Impact of heavy metal pollution on growth, biochemical composition and nutrition quality of Spirulina platensis

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Article

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Abstract

This study focused on the effect of five different concentrations of three heavy metals (Nickel, Copper and Zinc) on growth, IR spectra, the content of fatty acids and total soluble protein profile in *Spirulina platensis*. *Spirulina platensis* was selected for this study because of its high nutritional value as an important form of algae that is used commercially (especially for fish feeding) as a source of proteins, minerals, amino acids, vital unsaturated fatty acids, and a range of vitamins. EC50 of the three heavy metals was recorded nearly at concentration 2.0 mg/l. The inhibitory effect of growth measured in optical density was more prominent in case of copper than zinc and nickel. IR spectra showed formation of new compounds and absence of other compounds when compared to untreated cells, it was clearer in case of Cu^{2+} than in case of Zn^{2+} and Ni^{2}. Grand total fatty acids decreased under all the tested concentrations, saturated fatty acids were more dominant than unsaturated ones under stress conditions. The destructive effect of the heavy metal ions on protein profile was more prominent under the stress effect of Cu^{2+} than in case of Zn^{2+} and Ni^{2+}.

Introduction

The Industrial Revolution resulted in heavy metal contamination. Pollution has an impact on both the quality of life and the environment's ecosystem. Heavy metals are the major components of several industries, as is their emission into the environment. Egypt has five Mediterranean Lakes (Burullus, Manzala, Edku, Mariut, and Bardawil). They are vital in supplying a valuable natural resource for the production of fish. Heavy metal pollution of the aquatic environment has long been seen as a significant threat to aquatic creatures, particularly fish. The levels of pollution in these lakes are Mariut > Manzalah > Edku > Burollus > Bardawil (**Saad, 2003**).

Phytoplankton is a varied group of microorganisms that are crucial systems for maintaining species variety since they are the foundation of the aquatic food chain and the primary producers, (**Hanan** et al., **2015**). Microalgae biorefinery systems have been thoroughly investigated in terms of resources, energy, biofuel production potential, and high-value products. *Spirulina platensis* stands out among commercially important microalgae because of its high protein, carotene, and fatty acid content, making it appropriate for use in animal and human nutrition, (**Nethravathy** et al., **2019**). *Spirulina platensis* and *Chlorella vulgaris* are the most commonly utilized species at a commercial level. Currently, the estimated global production of *Chlorella* and *Spirulina* are 6600 and 12,000 tons of dry matter per year, respectively (**Garcia** et al., **2017**). Besides protein, lipid content varies according on growth circumstances, with values ranging from 9 to 17 percent DW for S. platensis. **(Piorreck** et al., **1984**). Because of its excellent nutritional content, *spirulina* has grown in popularity as a promising and useful feed additive. Furthermore, its bioactive phytochemicals have important anti-inflammatory and antioxidant properties,(**Abdel-Latif** et al., **2022).**

Microalgae producers strive for the best biomass productivity and quality while avoiding nutrient constraints and other detrimental growth conditions. Because protein contains nitrogen as well as other

nitrogenous elements such as nucleic acids, amines, and cell wall material, a total nitrogen measurement overestimates the true protein level, (**Muys** et al., **2019**). Microalgae are known to be excellent bio accumulators of heavy metals (**Arunakumara and Xuecheng, 2008**). While some metals, such as As, Hg, Cd, Pb, and Ni, are toxic, others, such as Zn, Cu, and Cr, are needed in human nutrition but become harmful when intake levels are exceeded. Toxicity from heavy metals poses a global concern to humans and the environment. *Spirulina* is a powerful bioaccumulator of a variety of heavy metals., (**Sanjib, 2020**).

Spirulina platensis is today of global nutritional importance in the fight against malnutrition, particularly in children. This blue-green cyanobacteria algae is grown in temperate waters all over the world and is regarded a functional food because of its high content of proteins, vitamins, minerals, healthy fatty acids, and other healing phytonutrients like various active plant colours. The International Association of Applied Microbiology identified *Spirulina* as a future food source in 1967. (**Lupatini** et al., **2017**). Furthermore, *Spirulina platensis* has a high quantity of amino acids and important unsaturated fatty acids (such as -linoleic acid) in terms of nutritional value. Calcium, magnesium, iron, phosphorus, manganese, potassium, zinc, and selenium are among the minerals found in it. *Spirulina* also contains a number of vitamins, including B12, B1, B2, and E, (**Pyne** et al., **2017).** *Spirulina* can synthesize polyunsaturated fatty acids, which have significant structural, biochemical, and functional functions in human metabolic pathways. Besides their role as source of energy, they act as main constituents of cellular membrane structures, fatty acids, particularly omega-3 and omega-6, have become a popular point of reference for these illnesses, such as cardiovascular, neurologic, and endocrinologic diseases. (**Zheng** et al., **2012**).

In the last decade, the nutritional value of *spirulina* in aquafeeds has been intensively explored as a fishmeal replacer or as a functional feed addition to boost fish growth and health performance. Because of its high protein content (up to 70%) and abundance of essential fatty acids, antioxidant pigments (phycobiliproteins and carotenoids), and polysaccharides, commercial *spirulina* production has grown in popularity for use in nutritional supplements for humans and animals as well as pharmaceuticals. *Spirulina* is a potential replacement for animal-derived proteins in aquafeed in aquaculture, (**Ragaza** et al., **2020).**

Materials And Methods

I-Measurement of growth (Optical Density):

The studied biological material (*Spirulina platensis*) was obtained from Algal Culture Collection of Botany and Microbiology Department, Faculty of Science, Alexandria University. In *spirulina* medium (**Zarrouk, 1966**), *Spirulina platensis* was cultured. Measurements of optical density (turbidity technique) are particularly suitable for determination of *Spirulina platensis* growth. In this method the change in percentage of light transmittance (T) relative to un-inoculated control tubes set at 100% T by using Perkin Elmer (Lambada 1) ultra violet spectrophotometer at wave length of 560 nm. The optical density was calculated from the following equation (Robert, 1979): **Optical density (O.D.) = log I_0/I**

Where: I= the transmittance of sample. $I_{O=}$ the transmittance of blank adjusted to read 100%.

II-Preparation of different concentrations of nickel, copper and zinc

The following salts were obtained from Algmhoria Co. in Alexandria, Egypt: NiCl2, CuSO4.5H2O, and ZnCl2. The three heavy metals nickel, copper, and zinc were chosen for this study because of their abundance in industrial waste water and their negative ecological impact. The selected heavy metals' stock solutions were made from their salts in double distilled water and sterilised using 0.2 m nitrocellulose membranes. The varied quantities of selective heavy metals used in the metal bioassays were made by diluting them with doubly distilled water. The EC50 of the three heavy metals was found to be about 2.0 mg/l. To compare the results of five different concentrations of the three heavy metals, we chose four concentrations (two greater and two lower) for each element.

III-Measurements of infrared spectra (IR) of tested species cells

Preliminary trials were performed to determine the density of cell suspensions necessary to produce spectra with a good signal. Lugol's iodine solution was added to pellets of a known volume of algal culture according to **Kansiz *et al.* (1999)**. For IR analysis the dried cells were placed on the infrared microscope stage for spectral acquisition. The spectra were collected on Perkin Elmer 1430 ratio recording infrared spectrophotometer. The absorbance spectra were collected between 4000 - 500 cm^{-1} with 10 scans co-added and average.

IV-Measurements of total lipid extraction

The total lipid content of the tested alga *Spirulina* was extracted according to **Bligh and Dyer (1959)**.

The total lipids were exhaustively extracted by the addition of 50 ml of chloroform and methanol mixture (2: 1 v/v) to 50 ml of the algal culture in a separating funnel, after setting two layers formed, the aqueous layer containing the remaining of cells was discarded while the other layer containing the lipid extracts was washed with 0.4% $MgCl_2$ solution (100 ml) followed by distilled water (100 ml) for several times to get rid of all the excess of $MgCl_2$. This extract was then mixed with 1.5 N HCl for acidification. To separate fatty acids from oils, 50 ml of 1n-hexan was add to acidified extract in a separating funnel followed by 50 ml of I % freshly prepared sodium carbonate. This mixture was re- acidified by adding 2 ml of 1.5 N HCl.. The hexane layer contained the fatty acids was separated from the aqueous layer and dried over anhydrous Na_2SO_4 to get rid of water droplets then filtration take place and the obtained filtrate was evaporated using rotary evaporator. Residue obtained represents the total lipids.

V-Measurements of protein profile

Protein profile estimated by dialysis tubing (previously cut into short length approximately 15 cm each) were boiled for 15 minutes in a solution of 1% sodium bicarbonate and 10^{-3} M Na_2EDTA. The freshly

frozen cell pellet of algal material obtained by centrifuging 10 ml of culture was ground in a mortar using quartz sand and small volume of 0.5 M Tris-HCl buffer until homogenous was extracted several times with small volumes of the same buffer (PH 7.2). The suspension was centrifuged for 10 minutes at 500 rpm, and the supernatant was concentrated in pre-activated dialysis tubing over a sucrose bed. Concentrated samples were transferred into small tubes, few drops of glycerol were added to increase viscosity, and 2-3 drops promophenol-blue were also added as an electrophoresis marker. Samples were then kept in the freezer for total soluble protein profile analysis by Electrophoresis gel method.

Result And Discussion

A- Growth:

Optical density (O.D.) is an important parameter of growth of *Spirulina platensis* which is used indicator of risk assessment of different contaminants especially heavy metals. Results obtained of optical density after 8 days of culturing represented in **figure (1)** revealed that, the effective concentration (EC50) of Nickel, Copper, and Zinc was about 2.0 mg/l, according to the results obtained (theses results obtained after doing different experiments to determined EC50 of the three heavy metals). Consequently, in this work, five concentrations were chosen (two concentrations below and two higher than 2.0 mg/l) i.e. l.0, 1.5, 2.0, 2.5 and 3.0 mg/l for each element besides control. The results showed that copper had a stronger inhibitory effect on growth evaluated in optical density than nickel and zinc at all concentrations tested. The lowest concentration (1.0 mg/L) accelerated the growth but increasing concentration of copper inhibited algal growth. Our results go in agreement with those obtained by **(Budi *et al.,* 2020)** who indicated that, with the treatment of various levels of Cu, the extreme growth rate of *Spirulina plantesis* displays that the treatment by adding 1 mg/L of heavy metal is needed for increasing growth but the higher the concentration of Cu given, the lower the density of *Spirulina plantesis*.

We discovered that Zn^{2+} ions were more toxic than Ni^{2+} ions, that lower quantities of zinc and nickel accelerated development, and that they are considered growth accelerators of *Spirulina platensis* when utilized in the lowest concentrations. These findings are in agreement with authors **(Meenakshi *et al.,* 2007)** they observed that by utilizing 2 mg/l for both heavy metals, they discovered a considerable reduction in growth in *Spirulina platensis* culture due to copper toxicity, which was higher than zinc. Heavy metal toxicity can be observed by changes in growth circumstances and reduction in the growth of the test microorganism.

Akbarnezhad *et al.,*(2019) found that there was a gradual increasing on optical density of *Spirulina platensis* culture at low concentrations of zinc and cooper. On the contrary, another important fact obtained by **Zinicovscaia *et al.,* (2021),** who observed that *Spirulina platensis* has a higher bioaccumulation capacity for Zn than Ni and Cu ions, and they believe it is an attractive option for environmental bioremediation because of its strong capacity in biosorption and bioaccumulation for heavy metal ions.

B-Infrared spectroscopy:

Infrared spectroscopy has distinct advantages over other conventional methods of biochemical analysis in that it is rapid, reliable and requires a relatively small sample size and simple sample preparation procedure (**Kansiz *et al*. 1999**). Infrared spectra of the total cell constituents showed the band assignments, which are based on the studies on whole cells, organelles and macromolecules of *S. platensis* at the region 4000-250 Cm^{-1}. The data obtained revealed that there are a number of spectra regions that can account for the chemical differences in this species.

The obtained infrared peaks of the major cell constituents of *S. platensis* cultured for 8 days under the stress effect of different concentrations of Ni^{2+}, Cu^{2+} and Zn^{2+}ions metals were recorded in **figures 2,3 and 4**. It is clear – when compared to control – that some peaks disappeared, others appeared new and still others remained unchanged. The new peaks that appeared under the stress of these three elements may be either from the changes of the position of some side chains or from the degradation of some compounds having high molecular weight to others with low molecular weights. These concepts are in line with those obtained by **Al-Osaimi (2010)**.

