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# Nitrogen, Phosphorus and Minerals (Sodium, Potassium and Calcium) Contents of Some Algae's Species (*Anabaena and Spirulina platensis*)

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**A B S T R A C T**

Some of minerals contents (sodium, potassium and calcium ) and addition to phosphorus and total nitrogen were determined in two different algae species which grow thing in laboratory in different solutions media. The algae species including (*Anabaena and Spirulina platensis*).The results showed different results of the investigated algae species for the studied metals, the data can be summarized as following : Na ( 73.3 and 225 ppm ), K ( 12.3 and 9.5 ppm ), Ca ( 55 and 115 ppm ), N ( 0.73 and 0.23 ppm ) and P ( 3.95 and 5.807 ppm ) for the (*Anabaena and Spirulina platensis* ) species, respectively. The study concluded that most of the selected ions were high accumulated in the *Spirulina platensis* algae specie comparing with *Anabaena one.*

## Introduction

Algae comprise a complex and heterogeneous group of organisms characterized by their photosynthetic nature and their simple reproductive structures, According to their size, algae can be roughly divided into unicellular organisms, known as microalgae and multicellular organisms referred to as macro algae, Algae frequently live in extreme environments of light, salinity, and temperature, In order to adapt to these extreme conditions, most algae produce a high variety of secondary metabolites that often have potent biological activities, (Mattox *et al.*, 1984). The importance of metal ions biological systems is now well established, It is well known that a number of metal ions are essential for biological systems, in that they are absolutely necessary for life processes (Ghallab, 2000), These elements are usually known as essential elements and includes Na, K, and Ca, Storage implies concentration, and there must then be either a binding mode or an energized concentration gradient relative to the external solutions, Biological systems have selected certain elements for certain purposes and in so doing have reject others, The operation involved in concentrating an element, or rejecting it, will also involve steps using selective ion binding, The study of stability constants is therefore central to

our understanding, The simplest store acts as a buffer when vital supplies are low, The nutrient (Nitrogen and phosphorous) store in itself is not much use unless it also is connected to a specific distribution and utilization system, Furthermore, nutrition requires a steady supply of required chemicals, and storage is then a valuable buffer of steady state use**.**

The functions of some metals in biological systems are diverse and complex, and often associated with a specific protein, Metals can function together with proteins in irreversible complexes (metallo proteins), and in reversible complexes (metal protein complexes), but there is also an intermediate class of reversible protein complexes, The first class of proteins are usually enzymes, the second class trigger biological response and act in transport, and the class may well be regulators, (El –Khair,1993). The aim of the current manuscript is to evaluate the bioaccumulation of sodium, potassium, calcium. nitrogen and phosphorus in two different types of algae's growthing in laboratory in different types of saline media.

## Materials and Methods

The methods of preparation and the condition of grow thing the algae can be describing as following:

### Specific Medium of Anabaena

### Isolation of *Anabaena* sp.

The cultures were isolated and purified by repeated plating on solid Chu 10 medium and colonies of different morphologies were identified according to morphological properties and pigment composition The microscopic structure was also observed, Table (1).

The nutrient medium was prepared by using one ml of each of the stock macronutrient solution and one ml of the micronutrient stock solution and making it up to one liter by distilled water. The final pH was then adjusted to 7.2. Potassium phosphate solution was autoclaved separately and then added aseptically to the sterilized medium to avoid phosphate precipitation. The composition of the macronutrient solution was given in Table (2).

Algal culture was grown in Erlenmeyer pyres-glass flasks capacity of 250 ml containing 100 ml culture medium. The flasks were grown under controlled light and temperature culture chamber. Culture experiments were conducted under a regime of 16 h. light/ 8 h. darkness.

### Production of purified algal biomass

Pure culture was maintained by sub culturing in 250 ml Erlenmeyer flasks containing 100 ml of sterile Chu 10 medium (liquid) and incubated under florescent light (3000 lux) at a temperature of 25 ± 1°C. The culture was harvested by centrifugation (4000 rpm for 15 minutes) after 15 days of inoculation.

### Medium *Spirulina platensis*

### Algal growth media

*Spirulina platensis* was grown in *Spirulina* medium (Zarrouk 1966). It consists mainly as described in Table (3).

On the other side the micronutrient stock solution which used in growthing of *Spirulina platensis* was given in Table (4).

One ml from stock solution b + 1.0 ml from stock solution c were added to each 1000ml of solution a. *This salt was neglected in our

work; this is because EDTA is a strong chelator for metal cations and it can sequester metal toxicity very much. Actually EDTA is used for detoxification of metal poisoning.

