Spirulina* samples (n=3, RSD=3-7%) and reference values described in the literature*.

Elements	BGR	CHN	BEL	USA	Refs. (3, 18-21)
Mg, g/kg	2.57 ^d	2.76 ^c	3.80 ^b	6.69 ^a	0.67 - 9.49
Mn, mg/kg	55.0 ^b	55.4 ^b	49.7 ^c	141 ^a	5 - 554
Fe, mg/kg	317 ^d	868 ^b	447 ^c	1177 ^a	195 - 6500
Zn, mg/kg	242 ^a	18.0 ^d	25.8 ^c	35.4 ^b	3.8 - 375
Cu, mg/kg	25.4 ^a	3.97 ^d	5.23 ^c	11.9 ^b	2.63 - 69.6

*Means in a row with a common superscript letter (a-d) differ ($p < 0.05$) as analyzed by one-way ANOVA.



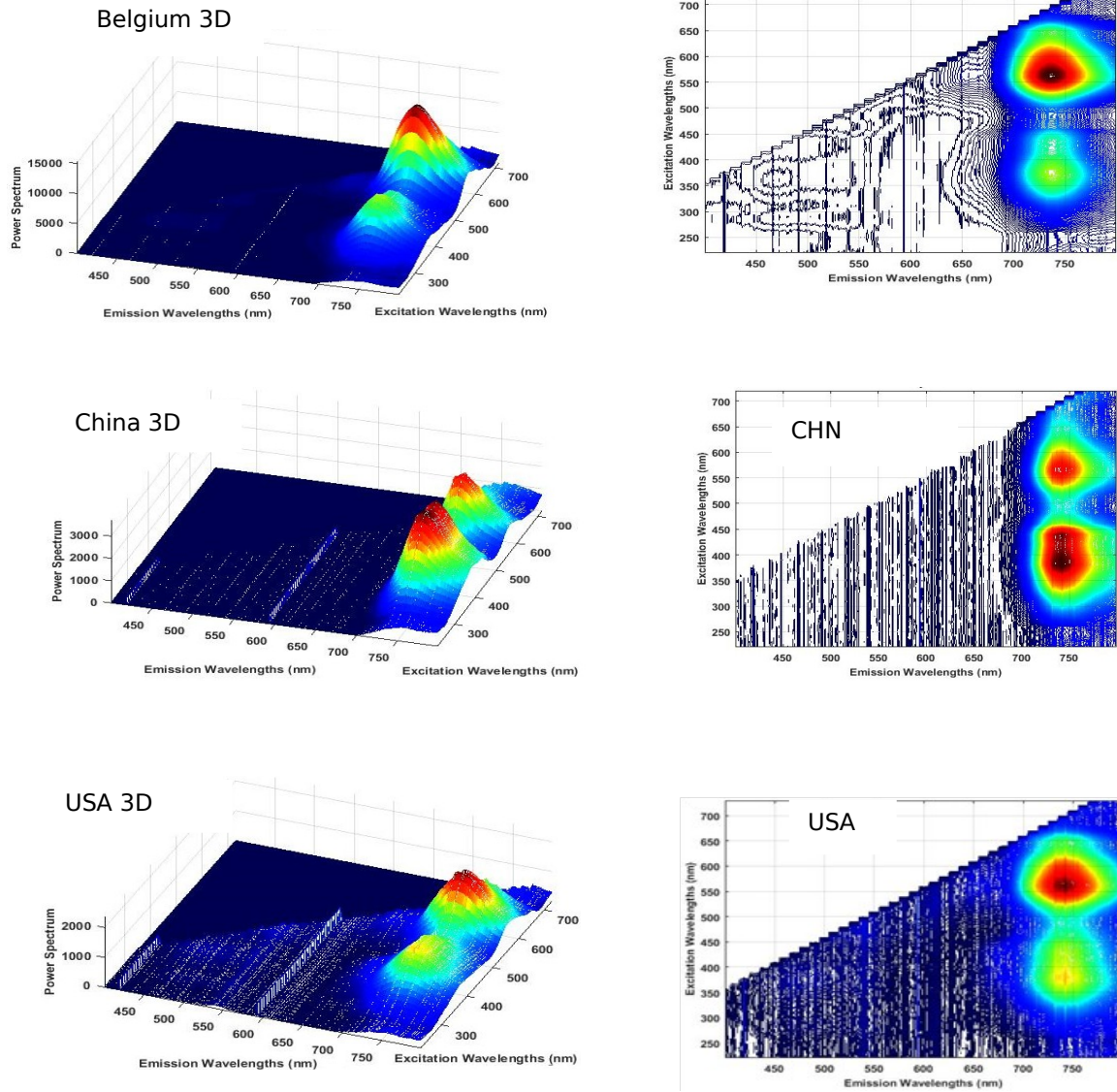
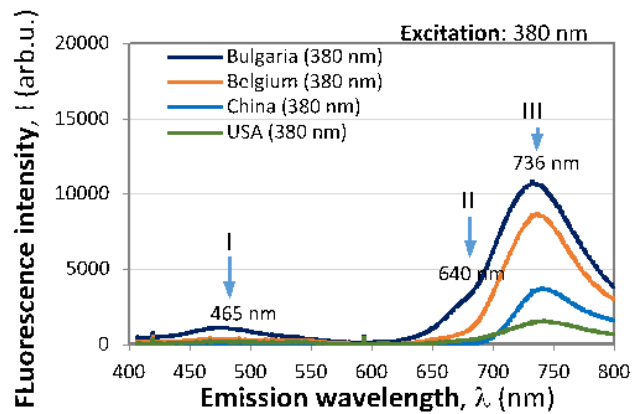
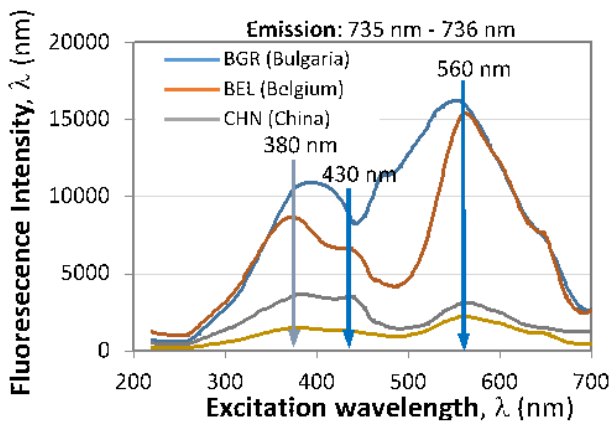