At 1.0 mg/l Ni^{2+} and Zn^{2+} the obtained number of peaks was higher than control (18 and 16 peaks, respectively) while in case of Cu^{2+} under the same concentration, the number of peaks decreased to 14 peaks as in case of control. However, at the concentrations more than 1.0 mg/l of Ni^{2+} (1.5, 2.0, 2.5 and 3.0 mg/l), the number of peaks decreased but still higher than control. Under this concentration (1.0 mg/l) in case of Ni^{2+}, 9 new peaks appeared at frequencies; 4000-3500, 2500-2000 and 1500-1000 cm^{-1}; and five peaks disappeared. The disappeared peaks were at frequencies 4500-4000 and 3500-3000 cm^{-1} which contained the asymmetric and symmetric C-H of methylene groups. At concentrations 1.0 and 1.5 mg/l Cu, the number of peaks are the same as at control (14 peaks); while at concentrations 2.0, 2.5 and 3.0 mg/l, the number of peaks decreased to 13, 12 and 7 peaks, respectively. Also, the total disappeared peaks were 5, 5, 6, 8 and 7 peaks at 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l respectively. The new peaks that appeared at concentrations from 1.0 to 2.0 mg/l are five peaks while at concentration 2.5 mg/l they reached six peaks. At concentration 3.0 mg/l Cu only one peak appeared. Although zinc shows evidence of some toxicity but appears to be only about one-fifth as toxic as copper (**Miller, 1946**).

It is clear therefore that the tested algal species *S. platensis* cultured at different concentrations of Ni, Cu and Zn that there are new compounds, and hence new peaks in the spectra appeared while other compounds disappeared when compared to untreated cells. These peaks may be resulted from the disappearance of some compounds, the weak rate of their synthesis, and the changes in the position of some side chains of the same compound and/or to the dissociation of complex compounds to simple one. Our findings are consistent with those obtained by **El-Agawany and Kaamoush,(2022)** who found that, the obtained infrared peaks of the major cell constituents of *Dunaliella tertiolecta* showed the appearance of new peaks and the disappearance of others which indicates changes in cell constituents due to the presence of different concentrations of zinc element.

In all of the tested elements the new peaks appeared at frequencies from 2500 – 1000 cm^{-1} which represents the amides associated with proteins together with the function groups of others from lipids and fatty acids (**Williams and Feleming 1996**). The higher number of peaks that appeared in case of control and the amended cultures with Ni^{2+}, Zn^{2+} and Cu^{2+} under all the tested concentrations were at frequencies 1500 - 1000 cm^{-1}. These frequencies represent the amides associated with protein and the phosphodiester back bone of nucleic acids (**Noctor and Foyer, 1998**). Finally, it could be concluded that Cu^{2+} metal ion is more toxic than Ni^{2+} and Zn^{2+} metal ions and the degree of stress depends mainly on the concentration and type of the element together with the length of the culture period. This could be proved from the results obtained by (**El-Sheikh *et al.,* 1999)** which revealed that toxic effects of heavy metal depend on the type of the element and its concentration**.**

C-Fatty acids:

Environmental conditions can affect both the relative proportions of fatty acids as well as the total amounts of lipids. Also, Lipid class and fatty acids composition of microalgal cells at different growth phases can differ significantly (**El-Maghrabi 2002**). Fatty acids have an important role in prevention and modulation of certain diseases like coronary heart disease, (**William, 2000**). The fatty acids composition of dietary microalgae is linked to the growth and survival of aquaculture. It is well known fact that lipid production usually differed between genera, species and strains of microalgae. However, total lipid fractions in healthy phytoplankton vary substantially from less than 1% to more than 40% of dry weight (**Dubinsky *et al*., 1978).** *Spirulina* is a plentiful source of highly valuable phytocompounds with unique functional characteristics including phenolic acids, carotenoids, and both omega-3 and omega-6 polyunsaturated fatty acids (**Pyne *et al.,* 2017**).

Concerning total fatty acids content of the tested alga *S. platensis* after 10 days of incubation in relation to the five concentrations of the chosen elements, it is quite evident that all the three groups of fatty acids are greatly affected especially at high concentrations of the elements. However, the toxic effect of these elements differed according to type of fatty acids group. So, by increasing the concentration of the element, the total content of the three groups of fatty acids decreased. This decrease was found to be very highly significant. The toxic effect of Ni^{2+}, Zn^{2+} and Cu^{2+} was found to be concentration dependent i.e. the toxic effect increased with increasing the element concentration. **El-Sheikh *et al.* (1999)** recorded that toxicity of the element was a concentration dependent. *Spirulina platensis* suffered greatly from Cu^{2+} concentration than both Zn^{2+} and Ni^{2+}. The toxic effect of all groups of fatty acids ranged from highly significant at low concentrations to very highly significant at higher ones. At higher concentrations, the synthesis of all groups of fatty acids was inhibited by the five concentrations used, but the degree of inhibition was more prominent at higher concentrations especially in the content of polyunsaturated fatty acids. The results prove that the toxic effect of the tested element on fatty acids content in *S. platensis* was more prominent in case of mono-unsaturated and poly-unsaturated fatty acids than saturated ones.

Under normal conditions, the organism synthesizes 27 fractions of fatty acids at the 10^{th} day of culturing. **Dempester and Sommerfeld, (1998)** reported that nutrient deficiencies may lead to an increase

in the cell lipid content and consequently cells of old healthy cultures are richer in fatty acids than those cells grown at the lag phase of growth. The mono unsaturated fatty acid C18:1 and the poly-unsaturated fatty acid C18:2 were the most dominant ones. **Cohen,(1991**) reported that microalgae are excellent sources of polyunsaturated fatty acids such as lindonic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. It was reported by many authors that linolinic acid (C18:2) fatty acid is essential for the survival and growth of many juvenile aqua culture organisms, (**El-Maghrabi, 2002**).

Anent the results recorded in **table (1)** and graphed in **figures (5&6)** which explained the effect of different concentrations of Ni^{2+} ions (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) on the content of the three groups of fatty acids fractions (saturated, mono- and poly- unsaturated fatty acids) in *S. platensis*, it is clear that Ni^{2+} ions have weak toxic effect on the content of fatty acids in *S. platensis* compared to control. The content of saturated fatty acids at all the tested concentrations of Ni increased by 12.36, 10.01, 10.66, 9.02 and 18.40% compared to control. Also at concentration 2.0 and 2.5 mg/l Ni^{2+} the saturated fatty acids (C20:0 and C21:0) disappeared completely. However, total saturated fatty acids increased under the effect of the different concentrations of Ni ions.

It is clear from data recorded in **table (2)** and graphed in **figures (7&8)** that, at concentration 1.0 mg/l Cu^{2+} the total content of the saturated fatty acids decreased by 8.51% compared to control. These results may confirm those results obtained for growth where the organism showed weak growth for the same concentration compared to control. The saturated fatty acid C6:0 at this concentration (1.0 mg/l) increased by 287.56% over control. The results cleared also that the mono-unsaturated fatty acids decreased under all the tested Cu^{2+} concentrations with different values which depends on the concentration of the element. On the contrary, although the total poly-unsaturated fatty acids increased under the effect of all the studied concentrations of Cu yet C20:5 was not detected at concentrations 2.5 and 3.0 mg/l, C20:3 at control and at concentration 3.0 mg/l, C20:2 and C22:6 was not detected at concentrations 1.5, 2.5 and 3.0 mg/l. C22:2 which was detected at concentrations 1.0 and 2.0 mg/l but was not detected at control and 1.5, 2.5 and 3.0 mg/l.However, the grand total fatty acids decreased under all the concentrations tested.

Taking into consideration the effect of the five tested concentrations of Zn^{2+} on the content of the three groups of fatty acids (saturated, mono- and poly- unsaturated fatty acids) in *S. platensis*, it is clear from **table (3)** and **figures (9&10)** that the grand total of the three groups of fatty acids increased at concentration 1.0, and 1.5 mg/l Zn^{2+} (4.993 and 0.006% over control) while at concentrations 2.0, 2.5 and 3.0 mg/l Zn the total content decreased by 0.009, 0.009 and 0.005% below control, respectively. The content of saturated fatty acids increased under all the tested concentrations of Zn ions and the percent of increase depended on the concentration of Zn^{2+}. At concentration 1.0 mg/l Zn^{2+} the percent of increase reached 16.74% while at concentration 3.0 mg/l Zn reached 0.74% over control. Concerning the mono-unsaturated fatty acids. Our results go with harmony with those obtained by **Alam *et al.* (2010)** and **Balaji, (2015)** who observed that, heavy metals have the potential to alter the rate of photosynthesis by disturbing chloroplast structure leading to the changes in the fatty acid composition. Another fact which

support our results obtained by (**El-Agawany and Kaamoush, 2022**) who observed that in *Dunaliella tertiolecta* culture by increasing zinc, the total content of the three fatty acid groups decreased and the toxic effect of Zn^{+2} was more prominent in the case of mono-unsaturated and poly-5-unsaturated fatty acids than in the case of saturated ones.

It was reported by **Dempester and Sommerfeld (1998)** that neutral lipid production of some diatoms was noticeably influenced by specifically altering the $MgCl_2$ concentration in the culture medium. **Roessler, (1989)** reported that the activity of acetyl-CoA carboxylase, an enzyme utilized early in fatty acid synthesis, was dependent on the presence of divalent metal cations especially magnesium (Mg^{2+}). The same author observed reduced acetyl-CoA activity when manganese (Mn^{2+}) was the only divalent metal present and no acetyl-CoA activity when only cobalt (Co^{2+}) was present. Increased lipid yield was observed with increasing salt concentration, which may cause physiological stress in *Botryococcus braunii* and *Isochrysis* species (**Ben-Amotz *et al*. 1985**) and in *Chlorella* species (**Tadros 1985**). **El-Maghrabi, (2002)** recorded that one of the major factors that enhanced lipid biosynthesis may be nutrient limitation. The same results were also obtained in our study. However, cyanobacteria do not show significant changes in their lipid content and fatty acid composition in response to nitrogen supply (**Becker, 2004**). It was found that nitrogen limitation is an effective method to increase lipid content, mostly at the expense of protein (**Piorreck *et al*., 1984**).

Simonopoulos, (1991) found that microlagae were a good source for Omega-3 fatty acids which are protective factor against chronic diseases, coronary-heart diseases, diabetes and cancer. **Chu and Dupuy, (1980)** concluded that the changes in the relative amounts of polyunsaturated fatty acids may be attributed to effects on the desaturation pathways of fatty acids. **Xu *et al*. (1997 and 1998),** reported that the reduction in polyunsaturated fatty acids fractions might be due to reduction in membrane fluidity and permeability. **Dowidar, (1983)**, mentioned that saturated fatty acids were more dominant than unsaturated ones under stress conditions. The same conclusion was also reported in our results.

D-Protein profile:

The gel plate of the total soluble protein profile for control and the treated organism with different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) of Ni^{2+}, Cu^{2+} and Zn^{2+}showed bands distributed through gel plates were illustrated in **tables (4 – 9)** and **plates (1- 3)**. The sum of the bands that appeared on the gel plate and confirmed by scanning using the band peaks were 20 bands for Zn and 17 bands for both Ni and Cu. Some of these bands were common in the control and the treated organism; others were common only in the treated organism under the effect of the five different concentrations of the three tested elements (Ni^{2+}, Cu^{2+} and Zn^{2+}). Most of them appeared in the region between 25 KDa and 212 KDa in nearly all the lanes. However, the number of these bands at all the concentrations of Ni^{2+}, Cu^{2+} and Zn^{2+} usually increased with the increase in the concentration of the element.

It is clear also in **figures (11, 12 and 13)** that, the destructive effect of the heavy metal ions on protein profile was more prominent under the stress effect of Cu^{2+} than in case of Ni^{2+} and Zn^{2+}. These results

seemed to be in conformity with findings of many authors (**Ahmed, 2010 ; El Taher, 2012** and **El-Agawany and Kaamoush, 2022**). It is clear from these results that the obtained bands of the protein profile are distributed throughout the gel plate. Some bands are cathodic, others are anodic, but most of bands are cathodic anodic symmetry. The sum of the bands that appeared on the gel plate and confirmed by scanning using the band peaks were 20 bands for Zn and 17 bands for both Ni and Cu. Some of these bands were common in the control and the treated organism; others were common only in the treated organism under the effect of the five different concentrations of the three tested elements (Ni^{2+}, Cu^{2+} and Zn^{2+}). Another important fact, The total protein content of *Spirulina platensis* was 82.63 % in 50 % effluent of wastewater, it was suggested that heavy metals in wastewater at low concentrations accelerate protein production in *S. platensis*, (**Balaji *et al.*, 2015**).