## Culture conditions

*Spirulina platensis* was grown in conical Erlenmeyer pyrex-glass flasks (capacity 250 ml). Each flask contained 50 ml culture medium. The inoculated medium was adjusted to optical density above 0.1 unit in order to yield a linear growth curve with a lag phase. The cultures were grown under controlled laboratory conditions (temperature at 25˚C ± 1˚C and light at 80 m mol $m^{-2}$ $s^{-1}$) in a controlled culture chamber. Temperature inside the growth chamber was kept as possible within 25˚C through periodical ventilation.

Culture experiments were conducted under a regime of 16 hour light/ 8 hour dark. Each experimental culture flask was regularly swirled daily by hand to detach adhered algal cells from the walls of the flask. After each mixing, the flasks were returned back to a different position on the glass shelves inside the controlled chamber to remove any bias due to the illumination or temperature gradient on the shelves. The culture period lasted for 18 days. The number of replicates were three separate conical flasks for each measure.

## Harvesting of cultures for analyses

At different periods of culturing which depend on the type of the tested metabolic compound, the cells of *Spirulina platensis* were harvested by centrifugation at 5000 r p m for 30 min using angle rotor centrifuge. The supernatants were discarded and the remaining pellets were used for the determination of the selected ions.
dry weight method

Algal samples from the different salt concentrations (10 ml) were filtered under vacuum through 0.45 µm filter membrane and washed several times with distilled water. Then, algal cells were dried at 100°C for 30 min and weighed (Abd El-Baky *et al*., 2003).

## Elemental analysis

The sodium,calcium and magnesium contents were measured by Flamphotometer (Type JENWAY ). The phosphorus levels were determined according to molybedate and vanadate , while the nitrogen contents were measured by spectrophotometric method using Nesler reagent.

## Results and Discussion

The contents of the selected minerals were given in Table (5). The data showed that the contents were fluctuated as following: Na (73.3 and 225 ppm), K (12.3 and 9.5 ppm), Ca (55 and 115 ppm), N (0.73 and 0.23 ppm) and P (3.95 and 5.807 ppm) for the (*Anabaena and Spirulina platensis*) species, respectively.

**Table.1** The saline structure of media according to (Chu 10 )

| Salts | $gl^{-1}$ | Salts | $gl^{-1}$ |
|---|---|---|---|
| $Ca(NO_3)_2$ | 0.04 | $Na_2CO_3$ | 0.02 |
| $K_2HPO_4$ | 0.01 | $Na_2SiO_3.5H_2O$ | 0.025 |
| $MgSO_4.7H_2O$ | 0.025 | $FeCl_3$ | 0.0008 |

**Table.2** Composition of the macronutrient solution

| Salts | $gl^{-1}$ | Salts | $gl^{-1}$ |
|---|---|---|---|
| $NaNO_3$ | 1.5 | Ferric ammonium citrate | 0.006 |
| $K_2HPO_4$ | 0.04 | EDTA (disodium-salt) | 0.001 |
| $MgSO_4.7H_2O$ | 0.075 | $Na_2CO_3$ | 0.02 |
| $CaCl_2.2H_2O$ | 0.036 | *Micronutrient solution | 1 ml |
| Citric acid | 0.006 | Distilled water | One liter |

**Table.3** The macronutrients solution of Spirulina platensis.

| Macronutrients (Solution a) | Quantity (g.) |
|---|---|
| 1- NaCl | 1.0 |
| 2- $MgSO_4$. $7H_2O$ | 0.2 |
| 3- $CaCl_2$. 2 $H_2O$ | 0.04 |
| 4- $FeSO_4$.7 $H_2O$ | 0.01 |
| 5- Na-EDTA* | 0.08 |
| 6- $K_2HPO_4$ | 0.5 |
| 7- $NaNO_3$ | 2.5 |
| 8- $K_2SO_4$ | 1.0 |
| 9- $NaHCO_3$ | 16.8 |
| 10- Distilled $H_2O$ | 1000ml |

**Table.4** Micronutrient stock solution contents

| **Micronutrient stock solution** | | | |
|---|---|---|---|
| **Stock solution b** | | **Stock solution c** | |
| **1- $NH_4NO_3$** | 0.023 g | 1- $H_3BO_3$ | **2.82 0g** |
| **2- $K_2Cr_2$ $(SO_4)_2$. 27 $H_2O$** | 0.096 g | 2- $MnCl_2$. $4H_2O$ | **1.810 g** |
| **3- $NiSO_4$. $7H_2O$** | 0.044 g | 3- $ZnSO_4$. $7H_2O$ | **0.222 g** |
| **4- $Na_2SO_4$. $7H_2O$** | 0.018 g | 4- $CuSO_4$. $5H_2O$ | **0.077 g** |
| **5- Ti $(SO_4)_3$** | 0.040 g | 5- $MoO_3$ | **0.015 g** |
| **6- Co $(NO3)_2$. $6H_2O$** | 0.044 g | 6- Distilled $H_2O$ | **1000ml** |
| **7- Distilled $H_2O$** | **1000ml** | | |

