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# Isolation and Identification the Cyanobacterium: *Scytonema hofmanni* var. *calcicolum* as New Record in Iraqi Drinking Water.

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**Abstract**

The aim of this study was to isolate and identify the cyanobacterium *Scytonema hofmanni* Var. *calcicolum* from the domestic drinking tanks as a new record in Iraqi drinking water. *Scytonema hofmanni* var. *calcicolum*, a filamentous freshwater cyanobacterium (blue-green alga). This alga was isolated from the walls of the domestic plastic water tanks in Al- karkh/ Baghdad city on July 2014. The sampling was performed by collecting three samples from this tanks, the three examined samples microscopically revealed the dominance of this cyanobacterium as unialgal in the studied samples. The results showed this alga has the ability to tolerate high temperature up to 42 $C^o$ and very low light intensity inside the tanks which up to 10 $\mu E/m^2/s$.

**Keywords:** New record, *Scytonema hofmanni var. calcicolum*, Iraqi freshwater.

# عزل وتشخيص الطحلب الخيطي *Scytonema hofmanni* var. *calcicolum* كنوع جديد في مياه الشرب العراقية

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**الخلاصة**

هدفت هذه الدراسة الى عزل الطحلب *Scytonema hofmanni* var. *calcicolum* من خزانات المياه المنزلية كنوع جديد في المياه العذبة العراقية. *Scytonema hofmanni Var. calcicolum* طحلب خيطي (طحلب أخضر مزرق) يعيش في المياه العذبة. عُزل هذا الطحلب من جدران الخزانات البلاستيكية لمياه الخزن المنزلية في جانب الكرخ من بغداد في شهر تموز سنة 2014. تم جمع العينات بواقع ثلاث عينات من هذه الخزانات. العينات الثلاث التي فحصت مجهريا اظهرت السيادة لهذا الطحلب في مياه هذه الخزانات كطحلب وحيد في العينات المدروسة . اظهرت نتائج الدراسة ان هذا الطحلب له القابلية على تحمل درجات حرارة عالية وصلت الى 42 درجة مئوية وشدة اضاءة منخفضة جدا وصلت الى 10 مايكرو اينشتاين.

## Introduction

Iraqi freshwater algae composed of three main prevalent groups involve Bacillariophyta, Chlorophyta and Cyanophyta [1, 2]. Cyanobacteria, also known as blue-green algae, Cyanophyta or cyanophyceae, are autotrophic prokaryotic micro-organisms. In evolutionary terms they represent a link between bacteria and higher plants [3]. *Scytonema* is a filamentous freshwater cyanobacterium (blue-green alga), predominantly a sub-aerial and aquatic mostly freshwater or occurs in damp terrestrial situations. Forty species of this genus have been reported from India [4]. In Iraqi freshwater there are 9 species have been reported according to the checklist of algal flora in Iraq [5]. *Scytonema*

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*spp* vary in color from blue green, dirty blue green, blackish green and yellowish brown to brown. They may float freely in the water ponds, lakes and tanks. Some may grow submerged along the walls of the tanks [6].

*Scytonema hofmanni* Var. *calcicolum*, the stratum of this alga is smooth brown or blackish, rarely almost soft gelatinous, often expanded, growing on calcareous rocks; filaments more or less branched, curved and aggregated into dense floccose masses; false branches somewhat narrower, single or geminate, erect, arising in between heterocysts; sheath close, somewhat thick with age, yellow or yellowish orange, rarely nearly colourless; trichomes 4-6 µ broad, blue-green or yellowish ; cells subquadrate or ½ as long as broad; heterocysts subquadrate, single or in two, a little shorter or longer than broad; contents yellowish [4]. Taxonomic classification is a method to obtain a review of the studied organisms in their whole variation possibilities and their relations. The system of cyanobacteria is constantly in process [7- 11]. The modern algologists classified the genus *Scytonema* to a new order namely Scytonematales [6].

The aim of this study was to isolate and identify the cyanobacterium *Scytonema hofmanni* var. *calcicolum* from the domestic drinking tanks as a new record in Iraqi freshwater.

## Materiales and Methods

### The site description and sampling

Three samples were collected from three plastic water tanks in Al- karkh/ Baghdad city on July 2014.


**Figure 1-** Map of Baghdad city: Site description of the study.

### Morphological characterization

The samples were observed under microscope. The cell shape and size were observed, measured by micrometry and documented as microphotograph. Identification of specimens was carried out using the taxonomic publication [4].

### Algal cultures

The cyanobacterium *Scytonema hofmanni* was grown in sterilized BG-11 in 250-ml flasks. The culture was incubated in a controlled-environment cabinet at 30 C° with cool white fluorescent lights 10 µE/m²/s 12 h light/12 h dark). The above- growth conditions was adjusted according to the studied environment.

### Physio-chemical Parameters

### Field Work

Water samples (500 ml) were taken from inside the tanks. Containers non-metallic bucket were used to avoid metallic contamination.

**Temperature**

Water temperature was measured immediately in the tanks by placing a precise clean mercury thermometer (range 10 to 60 C°) graduated up to 0.1Cº.