However, in nearly all the lanes most of the bands appeared in the region between 25 KDa and 212 KDa. A glimpse at the number of bands obtained for control only 13 bands were observed, while this number of bands increased or decreased depending on type and concentrations of the tested element. In case of Ni, the sum of bands increased by increasing the concentration of the element. At 1.0 and 1.5 mg/l Zn the percent of increase in the sum of bands reached 15.4%, while at concentrations 2.0, 2.5 and 3.0 mg/l Ni, the percent of increased of bands reached 30.8% over control for all the three tested concentrations of Ni. The newly formed bands at concentrations 1.0 and 1.5 mg/l Ni were 3 bands while at concentrations 2.0, 2.5 and 3.0 the number of the newly formed bands increased to 4 bands. It is clear from this data that most of the newly formed bands appeared at the region having low molecular weight, while most of the disappeared bands were recorded at the region of the high molecular weight.

It is clear also that by increasing the concentration of Cu from 1.0 mg/l to 2.0 mg/l, the newly formed bands were 3 bands, while at 2.5 mg/l the newly formed ones were 2 bands while at 3.0 mg/l the newly formed bands was one band only. Also the total bands in case of Cu at 1.0, 1.5 and 2.5 mg/l increased to 15 bands while at concentration 2.0 mg/l Cu the bands increased to 16 bands. At concentration 3.0 mg/l Cu; the number of bands decreased to 14 bands. In case of Zn the number of bands increased by increasing the concentration of the element. The newly formed bands increased by increasing the concentration of the element nearly 5, 3 and 4 bands at concentrations 2.0, 2.5 and 3.0 mg/l Zn, respectively. Only one band disappeared at concentrations 2.0 and 2.5 mg/l Zn. This map could explain the fact that *Spirulina platensis* is more sensitive to Cu ions followed by Zn then Ni ions. **El-Agawany and Kaamoush, (2022)** confirmed that, in *Dunaliella tertiolecta* culture, the percentage of increase in the number of bands was found to depend on the toxicity of zinc element. It is clear also that at concentration 25 mg/L, the organism greatly suffered from the toxic effect of zinc element.

In addition, some heavy metals can affect protein profile in algae. **Chernicova *et al.*, (2006)** observed that increasing of concentrations of manganese does not inhibit growth considerably altered cell ultrastructure and changed the protein profile in *Spirulina platensis*. **Sinha and Hader, (1996)**, found that *Anabaena* species cultured at stress conditions did not show any changes in protein pattern. On the other hand, **Fulda *et al.*, (1999)** found that the composition of periplasmic proteins obtained from cells of *Synechocystis* species grown under stress showed clear differences. **Hoyos and Zhang, (2000)** are in

agreement with those the above mentioned results, they found that reversible protein phosphorylation/dephosphorylation plays an important role in signaling the plant adaptive response to stress. The results of **Salah El-Din, (1994)** confirmed that, most of the algal species have similar physiological functions which are related to biosynthesis or biodegradation of some macromolecules. This conclusion seems to explain the different changes of the amount of total soluble protein bands in stressed algae.**). Ahmed, (2010) and El Taher, (2012)** reported that, the tolerance of an organism to stress conditions could be achieved through synthesis or accumulation of new proteins. These results are nearly in harmony with our results for *Spirulina platensis*. Protein deficiency in human nutrition is a major concern for most of developing countries; so there is a need to develop protein sources a new unconventional ones. The high protein concentration of various microalgal species specially *Spirulina platensis* makes them an excellent source in the supply of this nutrient,(**Anne *et al.*, 2016**).

Conclusion

The result obtained from the effect of different concentrations of Ni^{2+}, $Cu^{2+}$and Zn^{2+} on growth, IR spectra, the content of fatty acid fractions and total soluble protein profile of *Spirulina platensis* revealed that, EC50 of the three heavy metals was recorded nearly at concentration 2.0 mg/l. Inhibitory effect of copper on the growth measured in optical density was more pronounced in case of copper than zinc and nickel under all the concentrations used. Data obtained from IR spectra showed formation of new compounds, and hence new peaks in the spectra appeared while other compounds disappeared when compared to untreated cells, it was clearer in case of Cu^{2+} than in case of Zn^{2+} and Ni^{2+}. Toxic effect of the three elements differed according to type of fatty acids group. So, by increasing the concentration of the element, the total content of the three groups of fatty acids decreased. Grand total fatty acids decreased under all the tested concentrations, saturated fatty acids were more dominant than unsaturated ones under stress conditions. The destructive effect of the heavy metal ions on protein profile was more prominent under the stress effect of Cu^{2+} than in case of Zn^{2+} and Ni^{2+}. The tolerance of an organism to stress conditions could be achieved through synthesis or accumulation of new proteins. Finally, it could be concluded that Cu^{2+} metal ion is more toxic than Zn^{2+} and Ni^{2+} metal ions and the degree of stress depends mainly on the concentration and type of the element together with the length of the culture period, (Order of toxicity of the three heavy metal is $Cu^{2+} > Zn^{2+} > Ni^{2+}$ under all tested concentrations). Protein deficiency in human nutrition is a major concern for most of developing countries; so, there is a need to develop protein sources a new unconventional ones. The high protein concentration of various microalgal species specially *Spirulina platensis* makes them an excellent source in the supply of this nutrient.

Declarations

Ethical Approval: Not Applied

Consent to Participate: I voluntarily agree to participate in this research study. I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.

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References

1. **Abdel-Latif, H.M.R. ; El-Ashram, S,; Sayed, A.E.H.; Alagawany,M. Shukry,M.; Dawood M.A.O. Kucharczyk,D.(2022).** Elucidating the ameliorative effects of the cyanobacterium Spirulina (*Arthrospira platensis*) and several microalgal species against the negative impacts of contaminants in freshwater fish: A review. Aquaculture. Vol. 554:738155.
2. **Ahmed E. A. M. (2010).** Impact of Tributyltin (TBT) on metabolism of some marine algae. Ph.D. Thesis. Faculty of science, Alazhar University. Egypt.
3. **Akbarnezhad M. , Mehrgan M.S., kamali A.; Baboli M.J.(2019).** Effects of microelements (Fe, Cu, Zn) on growth and pigment contents of Arthrospira *Spirulina platensis* .Iran Hournal of fisheries Sciences.vol.19: (2).pages 653-668.
4. **Alam, M. Z., Ahmad, S., Malik, A., & Ahmad, M. (2010).** Mutagenicity and genotoxicity of tannery effluent used for irrigation at Kanpur, India. *Ecotoxicology and Environmental Safety, 73*(1620), 1628.
5. **Al-Osaimi M. (2010).** Impact of salinity stress on growth and some important metabolites of *Spirulina platensis* (A cyanobacterium). M.Sc. Thesis. Faculty of Science. Alex. University.

6. **Anne L., Luciane Maria C., Cristiane C., Eliane C.(2016).** Potential application of microalga *Spirulina platensis* as a protein source. Review. wileyonlinelibrary.com. DOI 10.1002/jsfa.7987.
7. **Arunakumara K.K.I.U. and Xuecheng Z.(2008).** Heavy metal bioaccumulation and toxicity with special reference to microalgae.J. Ocean Univ. China., 7 (1):pp. 25-30.
8. **Balaji S. , Kalaivani T. , Rajasekaran C. , Shalini M. , Vinodhini S. , Sunitha Priyadharshini S. and Vidya A. G. (2015).** Removal of heavy metals from tannery effluents of Ambur industrial area, Tamilnadu by *Arthrospira* (*Spirulina*) *platensis* .Environmental Monitoring and Assessment. **Vol. 187**, 325.
9. **Becker W.(2004).**Microalgae in human and animal nutrition. A. Richmond (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Blackwell Publishing Ltd.
10. **Ben-Amotz A., Tornabene T. G. and Thomas W. H. (1985).** Chemical profile of selected species of microalgae with emphasize on lipids. J. Phycol., 21**:** 72-81.
11. **Bligh E. G. and Dyer W. M. (1959).** Rapid method for lipid extraction can. J. Bio Chem. Physiol. 35: 911 - 915.
12. **Budi R. M. S., Rahardja B. S. and Masithah E, D. (2020).** Potential concentration of heavy metal copper (cu) and microalgae growth *Spirulina plantesis* in culture media. IOP Conf. Series: Earth and Environmental Science 441: 012147.
13. **Chernikova A. A. , Tsoglin L. N. , Markelova A. G. , Zorin S. N. , Mazo V. K. and Pronina N. A. (2006).** Capacity of *Spirulina platensis* to accumulate manganese and its distribution in cell. *Russian Journal of Plant Physiology* . vol. 53, pages 800–806.
14. **Chu F. E. and Dupuy D. J. (1980).** The fatty acid composition of three unicellular algal species used as food sources for larvae of the American oyster. Lipids. 15: 356 - 364.
15. **Cohen Z. and Cohen S. (1991).** Preparation of eicosapentaenoic acid (EPA) concentrate from porphyridium cruentun. JAOCS. 68: 16 - 19.
16. **Dempester T. A. and Sommerfeld M. R. (1998).** Effects of environmental conditions on growth and lipid accumulation in *Nitzclive communiz*. (Bacterio phyceae).J. Phycol. 34: 712 - 721.
17. **Dowidar N. M. (1983)** Primary production in the central Red Sea off Jeddah. Bull. Nat. Inst. Oceanogr. And Fish., AR.E. 9: 160 - 170.
18. **Dubinsky Z., Berner T. and Aaronson S. (1978).** Potential of large-scale algal culture for biomass and lipid production in arid lands. Biotechnology and Bioengineering Symposium. 8: 51- 68.
19. **El-Agawany N. I. · Kaamoush, M. I. A.(2022).** Role of zinc as an essential microelement for algal growth and concerns about its potential environmental risks. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-022-20536-z
20. **El Taher A. M. (2012).** Copper and zinc toxicity in *Chlorella vulgaris*: Response of growth; some metabolic and antioxidants activity. M.Sc. Thesis. Fac. of Sci. Alex. Univ. Egypt.
21. **El-Maghrabi D. M (2002):** Studies on the production of some important fatty acids from Algae. Ph.D. Thesis. Fac. of Sci. Alex. Univ. Alex. Egypt.