Figure 2: Excitation emission 3D and topographic presentations.



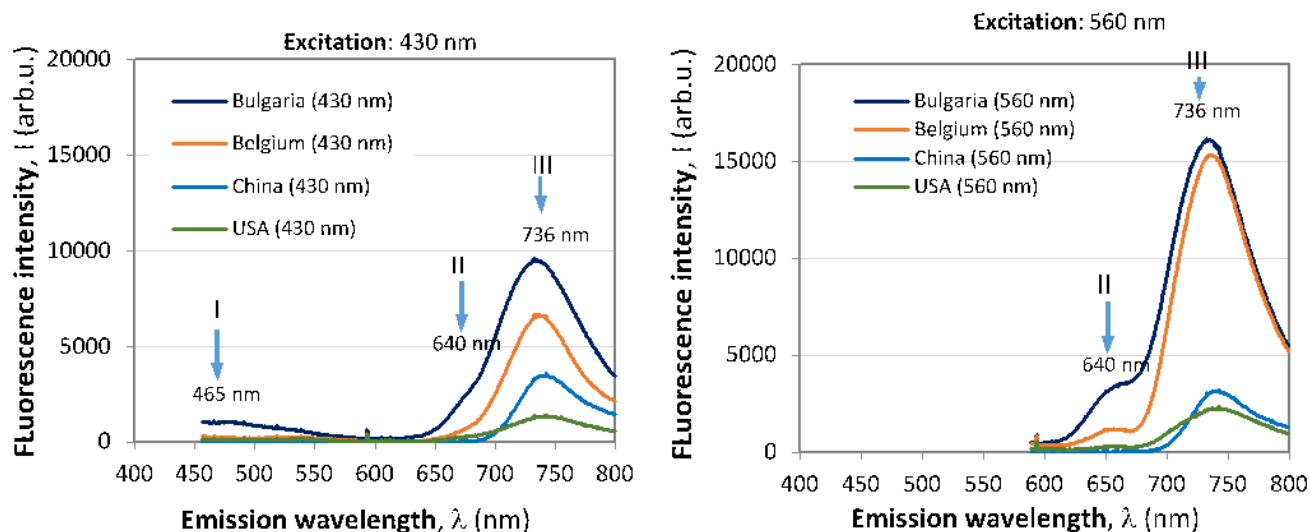


Figure 3: Sections of the 3D EEM plots: a) Dependence on the excitation wavelength for emission at 736 nm; Emission spectra for excitation at b) 380 nm; c) 430 nm; d) 560 nm.