**Light intensity**

Light intensity was measured by using the Lux -meter (Milwaukee, Italy).

**Conductivity (µS/cm), TDS, and pH**

The electrical conductivity, TDS and pH were measured by using pH-EC-TDS meter (HANNA Instruments). The expression of results was µS/cm for conductivity and mg/L for TDS.

**Salinity**

Salinity was determined by depending on the electrical conductivity values [13], Salinity expressed with $^{0}/_{00}$ as follows:

$$\textbf{Salinity } {}^{0}/_{00} = \frac{Ec - 1478}{1589.08}$$

EC= Electrical Conductivity.

**Turbidity**

Turbidity was determined by using Turbidometer (HACH Instruments) model 2100A after instrument calibration by known turbidity stander solution. Turbidity expressed with Nephlometric Turbidity Unit (NTU).

**Nitrate (NO3)**

Nitrate estimated by taking 5ml of sampled water which diluted to 50 ml with distilled water .One ml of HCl (1 M) was added to the sample, the solution was measured in 1cm cuvette using a wavelength of 220nm .The water sample estimation was repeated in a wavelength of 752 nm, the differences between two measures had been depended [12] Nitrate concentration was estimated by this expression:

µg NO3-N/L= (mgNO3 _ N) (4.43)/ml of sample.

**Nitrite (NO2)**

Fifty ml from the water sample was taken and one ml Sulphanilamide solution was added with shaking. After two minutes 1ml n-(1-naphthyl)-ethelene-diamine solution was added. After 15-20 minutes the absorbance of the light pink color of samples were measured by a Cintra-5 UV-Visible Spectrophotometer at wavelength 543 nm [12]. Results were stated in the unit µg nitrogen- nitrite /l.

**Results and Discussion**

As stated in the figure -2A, the structure of the trichome in *Scytonema* composed of a single row of cells lying end to end to form a longer or short thread-like structure. In some species the cells may be descoid, squarish, cylindrical or rectangular in shape. The trichome has heterocysts distributed at regular or irregular intervals throughout its length. The trichome increase in size by the repeated division of its cells. The cell division is mainly restricted to the apical or sub-terminal cells. The apical cell is generally hemispherical and flattened as shown in figure-2D. In general, *Scytonema* exhibits false branching. The branches may be single or geminate as stated in figure-2C. This type of branching starts with the breaking of the trichome at a certain point. One or both the ends may then perforate the sheath and grow out as branches (6).

The Check list of Algae of Maulood *et al*. [5] showed that *Scytonema hofmanni* var. *calcicolum* is not recorded in Iraqi freshwater; however, this cyanobacterium was identified as new record species in Iraqi freshwater belonged to the order of Nostocales [4]. The modern algologists classified the *Scytonema* with new order: Scytonematales [6].therefore, the modern classification of this alga was as the following:

- **Division**: Cyanophyta
- **Class**: Cyanophyaceae
- **Order***:* Scytonematales
- **Family**: Scytonemataceae
- **Genus**: Scytonema
- **Species**: *hofmanni* var. *calcicolum* Hansg

In this study this alga was isolated as unialgal from the sampling area (domestic water tanks) and there was no need to purify this alga which isolated from the studied tanks, this related to the absolute prevalence of *Scytonema hofmanni* in environment in which it is collect. As it is well known, this

cyanobacterium is a filamentous freshwater cyanobacterium (blue-green alga), produces secondary metabolites which inhibit the growth of other cyanobacteria and green algae (15). In a study by Mason *et al.* [14] has led to the isolation and characterization of an antibiotic (named cyanobacterin) from *Scytonema hofmanni*. This chlorine-containing antibiotic has a molecular weight of 430 and an empirical formula of C23H23O6Cl and contains a gamma-lactone and a chlorinated aromatic nucleus.

It inhibits the growth of various algae but has limited effect on non -photosynthetic bacteria or protozoans and thus may have potential use as a specific algicide, therefore the prevalence of *Scytonema hofmanni* attributed to the impact of allelopathy of this antibiotic. Allelopathy may lead to the formation of harmful phytoplankton blooms and the spread of exotic species into new habitats [15].