22. **El-Sheikh M. M., El-Naggar A. H., Osman M. E. H. and Haider A. (1999).** Comparative studies on the green algae *Chlorella homosphaera* and *Chlorella vulgaris* with respect to oil pollution in the River Nile. J. Union arab Biol. Cairo. 7(B): Physiology and algae, 117 - 136.
23. **Fulda S., Mikkat S., Schroder W. and Hagemann M. (1999).** Isolation of salt – induced periplasmic proteins from *Synechocystis* sp. Strain pcc 6803. Arch. Microbiol. 171: 214 - 217.
24. **Garcia J.L., DeVicente M., Galan B.(2017).** Microalgae, old sustainable food and fashion nutraceuticals Microb. Biotechnol., 10 (5):pp. 1017-1024.
25. **Hanan M. K., Kamal H. S., Mostafa M. E., and Dorea I. E.(2015).** Algal Diversity of the Mediterranean Lakes in Egypt. International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES-2015) July 22-23, 2015 London.
26. **Hoyos M. E. and Zhang S. (2000).** Calcium independent activation of salicylic acid-induced protein Kinase and 40- Kilodalton protein Kinase by hyperosmotic stress. Plant Physiol. 122: 1355 - 1363.
27. **Jorge A. V.; Barbara C. B.; Gabriel M. ; Luiza M.; Michele G. ; B. Greg M.(2019).** Operational and economic aspects of *Spirulina*-based biorefinery. Bioresource Technology.Vol.(292), 121946.
28. **Kansiz M., Heraud P., Wood B., Burden F., Beardall J. and Mc Naughton D. (1999).** Fourier transform infrared microspectroscopy and chemometrics as a tool for the discrimination of cyanobacterial strains. Phytochemistry. 52: 407 - 417.
29. **Lupatini, A.L.. Colla, L.M Canan, C. Colla E.(2017).** Potential application of microalga *Spirulina platensis* as a protein source.J. Sci. Food Agric., 97.pp. 724-732.
30. **Meenakshi B. (2007).** Bioremediation of oils: Role of Cyanobacteria. In Biotechnological Applications of Microalgae. Narosa Publication House New Delhi. 211-243.
31. **Miller M. A. (1946).** Toxic effect of copper on attachment and growth of *Bugula neritina*. Biol. Bull. 90: 122 - 140.
32. **Nethravathy M. U., Jitendra G. Mehar, Sandeep N. Mudliar, Ajam Y. Shekh.(2019).**Recent Advances in Microalgal Bioactives for Food, Feed, and Healthcare Products: Commercial Potential, Market Space, and Sustainability. Vol. 18, Iss. 6. P: 1882-189.
33. **Muysa M. ; Sui Y. ; Schwaiger B. ; Lesueur C. ; Vandenheuvel D. ; Vermeir P. ; and Siegfried E.Vlaeminck.(2019).** High variability in nutritional value and safety of commercially available *Chlorella* and *Spirulina* biomass indicates the need for smart production strategies. Bioresource Technology. Vol. (275), Pages 247-257.
34. **Noctor G. and Foyer C. H. (1998).** Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Mol. Biol. 49: 249 - 79.
35. **Piorreck M., Baasch K.H., Pohl P.(1984).**Preparatory experiments for the axenic mass-culture of microalgae. 1. Biomass production, total protein, chlorophylls, lipids and fatty-acids of fresh-water green and blue green-algae under different nitrogen regimes. Phytochemistry, 23 (2):pp. 207-216.
36. **Pyne,S.; Bhattacharjee,P.; Srivastav, P. (2017).**Microalgae (*Spirulina platensis*) and its bioactive molecules: review. Indian J. Nutr., 4 :pp. 1-6.

37. **Radwan S. S. (1978).** Sources of C20 polyunsaturated of fatty acids for Biotechnological use. Appl. Microbiol. And Biotechnol. 35: 421 -430.
38. **Ragaza,J.A.; Sakhawat H. M. Meiler,K.A.; Velasquez,S.F.; Kumar,V.(2020).** A review on Spirulina: alternative media for cultivation and nutritive value as an aquafeed. Aquaculture:12, 2371–2395.
39. **Roessler P. G. (1989).** Purification and characterization of acetyl. CoA carboxylase from the diatom *Cyclotella cryptica*. In aquatic species Program Annual Review Meeting. Solar Energy Research Institute, Golden, Colorado, PP. 125 - 138.
40. **Saad, L. (2003).** Environmental concern down this earth day. Gallup News Service. Poll Analyses, 17 April. Available at http://www.gallup.com/poll/releases.
41. **Salah El-Din R. A. (1994).** Contribution to the biological and phytochemical studies of marine algal vegetation on the coasts of Red-Sea and Suez-Canal (Egypt). Ph.D. Thesis. Botany Department. Faculty of Science. Al-Azhar University, Cairo Egypt.
42. **Sanjib Bhattacharya.(2020).** The Role of *Spirulina (Arthrospira)* in the Mitigation of Heavy-Metal Toxicity. Journal of Environmental Pathology, Toxicology and Oncology. pages 149-157.
43. **Simonopoulos A. P. (1991).** Omega-3 fatty acids in health and disease and in growth and development. Am. J. Chin. Nutr. 54: 438 - 463.
44. **Sinha R. P. and Hader D.P. (1996).** Response of a rice field cyanobacteria *Anabaena* sp. To physiological stressors. Environ. Exp. Bot. **36(2):** 147-155.
45. **Tadros M. G. (1985).** Screening and characterizing oleaginous microalgal species from the Southeastern United States. In Mc-Intosch R.P. (Ed). Aquatic Species Program Review: Proceedings from the March 1983 Principal Investigators Meeting, Publ. SERI/ CP-231-2700. Solar Energy Research Institute, Golden, Colorado, pp. 28 - 42.
46. **William E Connor.(2000).** Importance of n−3 fatty acids in health and disease. The American Journal of Clinical Nutrition, Volume 71, Issue 1. Pages 171S–175S.
47. **Williams D. H. and Feleming I. (1996):** Spectroscopic methods in organic chemistry (5^{th} ed). London: Mc Graw- Hill International Ltd.
48. **Xu X. Q., Tran V. H., Kraft G. and Beardall J. (1998).** Fatty acids of six *Codium* species from South East Australia. Phytochemistry. 84: 1335 - 1339.
49. **Xu X., Haallett S. G., Sheppard J. and Watson A. K. (1997).** Application of the Plackett-Burman experimental design to evaluate nutritional requirements for the production of *Colletotrichum coccoides* spores. Appl. Microbiol. Biotechnol. 47: 301 - 305.
50. **Zarrouk C. (1966).** *"Contribution a l'Etude d'une Cyanophycre sur la Croissance de la Photosynthrse de Spirulina maxima"*. Stech et Gardner (ed.), Geitler, These, Paris.
51. **Zheng G. , Li C. , Guo L. , Ruo W. , Wang S.(2012).** Purification of Extracted Fatty Acids from the Microalgae *Spirulina.* Journal of American Chemist's Society.Vol.(89), Issue 4. Pages :561-566.
52. **Zinicovscaia I., Cepoi L., Rudi L., Chiriac T., Grozdov D., Vergel K.(2021).** Effect of zinc-containing systems on *Spirulina platensis*: bioaccumulation capacity and biochemical composition.

Environmental Science and Pollution Research. 14457-6.

Tables

Table 1 :Effect of different Ni^{2+} concentrations on the content of fatty acid fractions (µg/ml) of *Spirulina platensis* cultured for 10 days.

Fatty acids		Control	Different Ni^{2+} concentrations (mg/l)				
			1.0	1.5	2.0	2.5	3.0
Saturated fatty acids	C6:0	0.788	1.489	1.113	8.645	9.645	12.313
	C8:0	1.360	2.476	0.756	0.434	0.262	0.694
	C10:0	0.417	1.057	0.424	0.240	0.193	0.430
	C11:0	0.914	1.485	0.890	0.584	0.469	0.539
	C12:0	0.Q337	0.925	0.563	0.257	0.272	0.404
	C13:0	2.641	3.643	2.593	2.017	1.385	1.595
	C14:0	2.019	2.033	0.319	1.258	1.479	1.662
	C15:0	1.233	1.157	1.068	0.881	0.607	0.668
	C16:0	35.323	40.648	47.296	43.170	40.928	41.182
	C17:0	0.162	0.045	0.334	0.312	0.309	0.268
	C18:0	9.427	4.334	5.112	3.912	5.242	5.346
	C20:0	0.485	0.147	0.458	–	–	0.546
	C21:0	0.657	0.217	0.420	–	–	0.375
Total		55.763	59.656	61.346	61.71	60.791	66.022
% of increase		—	(+)6.98	(+)10.01	(+)10.66	(+)9.02	(+)18.40
Mono- unsaturated fatty acids	C14:1	6.570	14.182	1.627	9.476	10.215	10.164
	C15:1	2.116	1.787	2.114	1.830	1.600	2.577
	C16:1	2.021	3.653	4.465	4.643	4.673	2.794
	C17:1	0.395	0.130	0.353	0.222	0.215	2.448
	C18:1	16.064	2.869	6.831	4.266	7.046	5.041
	C20:1	0.252	0.436	0.397	–	–	–
	C22:1	2.372	4.071	1.936	0.487	0.400	1.422
Total		29.79	27.128	17.723	20.924	24.149	24.446
% of decrease		—	(-)8.94	(-)40.51	(-)29.76	(-)18.94	(-)17.94
	C18:3	5.942	4.091	9.610	8.722	6.705	4.304
Poly- unsaturated fatty acids	C18:2	7.784	4.870	8.328	7.277	6.307	4.154
	C20:5	0.155	0.151	0.367	0.539	0.270	0.166

	C20:3	–	0.414	0.766	0.499	0.783	0.174
	C20:2	0.274	0.825	0.726	–	0.481	–
	C22:6	0.293	1.147	0.370	0.332	0.261	0.132
	C22:2	–	1.251	0.764	–	0.254	0.600
Total		**14.448**	**12.749**	**20.931**	**17.369**	**15.061**	**9.53**
% of increase or decrease		**—**	**(-)11.76**	**(+)44.87**	**(+)20.22**	**(+)4.24**	**(-)34.04**
Grand Total content		**100.001**	**99.533**	**100.000**	**100.003**	**100.001**	**100.091**
% of increase or decrease		**—**	**(-)0.47**	**(-)0.009**	**(+)0.002**	**0.000**	**(+)0.090**

Table 2: Effect of different Cu^{2+} concentrations on the content of fatty acid fractions (µg/ml) of *Spirulina platensis* cultured for 10 days.

Fatty acids		Control	Different Cu^{2+} concentrations (mg/l)				
			1.0	1.5	2.0	2.5	3.0
Saturated fatty acids	C6:0	0.788	3.054	4.633	0.512	1.051	1.512
	C8:0	1.360	1.060	1.077	1.849	0.275	1.878
	C10:0	0.417	0.364	0.642	0.473	0.164	0.446
	C11:0	0.914	0.942	0.917	0.699	1.119	0.771
	C12:0	o.337	0.376	0.782	0.640	0.542	0.590
	C13:0	2.641	2.456	2.726	1.920	3.673	2.865
	C14:0	2.019	1.738	2.051	1.662	2.162	1.395
	C15:0	1.233	0.242	0.221	0.882	0.237	1.022
	C16:0	35.323	31.800	38.409	40.201	39.112	39.028
	C17:0	0.162	0.267	0.378	0.336	0.272	0.548
	C18:0	9.427	6.879	7.392	7.109	6.787	5.172
	C20:0	0.485	0.760	–	0.792	0.295	–
	C21:0	0.657	1.077	0.580	0.796	0.758	0.663
Total		**55.763**	**51.015**	**59.808**	**57.87**	**56.447**	**55.89**
% of increase or decrease		**—**	**(-)8.51**	**(+)7.25**	**(+)3.78**	**(+)1.23**	**(-)1.21**
Mono- unsaturated fatty acids	C14:1	6.570	2.872	8.379	6.498	8.017	7.761
	C15:1	2.116	0.743	2.177	1.867	1.963	1.102
	C16:1	2.021	4.378	3.501	3.331	4.900	4.027
	C17:1	0.395	0.342	0.428	0.363	0. 459	2.489
	C18:1	16.064	12.788	9.629	10.062	6.107	10.711
	C20:1	0.252	0.292	–	0.297	0.436	–
	C22:1	2.372	3.244	1.181	3.353	1.665	1.519
Total		**29.79**	**24.659**	**25.295**	**25.771**	**23.547**	**27.609**
% of decrease		**—**	**(-)17.22**	**(-)15.09**	**(-)13.49**	**(-)20.96**	**(-)7.32**
	C18:3	5.942	7.737	6.646	6.461	8.680	7.884
Poly- unsaturated fatty acids	C18:2	7.784	8.680	7.803	7.299	11.055	8.613

	C20:5	0.155	0.244	0.257	0.205	–	–
	C20:3	–	0.758	0.190	0.277	0.272	–
	C20:2	0.274	0.823	–	0.649	–	–
	C22:6	0.293	0.496	–	1.042	–	–
	C22:2	–	0.070	–	0.231	–	–
Total		**14.448**	**18.808**	**14.896**	**16.164**	**20.007**	**16.497**
% of increase		**—**	**(+)30.18**	**(+)3.10**	**(+)11.88**	**(+)38.48**	**(+)14.18**
Grand Total content		**100.001**	**94.482**	**99.999**	**99.995**	**100.001**	**99.196**
% of decrease		**—**	**(-)5.52**	**(-)0.002**	**(-)0.006**	**0.000**	**(-)0.805**

Table 3: Effect of different Zn^{2+} concentrations on the content of fatty acid fractions (µg/ml) of *Spirulina platensis* cultured for 10 days.