**Table.5** The contents of the elements in the both studied algae species.

| Algae Species | | | |
|---|---|---|---|
| ***Anabaena* Algae** | | ***Spirulina platensis* Algae** | |
| **Sodium** | 7.33 ppm | Sodium | 225 ppm |
| **Potassium** | 12.33 ppm | Potassium | 9.5 ppm |
| **Calcium** | 55 ppm | Calcium | 115 ppm |
| **Phosphorus** | 3.58 ppm | Phosphorus | 5.80 ppm |
| **Nitrogen** | **0.37 ppm** | **Nitrogen** | 0.23 ppm |

**Fig.1** The percentage (% ) of the studied elements of Anabaena species

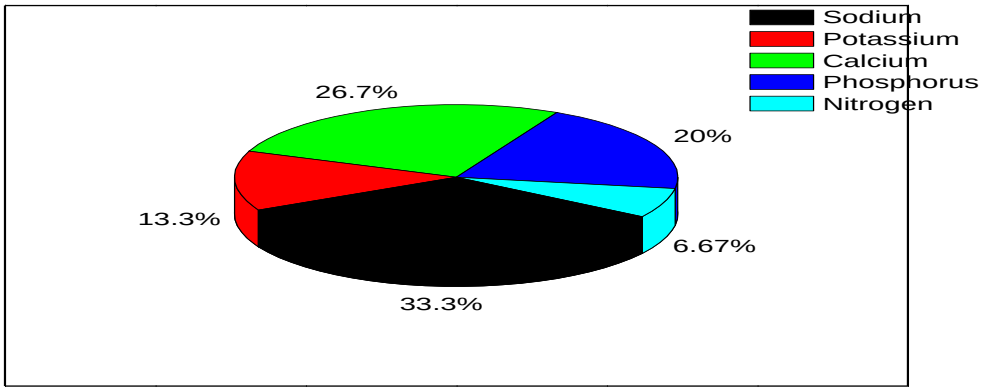
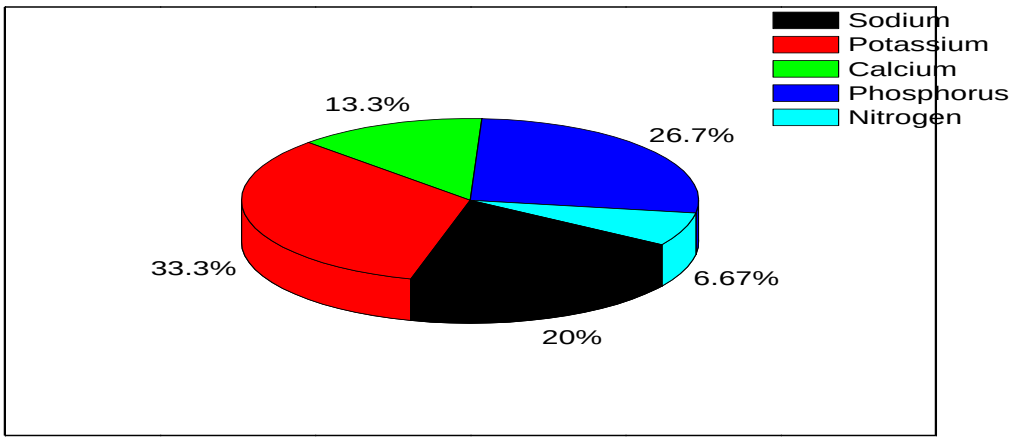



**Fig.2** The percentage (% ) of the studied elements of Spirulina platensis Algae species




The results indicated that there is relative variations in some contents of the studied elements especially for sodium and calcium On the other hand no different values were recorded for the other studied elements, the different mainly due to the stock solution of

the algae growth. The bioaccumulation of studied metals may be concerning with the nature of the physiological structure of the algae species.

In conclusion, from the data which recorded in this investigation we can say that the contents of the solutions are affecting on the accumulation of the elements in the studied species , the study highly recommend to study the other factors which may affecting on the distribution the metals as Temperature or $P^{H}$.

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