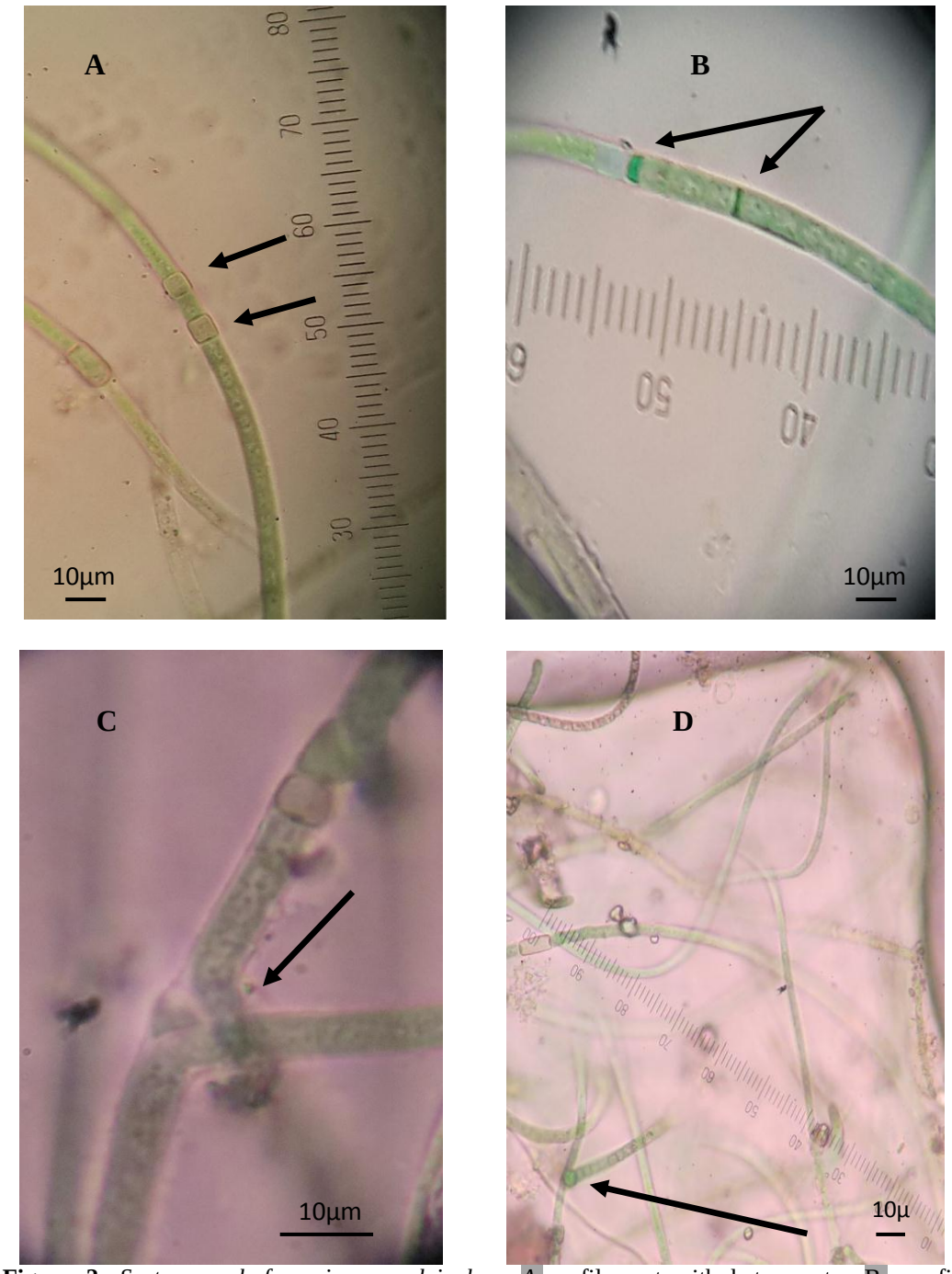


**Figure 2**- *Scytonema hofmanni* var. *calcicolum*. *A*: a filament with heterocyst. B: a filament showing hormogonia formation; C: a trichome exhibits false branching. D: the apical cell**.**

The results of the current study revealed that the water temperature was 42C° inside the tanks. Also, the light intensity was very low inside the tanks almost non-existent which up to 10 µE/m²/s. In addition to the allelopathy impact of this alga by its antibiotic (cyanobacterin); extreme conditions (high temperature and low light intensity) may play crucial role in the dominance and this explains the absolute prevalence of this alga in the studied tanks. The ability of blue-greens to out-compete other freshwater algae has been attributed to a range of characterstics, including low light tolerance, high temperature tolerance, and resistance to zooplankton grazing and depth regulation by buoyancy (16). The high temperature and low light intensity might be considered optimal growth conditions of *Scytonema hofmanni* var. *calcicolum* isolated from the domestic water tanks.

As shown in the table-1. The values of physio-chemical characteristics except the high temperature and the low light intensity revealed normal scales and exhibit Eutrifcation status due to the blooming. The high bloom of this alga might be related to the high temperature and the low light intensity inside the tanks.

**Table 1**-The physio-chemical characteristics of the studied water samples inside the water tanks.

| **Physio-chemical characteristics** | Water temperature | Light intensity µE/m²/s | E.C µS/cm | Salinty | Turbidity FTU | T.D.S mg/l | Nitrate (NO3) mg/l | Nitrite (NO2) mg/l | pH |
|---|---|---|---|---|---|---|---|---|---|
| **Scales** | 42 C° | 10 | 399 | 0.242 | non | 440 | 4 | 0.005 | 7.2 |

In conclusion, the findings of the current study revealed that the extreme environmental conditions such as the high temperature and the low light intensity may be play crucial role to dominating the cyanobacterium *Scytonema hofmanni* var. *calcicolum* as uni algal in some studied domestic water tanks.

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