Fatty acids		Control	Different Zn^{2+} concentrations (mg/l)				
			1.0	1.5	2.0	2.5	3.0
Saturated fatty acids	C6:0	0.788	1.648	6.769	36.455	4.974	1.570
	C8:0	1.360	1.463	1.172	3.056	1.606	0.961
	C10:0	0.417	1.022	0.866	4.293	1.036	0.063
	C11:0	0.914	1.421	0.733	1.947	0.924	1.015
	C12:0	o.337	1.281	0.772	0.217	0.438	0.584
	C13:0	2.641	4.809	1.617	5.240	3.400	2.993
	C14:0	2.019	0.696	1.432	4.632	2.255	1.938
	C15:0	1.233	2.072	0.74 7	2.081	1.637	2.130
	C16:0	35.323	36.646	33.580	15.876	32.457	35.603
	C17:0	0.162	5.492	0.138	0.802	0.321	0.206
	C18:0	9.427	6.325	7.629	3.152	9.934	8.404
	C20:0	0.485	1.242	3.751	0.949	0.924	0.173
	C21:0	0.657	0.980	0.417	0.310	1.600	0.534
Total		**55.763**	**65.097**	**59.623**	**79.010**	**61.506**	**56.174**
% of increase		**—**	**(+)16.74**	**(+)6.92**	**(+)41.69**	**(+)10.30**	**(+)0.74**
Mono- unsaturated fatty acids	C14:1	6.570	3.473	7.368	7.286	4.696	7.982
	C15:1	2.116	3.076	1.597	0.352	2.710	6.112
	C16:1	2.021	2.343	1.826	1.113	1.766	2.006
	C17:1	0.395	0.927	0.327	0.169	0.667	0.316
	C18:1	16.064	10.087	12.500	3.798	9.344	7.970
	C20:1	0.252	--	0.271	--	0.424	0.354
	C22:1	2.372	1.503	1.595	0.428	3.185	4.003
Total		**29.79**	**21.409**	**25.484**	**13.146**	**22.792**	**28.743**
% of decrease		**—**	**(-)28.13**	**(-)14.45**	**(-)56.14**	**(-)23.49**	**(-)3.51**
	C18:3	5.942	8.541	7.348	2.621	5.667	6.103
Poly- unsaturated fatty acids	C18:2	7.784	8.399	6.801	3.216	7.532	8.474
	C20:5	0.155	--	--	0.243	0.217	--

	C20:3	–	0.289	0.353	–	0.277	0.169
	C20:2	0.274	1.005	0.246	–	0.816	–
	C22:6	0.293	–	0.152	0.227	1.193	0.223
	C22:2	–	0.254	–	0.738	–	0.110
Total		**14.448**	**18.488**	**14.9**	**7.045**	**15.702**	**15.079**
% of increase or decrease		**—**	**(+)27.96**	**(+)3.13**	**(-)51.24**	**(+)8.68**	**(+)4.37**
Grand Total content		100.001	*104.994*	*100.007*	*100.000*	*100.000*	*99.996*
% of increase or decrease		**—**	**(+)4.993**	**(+)0.006**	**(-)-0.009**	**(-)0.009**	**(-)0.005**

Table 4: Soluble protein profile bands pattern of the studied *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Ni^{2+} concentrations.

+ = Band present – = Band absent

Table 5: Soluble protein profile pattern bands showing sum, unchanged, disappeared and newly formed bands at control and under the effect of different Ni^{2+} concentrations.

Treatment / Bands	Control	Different Ni^{2+} concentrations (mg/l)				
		1.0	**1.5**	**2.0**	**2.5**	**3.0**
Sum of bands	**13**	15	15	17	17	17
Unchanged bands	**–**	12	12	13	13	13
Disappeared bands	**–**	1	1	0	0	0
Newly formed bands	**–**	3	3	4	4	4

Table 6: Soluble protein profile bands pattern of the studied *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Cu^{2+} concentrations.

+ = Band present – = Band absent

Bands No.	Molecular weight KDa	Marker	Control	Different Ni^{2+} concentrations (mg/l)				
				1.0	1.5	2.0	2.5	3.0
1		-	+	+	+	+	+	+
2		-	+	+	+	+	+	+
3	212.0	+	+	+	+	+	+	+
4		-	+	+	+	+	+	+
5		-	-	+	+	+	+	+
6	116.0	+	+	+	+	+	+	+
7		-	+	+	+	+	+	+
8	66.0	+	+	+	+	+	+	+
9	45.0	+	+	+	+	+	+	+
10		-	+	+	+	+	+	+
11		-	+	+	+	+	+	+
12		-	-	+	+	+	+	+
13	25.0	+	+	+	+	+	+	+
14		-	+	+	+	+	+	+
15		-	+	-	-	+	+	+
16	18.4	+	-	-	-	+	+	+
17	14.4	+	-	+	+	+	+	+

Table 7: Soluble protein profile pattern bands showing sum, unchanged, disappeared and newly formed bands at control and under the effect of different Cu^{2+} concentrations.

Treatment Bands	Control	Different Cu^{2+} concentrations (mg/l)				
		1.0	1.5	2.0	2.5	3.0
Sum of bands	13	15	15	16	15	14
Unchanged bands	–	12	12	13	13	13
Disappeared bands	–	1	1	0	0	0
Newly formed bands	–	3	3	3	2	1

Bands No.	Molecular weight KDa	Marker	Control	Different Cu^{2+} concentrations (mg/l)				
				1.0	1.5	2.0	2.5	3.0
1		-	+	+	-	+	+	+
2		-	+	-	+	+	+	+
3	212.0	+	+	+	+	+	+	+
4		-	+	+	+	+	+	+
5	116.0	+	+	+	+	+	+	+
6		-	+	+	+	+	+	+
7	66.0	+	+	+	+	+	+	+
8	45.0	+	+	+	+	+	+	+
9		-	+	+	+	+	+	+
10		-	+	+	+	+	+	+
11		-	+	+	+	+	+	+
12	25.0	+	-	+	+	+	+	-
13		-	+	+	+	+	+	+
14		-	+	+	+	+	+	+
15	18.4	+	-	+	-	-	-	-
16		-	-	+	+	+		
17	14.4	+	-	-	+	+	+	+

Table 8: **Soluble protein profile bands pattern of the studied *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Zn^{2+} concentrations.**

+ = Band present – = Band absent

Table 9: Soluble protein profile pattern bands showing sum, unchanged, disappeared and newly formed bands at control and under the effect of different Zn^{2+} concentrations.

Bands No.	Molecular weight KDa	Marker	Control	Different Z^{2+} concentrations (mg/l)				
				1.0	1.5	2.0	2.5	3.0
1		-	+	+	+	+	+	+
2		-	+	+	+	+	+	+
3	212.0	+	+	+	+	+	+	+
4		-	-	-	-	-	-	+
5		-	+	+	+	+	+	+
6	116.0	+	+	+	+	+	+	+
7		-	+	+	+	+	+	+
8		-	-	-	-	+	-	-
9		-	-	-	-	+	-	-
10	66.0	+	+	+	+	+	+	+
11	45.0	+	+	+	+	-	-	+
12		-	+	+	+	+	+	+
13		-	+	+	+	+	+	+
14		-	+	+	+	+	+	+
15	25.0	+	-	+	+	+	+	-
16		-	+	+	+	+	+	+
17		-	+	+	+	+	+	+
18	18.0	+	-	-	-	-	-	+
19		-	-	-	+	+	+	+
20	14.4	+	-	-	-	+	+	+

Treatment / Bands	Control	Different Zn^{2+} concentrations (mg/l)				
		1.0	1.5	2.0	2.5	3.0
Sum of bands	13	14	15	17	15	17
Unchanged bands	–	13	13	12	12	13
Disappeared bands	–	0	0	1	1	0
Newly formed bands	–	1	2	5	3	4

Supplementary

Plate 1-3 are available in Supplementary Files section.

Figures

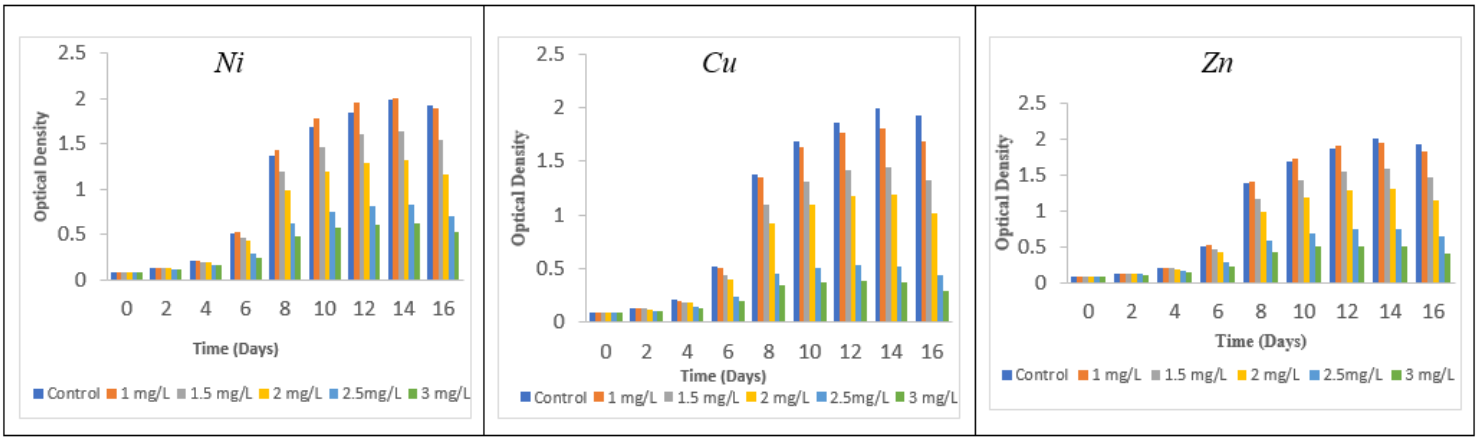

Figure 1

Effect of different concentrations of Ni^{2+} , Cu^{2+} and Zn^{2+}on growth of *Spirulina platensis* cultured for 16 days of culturing measured in optical density .

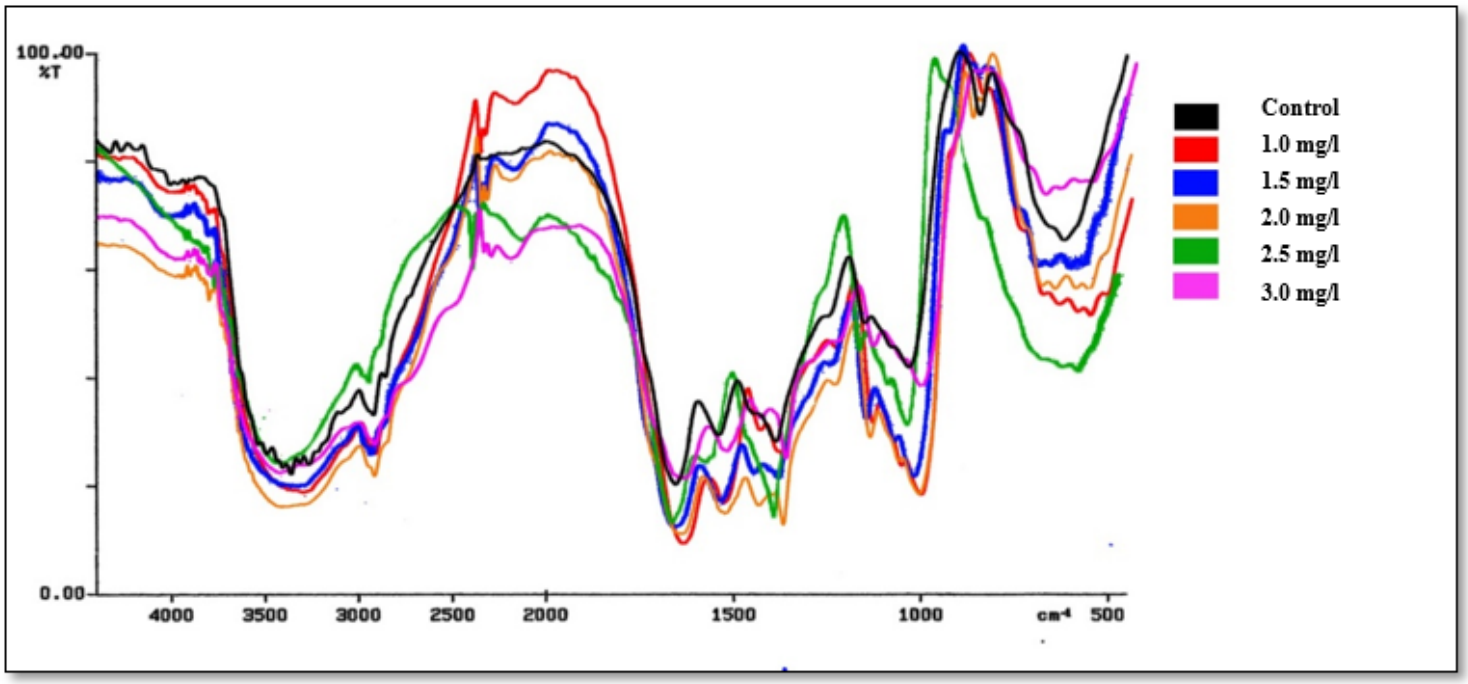

Figure 2

Infrared spectra of total cell constituents of *Spirulina platensis* cultured for 8 days under the effect of different Ni^{2+} concentrations.

