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Toxic effect of copper and nickel on biomass and omega-3 content in *Chroococcus* sp. (Cyanobacteria)

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Abstract

The current study included a statement effect of the toxicity of some heavy metals (Copper and Nickel) individually on the content of non-saturated fatty acids (Omega-3) in Micro-algae *Chroococcus* sp. (from blue green algae, Cyanobacteria), the content of these acids was determined after exposure to different concentrations (2, 4 and 8 ppm) of Copper(Cu^{+2}) and Nickel (Ni^{+2}) ions as for different periods of time. Results showed decreased growth rate (Biomass and Cell number) in alga both metals depending on the concentration and exposure period, the disappearance of omega-3 in the studied alga on (8) day at a concentration of (4) mg / l of Copper, and on (8) day at a concentration of (4) mg / l of Nickel.

Keywords: Algae, Heavy metals, Fatty acids.

Introduction

Micro Algae are one of the important components in different ecosystems, and their importance lies in being the primary products in the food chain on which the rest of the living organisms depend for their food. Also, through the process of photosynthesis, it supplies environment in which it grows with the oxygen necessary for the respiration of other organisms (Goldman, 1979), and given the importance of algae in the environment Therefore, it is suitable to study the toxic effects and their effects and their transmission to higher levels within this series, especially those resulting from pollution with an important factor, which is pollution with heavy metals (Stauber, 1995). Microalgae specifically produce fatty acids with chain lengths of sixteen and 18 carbon atoms, however a few species could make fatty acids of as much as 24 carbon atoms in length. Both saturated in addition to enormously unsaturated fatty acids are produced via way of means of microalgae (Breuer *et al.,*2013).

Algae product unsaturated fatty acids (UFAs), which humans and animals need in an essential way because they are unable to synthesize them vitally, so they must be obtained from food or the nutritional supplements they contain. These acids are used in the structural processes and biosynthesis of cells and body organs as well as the production of calories (Cleland *et al.*, 2005). These acids are also considered among the main components of cellular plasma membranes and their various organelles and work to regulate their structural shape, kinetics and permeability (Lauritzen *et al.,* 2001) and are of therapeutic and health importance because their loss causes various pathological conditions such as vascular and heart diseases (Zvicohen 1996, 1996).

Various studies dealt with biomass production from micro algae, especially the production of lipids and unsaturated fatty acids (UFAs) and determining the type and concentration of each acid.

Its physiological effects and its benefits to living organisms feeding on it because of its high content of these acids, its diversity and production within a short period of time and at an appropriate economic cost, and that the quantity and quality of unsaturated fatty acids (UFAs) varies from one alga to another according to the environmental conditions in which it lives In it, these acids are similar to animal and vegetable fats, meaning that they are edible by humans (Abubakar *et al.,* 2012).

Heavy metal pollution is one of the most dangerous types of pollution because it is one of the transitional elements that have the ability to form complex compounds that are stable with most of the organic and inorganic compounds found in the bodies of living organisms, including microalgae, which leads to their accumulation within these living ecosystems, and is characterized by the inability to decompose their compounds. By decomposing organisms and other natural processes as well as their stability in the environment, the effect of heavy metals on acid content was studied, unsaturated fatty acids (UFAs) in different algae included a study of the effect of cadmium on alga *Ulva* (Kumar *et al.,* 2012) .

Toxic effect of cadmium on the alga *Euglena gracilis* (Watanabe *et al.,*2002) and the effect of chromium on the alga *Chlorella pyrenoidosa* (Horcsik *et al.*, 2006), and the effect of the interaction between heavy metals and its effect on algae was studied, and it was noted that the toxic effect of these elements was not It depends on the amount of it accumulated in its cells, but rather on the abundance of free ions for it inside the cell, as it was found that cases of antagonistic or synergistic effect appeared when two or more elements were combined, depending on the type of algae and the element and its concentration (Al-Hajjaj, 1999; Ellwood and Hunter,2002). Kumar et al., (2012) studied the effect of the interaction between cadmium and copper on the unsaturated fatty acid content of *Ulva lactuca.*

The current study aims to show the effect of some heavy metals (Copper and Nickel) on the growth rate and the content of unsaturated fatty acids (3-Omega) in micro algae *Chroococcus sp.* isolated from our local environment.

Materials and Methods

Cyanobacteria *Chroococcus sp.* was isolated from Euphrates river in Nassiriya city, and then remove to the laboratory, the samples streaked on agar medium (Chu-10) plates and incubated under the air conditioned room which was illuminated with while fluorescent (50) micro-Einstein /m/sec and with a lighting system (16: 8) light:dark at room temperature (25±3)°C, until the appearance of algal colonies. Single algal; colonies were selected and re-streaked on agar medium plates several time to obtain unialgal cultures. Single colonies were maintained in 250 cm^3 classified according to Desikachary (1959), of Chu-10-D medium for stock culture under the same conditions classified according to Desikachary (1959), purified according to (Stein, 1973). Copper (Cu^{+2}) and Nickel (Ni^{+2}) ions as stock solutions (1000 ppm) were prepared separately by dissolved $Cu(NO_3).H_2O$ and $Ni(NO_3)2. H_2O$ respectively , in deionized distilled water. Different concentrations (2, 4 and 8ppm) of each stock solution were used (singly and in dual combinations).

The direct microscopic cell count by Thoma haemocytometer was performed with the optical microscope (Labomed, USA).