The dependencies of the average intensity around each of these maxima on the contents were plotted for each element for each of the maxima. As examples, the plot for Zn when excited at 430 nm for maxima I (465 nm) and II (640) were shown in fig 4a) and in Fig. 4b) - for Fe at 560 nm for maxima II and III. Two basic observations for all samples must be noted for the two plots in Fig. 4. First, for Zn from Fig. 4a), the intensities of both peaks increased with the content, while for Fe, the intensities decreased with the Fe content. Second, the intensity of the first maximum is higher than the second. These observations are reversed for Fe from Fig.4b). This implies positive and negative correlation between fluorescence intensity and content for the *Spirulina* samples of different origin. There were some peaks for excitation wavelengths of 380, 430, and 560 nm. The emission spectra of the samples for each of these three excitation wavelengths were shown in Fig. 3b) to 3d). The emission spectra exhibited maxima at three wavelengths: 465, 640, and 736 nm were labeled as I, II, and III.

The correlation between the content of each metal and the intensity of each maximum I, II, and III for each excitation wavelength were obtained, and the coefficient r was calculated. The results were summarized in Table 3.

Zn and Cu exhibit a very high positive correlation between their content and the intensity of the maxima I and II for 380 nm excitation and for maximum II at 560 nm. In contrast Fe exhibits very high negative correlation for maximum III and a high correlation for maxima I and II for all excitation wavelengths. Mn exhibits at best a moderate negative correlation for maximum III and low or weak for the other maxima at any excitation.

Figure 5 presents the FT-IR spectra of the *Spirulina* samples. The peak around 3290 cm^{-1} could be attributed to -OH and -NH groups (28-29). The C-H stretching vibrations could be found around 2920 cm^{-1} (28). These signals could be assigned to lipid and protein methylene vibrations (30). The adsorption peaks in the region $1700\text{--}1400\text{ cm}^{-1}$ could be assigned to -CO stretches aldehydes, ketones, and carboxylate groups. (31-32, 28). These vibrations could be attributed to functional groups present in proteins in the *Spirulina* sample (31). More specifically, these signals represent the vibrations of amides I and II, from the protein in *Spirulina* powder (30). The signals in the region $1100\text{--}1000\text{ cm}^{-1}$ belong to functional groups in the carbohydrate components in the samples (31-32).

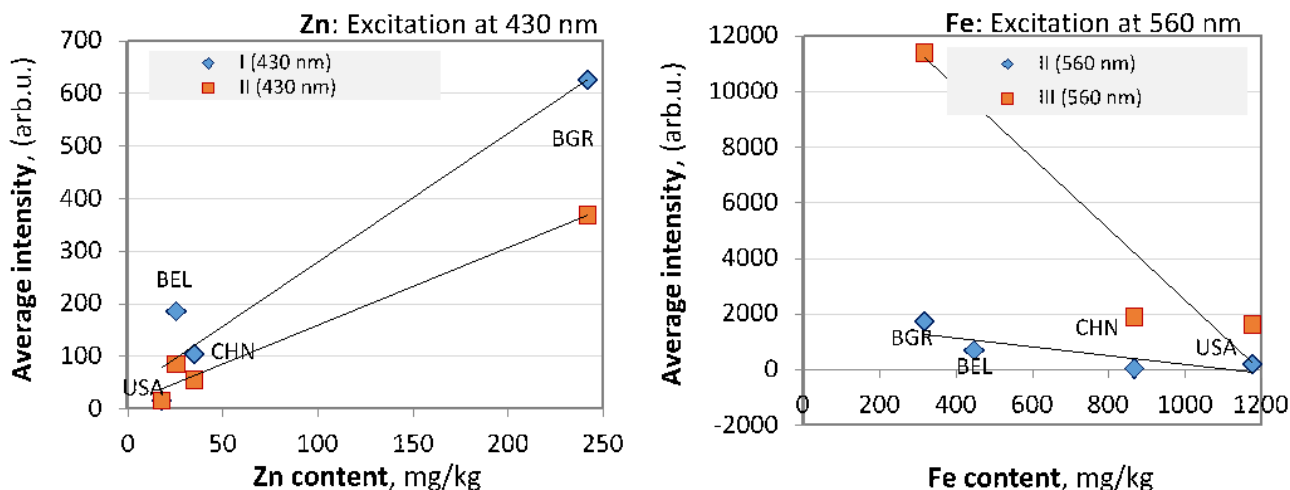


Figure 4: Plots of the average power at particular maxima vs. the content of a definite metal: a) Zn at 430 nm excitation for maxima I and II; Fe at 560 nm excitation for maxima II and III.