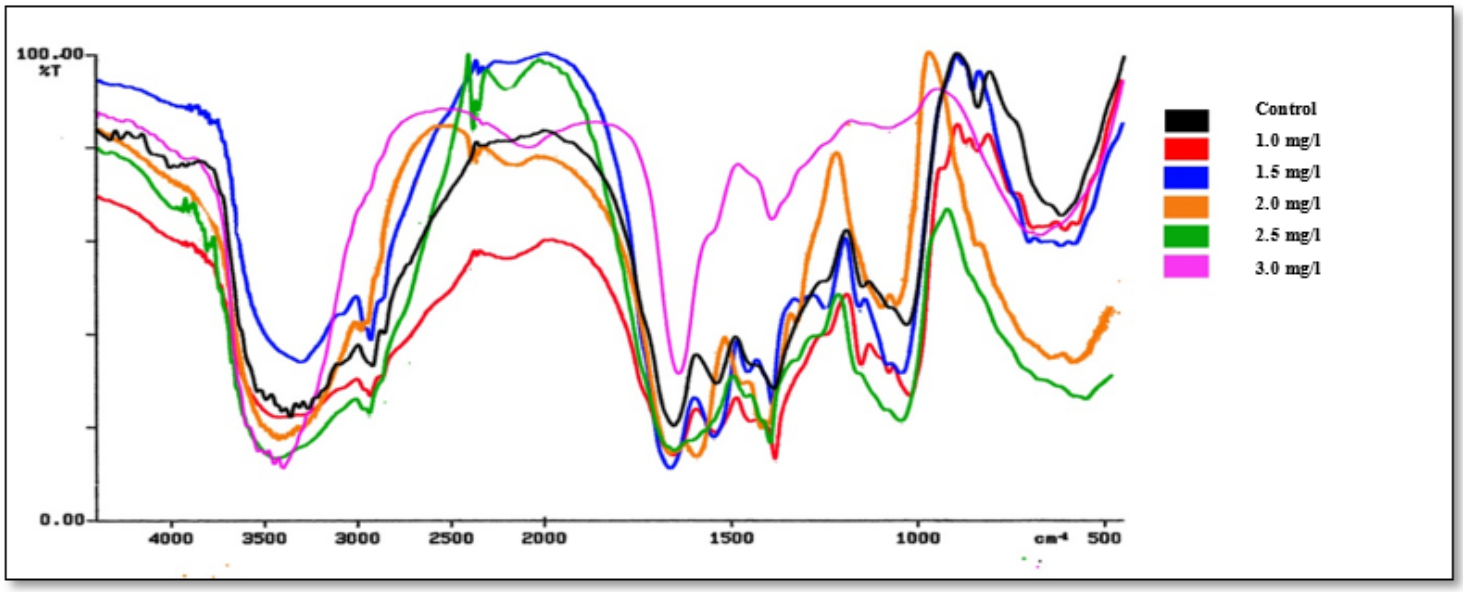

Figure 3

Infrared spectra of total cell constituents of *Spirulina platensis* cultured for 8 days under the effect of different Cu^{2+} concentrations.

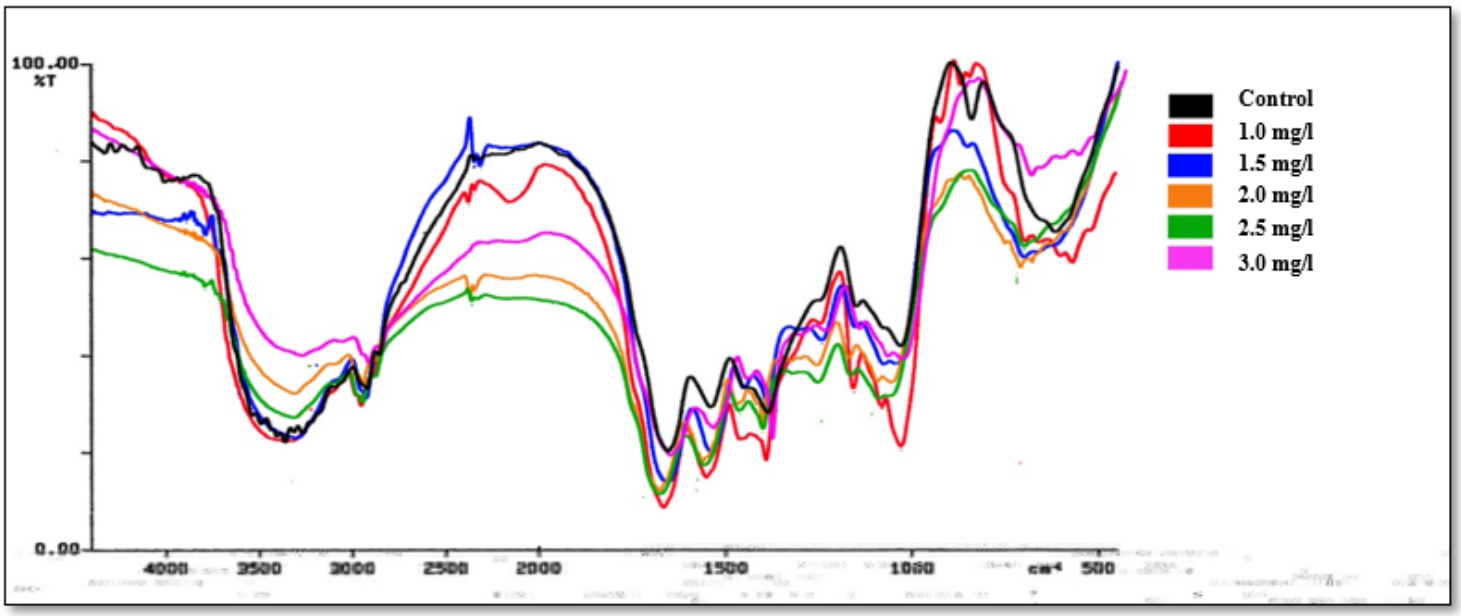

Figure 4

Infrared spectra of total cell constituents of *Spirulina platensis* cultured for 8 days under the effect of different Zn^{2+} concentration

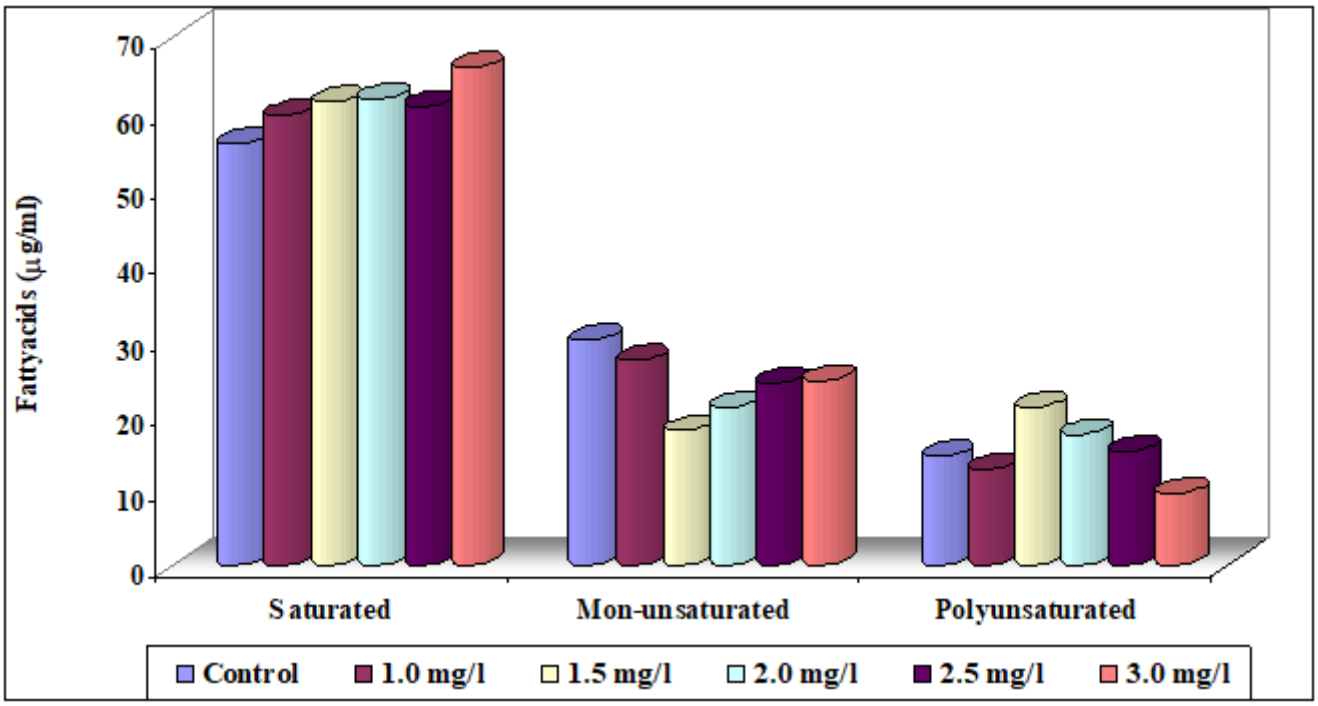

Figure 5

Effect of different Ni^{2+} concentrations on the content of fatty acids groups (mg/ml) of *Spirulina platensis* cultured for 10 days of culturing.

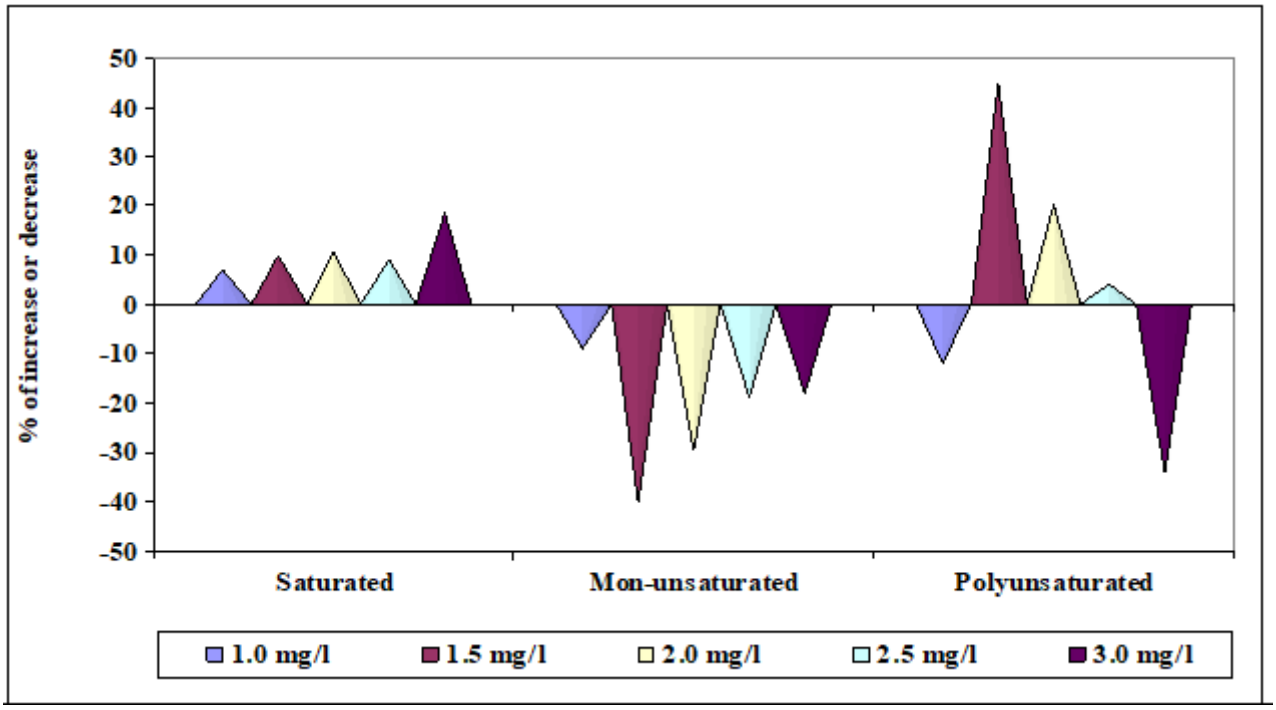

Figure 6

Percentage of increase or decrease in the content of fatty acids groups of *Spirulina platensis* cultured for 10 days under the effect of different Ni^{2+} concentrations compared to control.