Estimation of the biomass of algae used in the study:

The biomass of the algae used in the study was estimated by filtering the farm after the end of the incubation period using filter papers Whitman No.1 known weight, and after drying the samples using an oven at a temperature (60° C) and then weighed again and the difference between the two weights represents the dry algae biomass.

Content of acids (Omega-3) measure by using Gas Chromatography - Mass Spectrometry (GC-Mass) using a GC device type (Japan - Shimadzu – Mass).

All statistical analysis were performed by using ANOVA test.

Results and Discussion

The statistical analysis (ANOVA test) revealed a significant ($P<0.05$) growth response that caused by the different concentrations of Copper and Nickel ions, on the growth of a strain of blue-green algae *Chroococcus sp.* The effect of Copper and Nickel ions ware significant differences ($P>0.05$) compare with growth rate and Omega-3 content in control groups as show in table 1.

Table 2 showed that all the Copper concentrations (2, 4 and 8ppm) were lead to inhibition growth of *Chroococcus sp.* (Dry weight and Cell number), the maximum inhibition growth was appeared at 4and 8 ppm treatments, which had decreased in dry weight and cell number, at the first days exposure periods.

Omega-3 content was less than natural value in control groups (Table 1), lack of fatty acids was concurrent with the increase in the concentration of cobalt and period for exposure (Table 2).

Nickel ion was have inhibition growth of *Chroococcus sp*, these effects were clearly appeared at the first days for exposure (Table 3), then, the growth was increasing with the exposure period increasing (last days).

Effect of nickel element on the growth of the studied algae, where the dry weight and the number of cells decreased compared with Table 1, as well as the omega 3 content was less than the normal limit by increasing the exposure period and the concentration of the element as showmen in Table 3.

Table 1 :Growth rate and Omega-3 content of microalgae of *Chroococcus sp.*

Growth Rate	Times (day) 1	3	5	8
Biomass g/l	2.34	3.45	3.92	1.98
Number cell/l	$560*10^7$	$640*10^7$	$690*10^7$	$490*10^7$
Omega-3 % from total lipid	15%	18%	19%	13%

Table 2 : Effect of different concentrations of Copper on Growth rate and Omega-3 in *Chroococcus sp.*

Copper concentrations ppm	0	2	4	8
Growth Rate	Time 3 Days			
Biomass g/l	3.45	3.65	3.56	2.11
Number cell/l	$640*10^7$	$648*10^7$	$572*10^7$	$494*10^7$
Omega-3 % from total lipid	18%	18%	15%	13%
Growth Rate	Time 5 Days			
Biomass g/l	3.92	2.12	2.01	1.21
Number cell/l	$690*10^7$	$492*10^7$	$401*10^7$	$322*10^7$
Omega-3 % from total lipid	19%	12%	9%	7%
Growth Rate	Time 8 Days			
Biomass g/l	1.98	0.52	0.11	0.07
Number cell/l	$490*10^7$	$120*10^7$	$98*10^7$	$43*10^7$
Omega-3 % from total lipid	13%	1%	0%	0%

Table 3 :Effect of different concentrations of Nickel on Growth rate and Omega-3 in *Chroococcus sp.*

Nickel concentrations ppm	0	2	4	8
Growth Rate	3 Days			
Biomass g/l	3.45	3.25	3.55	1.99
Number cell/l	$658*10^7$	$647*10^7$	$569*10^7$	$489*10^7$
Omega-3 % from total lipid	18%	17%	15%	13%
Growth Rate	5 Days			
Biomass g/l	3.92	2.02	1.99	1.31
Number cell/l	$658*10^7$	$482*10^7$	$398*10^7$	$334*10^7$
Omega-3 % from total lipid	18%	12%	10%	7%
Growth Rate	8 Days			
Biomass g/l	1.78	0.5	0.09	0.02
Number cell/l	$487*10^7$	$122*10^7$	$96*10^7$	$33*10^7$
Omega-3 % from total lipid	13%	1%	0%	0%

The change in the concentration of Copper and Nickel and its exposure period, effects on the biomass and content of unsaturated fatty acids (UFAs) in algae. In this study decrease in biomass and omega-3 content occurs, perhaps because heavy metals are transitional elements that have the ability to form complex compounds that are stable with most of the vital components found in microalgae cells, which leads to their accumulation within cells (Akira *et al.,*2005).

Toxicity of heavy elements is due to their structure and physicochemical properties, as they are found in nature in the ionic form associated with water or in a complex form with organic or inorganic compounds in organism or its surrounding environment that contains sulfur (SH), nitrogen (NH) or oxygen (OH) (Abassi, 2004), or perhaps because it causes changes in the shape and components of cellular membranes, causing their small size and consequently the low content of algal cells of fatty acids Unsaturated (UFAs) (Horcsik et al., 2006), this is in agreement with Walsh *et al.* (1998), who found that the use of (3) mg/L of iron causes the disappearance of all acids.

Unsaturated fatty acids (UFAs) in *Microcystis aeruginosa*, meaning that the cells of this alga became containing saturated fatty acids (SFAs), and with a study (Watanabe *et al.,* 2003) who studied the effect of cadmium on the content of unsaturated fatty acids (UFAs) in alga *Euglena gracilis* and it was found that it causes the destruction of the song of many organelles and inhibits the growth of this alga by (5) % as it contains less unsaturated fatty acids (UFAs), Or perhaps because of the effect of heavy elements on building proteins, and because the enzymes responsible for the manufacture of these acids are of a protein nature, and therefore the chemical content in true algae decreased, especially its content of unsaturated fatty acids (Lavoie *et al.,* 2009).

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