Table 3: Correlation coefficient between metal contents and maximum intensity.

Max	380 nm excitation			430 nm excitation			560 nm excitation		
	I	II	III	I	II	III	I	II	III
Mg	-0.38	-0.45	-0.65	-0.41	-0.43	-0.68		-0.45	-0.50
Mn	-0.32	-0.35	-0.69	-0.31	-0.3	-0.66		-0.40	-0.61
Fe	-0.78	-0.77	-0.98	-0.74	-0.71	-0.95		-0.82	-0.95
Zn	0.93	0.97	0.75	0.97	0.99	0.84		0.93	0.63
Cu	0.88	0.90	0.58	0.92	0.93	0.67		0.85	0.40

Positive: Very High: 0.9-1 High: 0.7-0.9 Moderate: 0.5-0.7 Low: 0.3-0.5 Very low: 0-0.3

Negative: Very High: 0.9-1 High: 0.7-0.9 Moderate: 0.5-0.7 Low: 0.3-0.5 Very low: 0-0.3

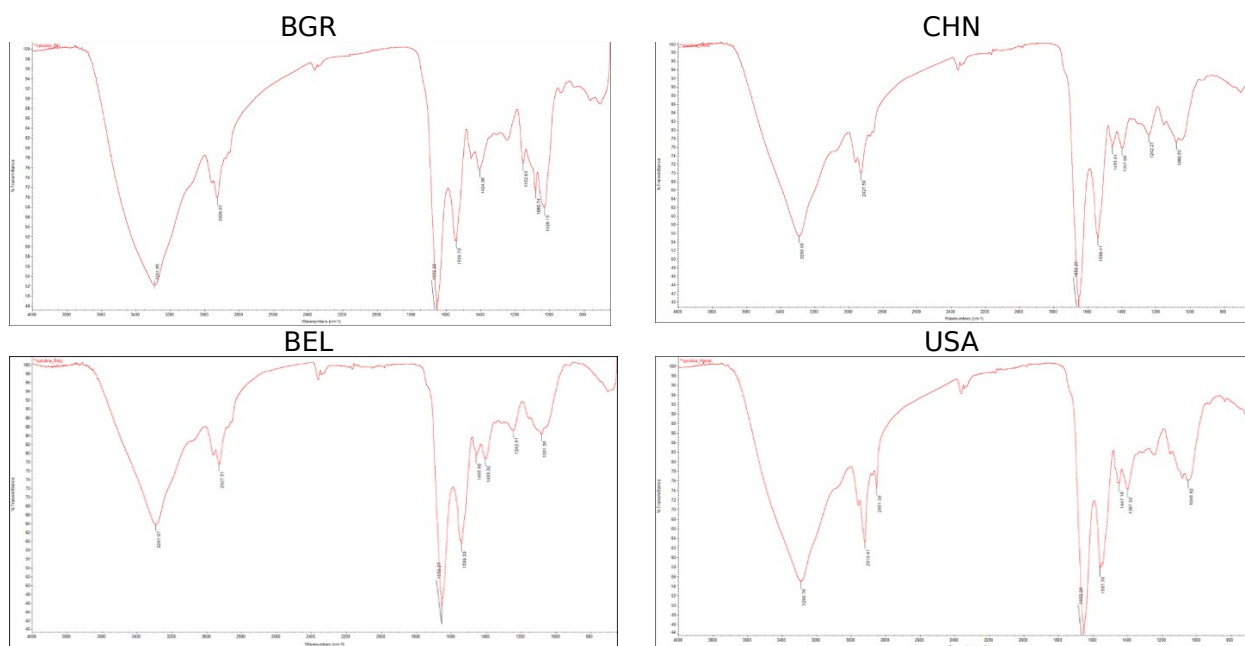


Figure 5: FT-IR spectra of the different *Spirulina* samples.

4. CONCLUSION

All investigated samples have a similar functional composition. The resulting signals in the different regions of the IR spectrum can be assigned to proteins, and carbohydrates. *Spirulina* is a good source of essential elements such as magnesium and iron, a trend was observed: $Mg > Fe > Mn > Zn > Cu$. Comparing the content of each element with the intensity of the maxima for the most effective excitations at 380, 430, and 560 nm show that the presence of Zn and Cu can be linked to the very high positive correlation, while that of Fe to the very high negative correlation with the intensity of fluorescence maxima.

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