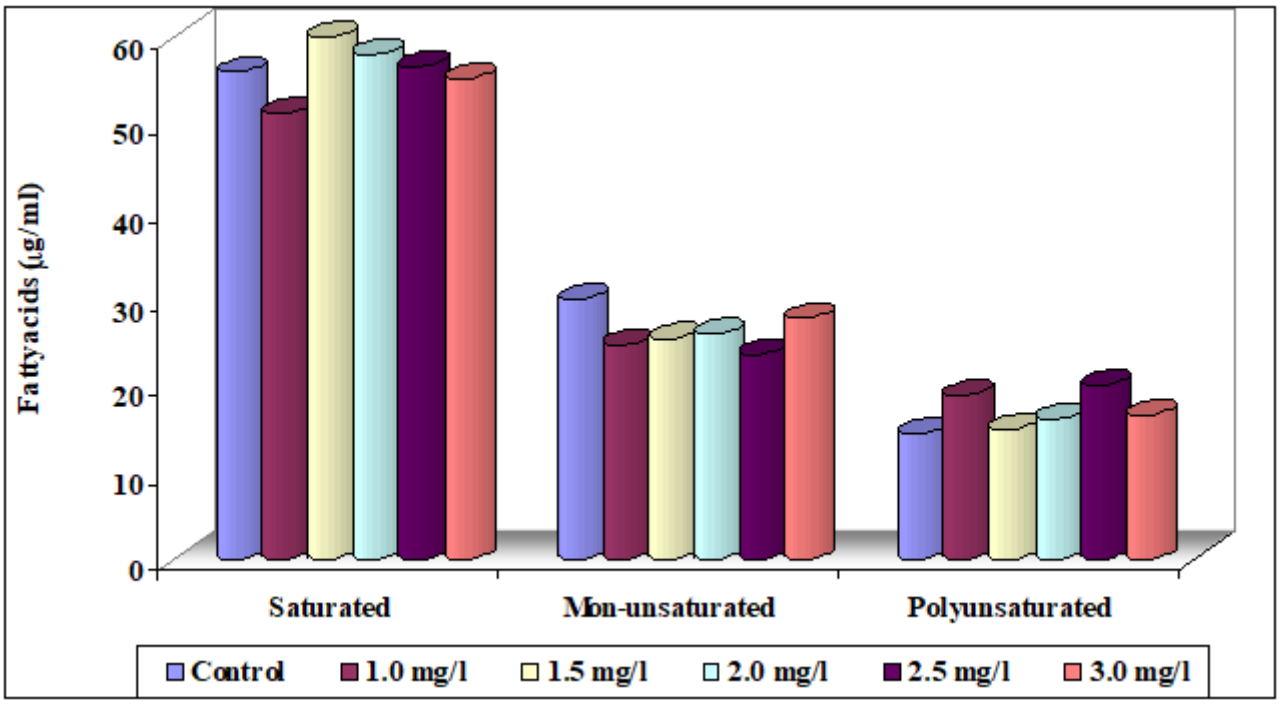

Figure 7

Effect of different Cu^{2+} concentrations on the content of fatty acids groups (mg/ml) of *Spirulina platensis* cultured for 10 days of culturing.

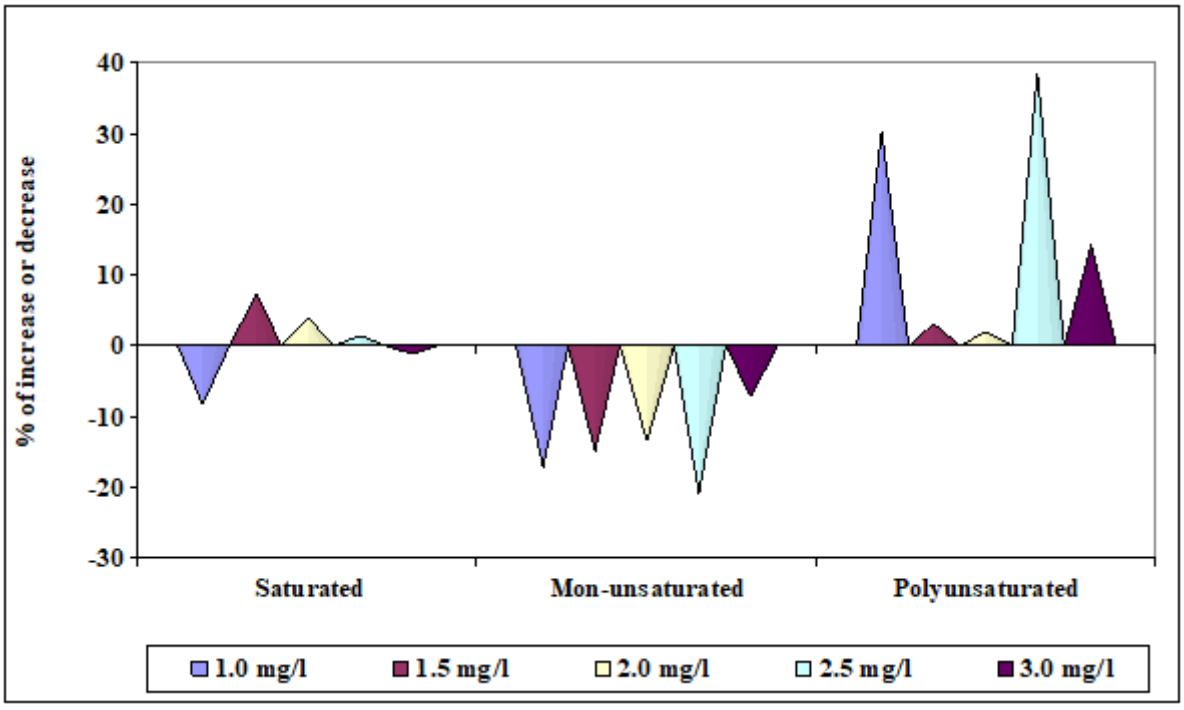

Figure 8

Percentage of increase or decrease in the content of fatty acids groups of *Spirulina platensis* cultured for 10 days under the effect of different Cu^{2+} concentrations compared to control.

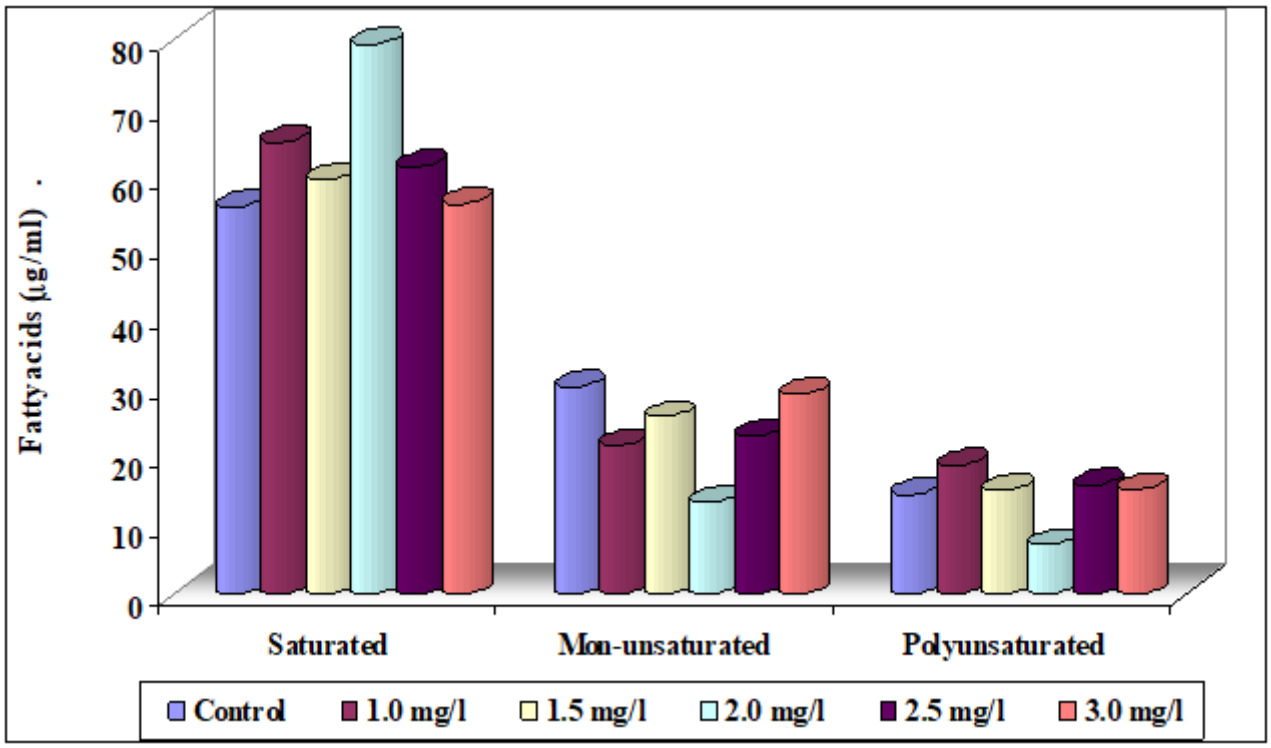

Figure 9

Effect of different Zn^{2+} concentrations on the content of fatty acids groups (mg/ml) of *Spirulina platensis* cultured for 10 days of culturing.

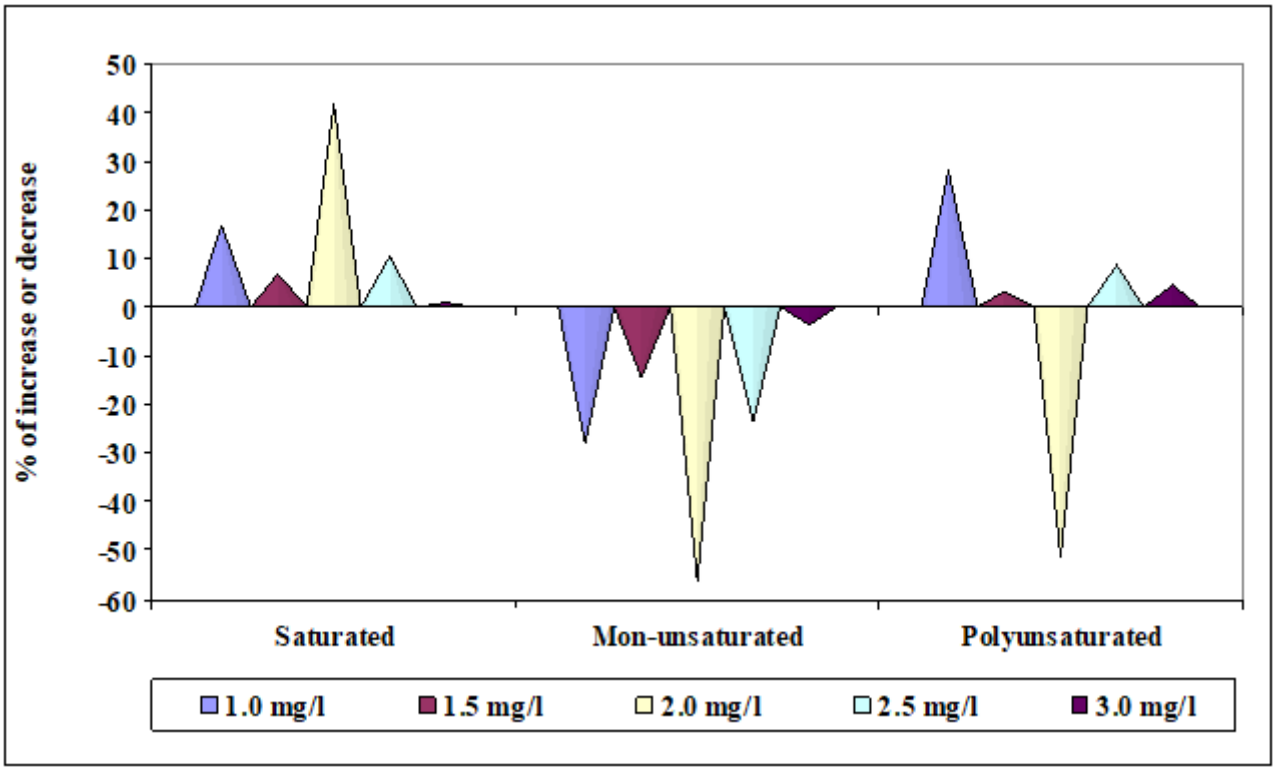

Figure 10

Percentage of increase or decrease in the content of fatty acids groups of *Spirulina platensis* cultured for 10 days under the effect of different Zn^{2+} concentrations compared to control.

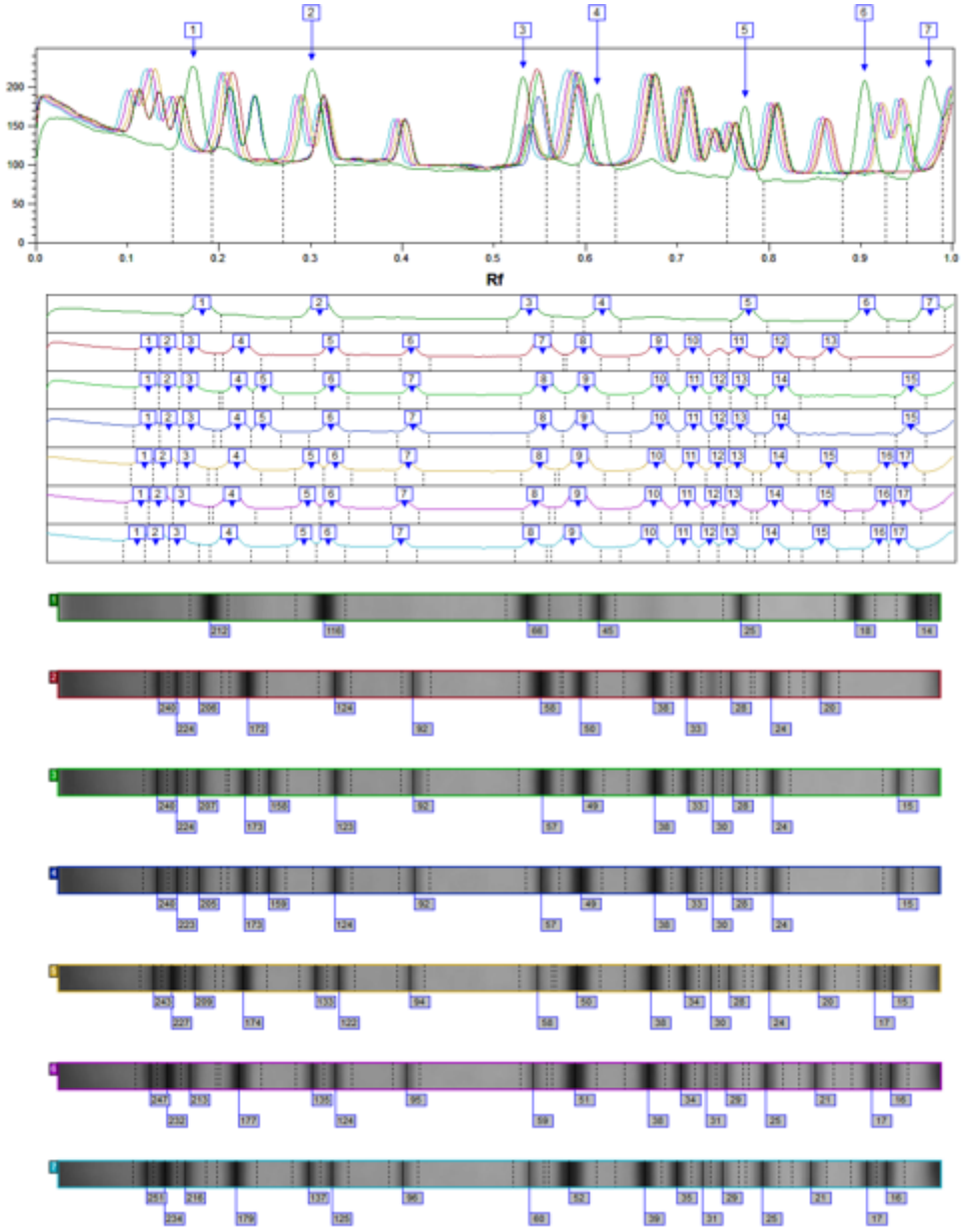

Figure 11

Electropherogram showing the results of scanning of protein profile bands of *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Ni^{2+} concentrations.

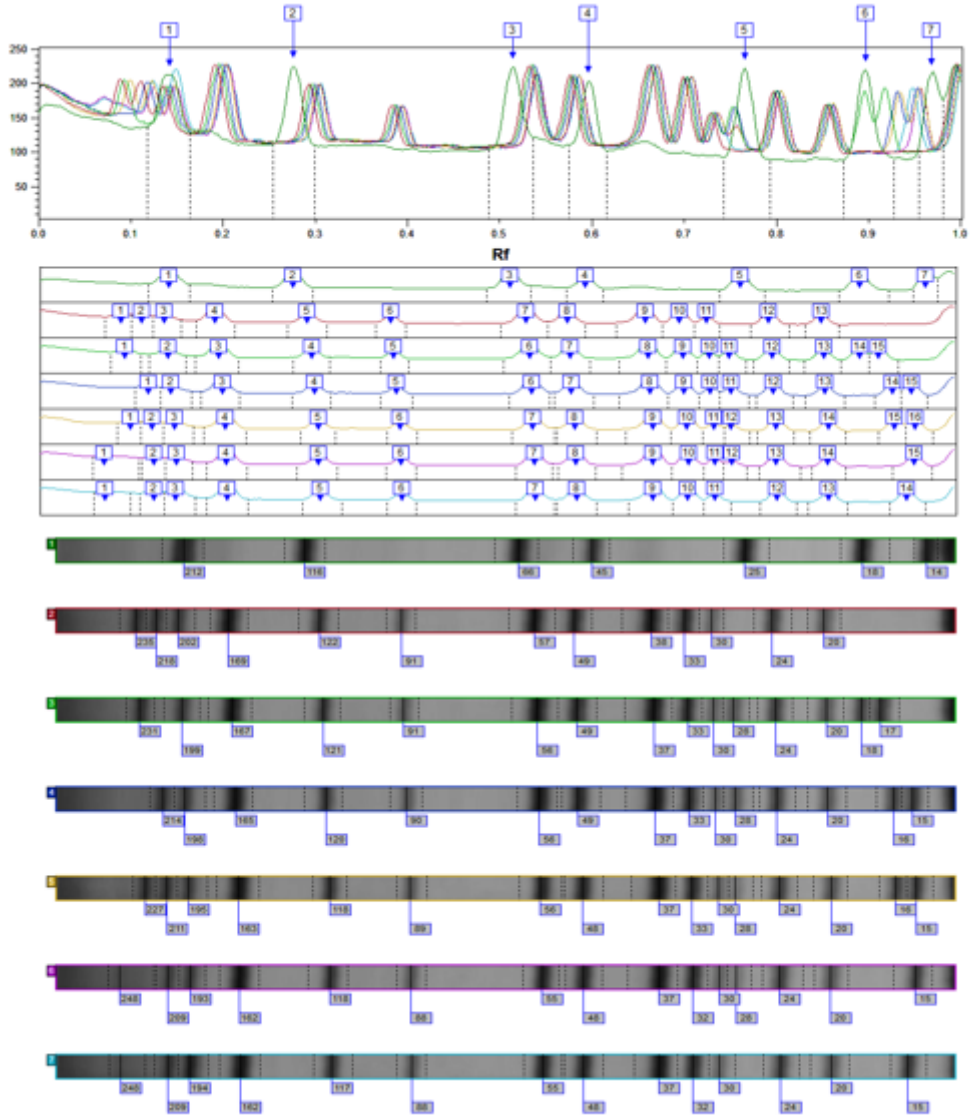

Figure 12

Electropherogram showing the results of scanning of protein profile bands of *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Cu^{2+} concentrations.

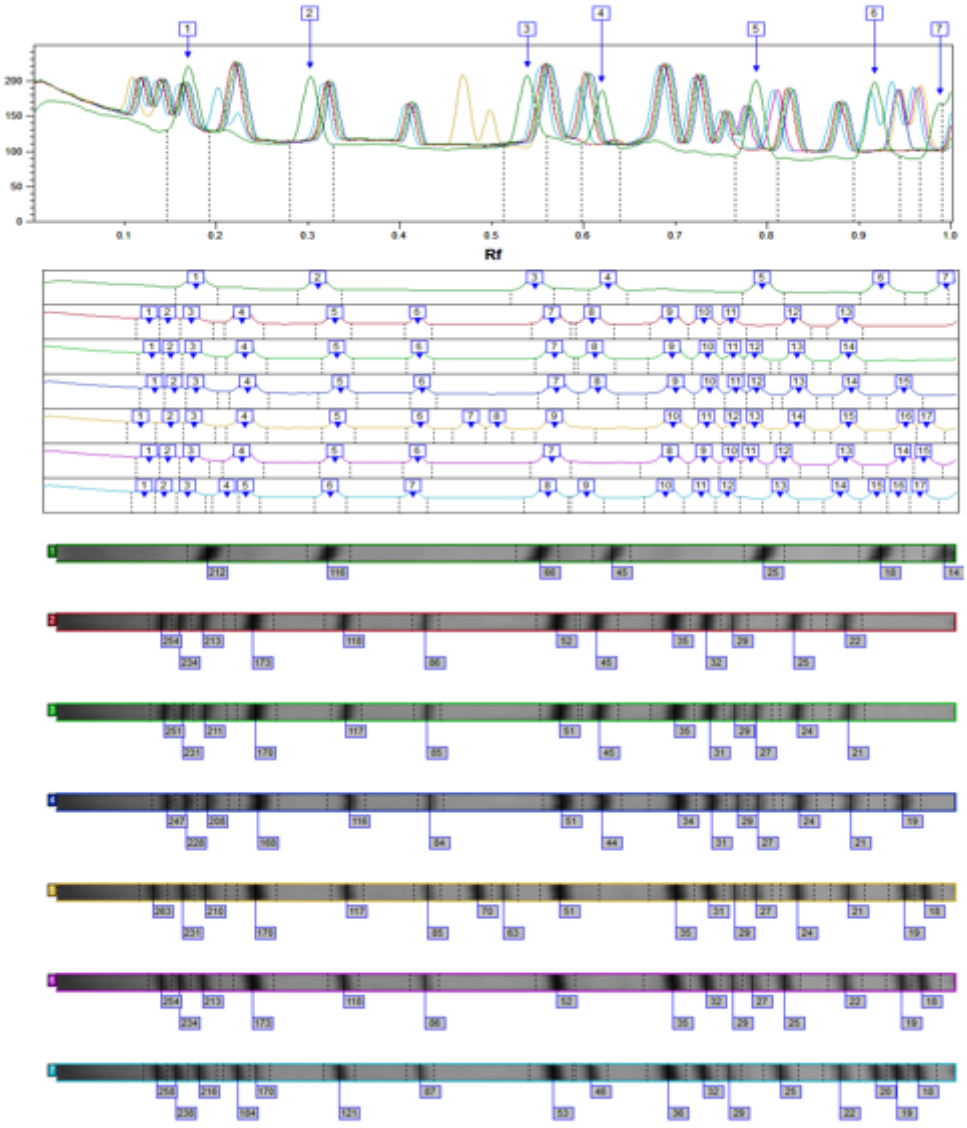

Figure 13

Electropherogram showing the results of scanning of protein profile bands of *Spirulina platensis* cells cultured for 10 days on control and under the effect of different Zn^{2+} concentrations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Plate1.png
- Plate2.png
- Plate3.png