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Original Research Article

The secret story of the farmer's friend: The case of *Anabaena cylindrica*

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Keywords

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A B S T R A C T

Anabaena cylindrica,Lemm. is a genus which consists 46 species. These are unbranched and uniseriate filamentous cyanobacteria that exists as plankton. It is well known for its nitrogen fixing abilities, they form symbiotic relationships with certain plants ex mosquito fern. Their cell structure is prokaryotic. There are two types of cells: vegetative cell & heterocysts. Sevin, 50% W.D.P. based on carbaryl. 1. Naphthylmethyl carbamate, is a broad spectrum pesticide is used to study the growth of the particular alga in laboratory condition. The three selected concetrations were exposed at,2.54ml/L, 3.01 ml/L,3.25 ml/L , and studied at 0,3,6.9,12 and 15 days. After exposure the alga were allowed to recover in normal condition in three consecutive periods of 5 days upto 15 days. Sevin did not bring about any significant morphometric change, in the exposed cultures. At higher concentration of the pesticide and at higher exposure period, bleaching of the filaments and total chlorosis of the filaments were observed. The exposed alga could not recover in recovery studies, even after prolonged recovery period. This indicated that the toxicant, Sevin caused permanent damage to the exposed algal system. But the recommended dose can prove more than handy for the farmer and can be considered as the success story of the farmer

Introduction

The BGAs are our friends but with use of pesticide the growth of these microorganisms is affected severely. These are also oxygen producing prokaryotes. It is small primitive group comprising of about 2500 species placed under 150 genera. They are mainly present in colony. A number of chains of bead like cells (trichomes) of different length are combined to form gelatinous matrix colony. Trichomes are enclosed in their own mucilaginous sheath called filament. A soft mucilaginous envelop holds the filament together by the fusion of individual trichomes sheath and secreted copiously by the component cell. Each colony is considered a bluish green or yellowish mass of jelly. Usually young colonies are small and microscopic, mature ones are large. A compound colony is

formed with the help of many macroscopic, mucilaginous lump or thallus. From these genera one of the genus i.e. *Anabaena cylindrica* is chosen. The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. A closely related group of insecticides are the carbamate esters first discovered by the Geigy Company in Switzerland in 1947, although the most generally effective member of the group carbonyl or Sevin (N - methyl a - naphthylcarbamate) was not introduced until nearly a decade later. This is becoming increasingly important as a possible replacement for DDT. From this point of view we select Sevin 50% W.D.P. which is a low mammalian toxicity and also it is generally regarded as one of the safer insecticides. This project was designed to show the growth pattern of this BGA with selected lethal concentration of Sevin.

Materials and Methods

Test organism: ***Anabaena cylindrica***, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO_2 and evolve O_2 where as heterocysts are unable to fix CO_2 or evolve O_2 but can fix nitrogen under aerobic condition (Stewart, 1976). The akinetes are perennating spores that develop between vegetative cells and heterocysts and obtain fixed carbon and nitrogen from them.

Selection of toxicant: SEVIN 50% W.D.P. based on carbaryl. 1. Naphthylmethyl carbamate, is a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets. It is relatively free from handling hazards and may be applied in the immediate pre-harvest period without concern for excessive residues. SEVIN 50% W.D.P. has a low mammalian toxicity. It is generally regarded as one of the safer insecticides Sevin 50% W.D.P. is normally non-phytotoxic Sevin 50% W.D.P. is compatible with most of the pesticides, except those of alkaline nature.

Selection of pesticide and duration: The selected concentrations were exposed at 2.13 ml/L,2.54ml/L, 3.01 ml/L, 3.25 ml/L, 3.35ml/L and studied at 0, 3, 6.9, 12 and 15 days interval. After exposure the algae were allowed to recover in normal condition in three consecutive periods of 5 days up to 15 days.

Growth measurement: Light scattering technique (Sahu, 1987) was used to monitor the growth of the alga in the culture medium. The pre-requisite for the use of light scattering to monitor the algal growth is that the microbes should give a stable and uniform suspension during optical density (O.D.) measurements and the advantage of this method is that an experimental culture can be used over any span of time and for any number of growth measurements without terminating a culture (Eyster, 1972). A suspension of the exponentially growing alga of the same age, having some biomass and same physiological conditions, was inoculated initially into the experimental flasks. Growth was estimated by withdrawing the cultures under aseptic conditions. On every third day interval, growth was measured in terms of optical density and dry weight. The optical density of the samples was measured in a spectrophotometer (Systronics) at 530 nm. Dry weight of the sample was determined by drying the material in an infra-red moisture

meter and weighing in a single pan electric balance.

Statistical analysis: The data was processed initially for determination of mean values and standard deviation. Correlation coefficient, regression analysis and analysis of Variance Ratio (ANOVA) tests were conducted to analyze the obtained data (Panigrahi & Sahu, 2000).

Results and Discussion

A graded series of concentrations of the pesticide, Sevin was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. Unialgal, axenic culture of *Anabaena cylindrica*, Lemm. was inoculated and the survival percentage was determined. Table 1 describes the toxicity values of the pesticide, Sevin on *Anabaena cylindrica*. The following concentrations were selected for further detailed studies pertaining to the effects of Sevin on the blue-green alga.

Table 1 indicated that with the increase in concentration of the toxicant (Sevin) the survival percent decreased significantly showing a negative correlation. The above three lethal concentrations were chosen to study the differential effects of different concentrations of the pesticide (Sevin) on the blue-green alga, *Anabaena cylindrica*, Lemm.

The control set showed a sigmoid growth curve with the increase in exposure period. At 2.54 ml l^{-1} (A) of Sevin concentration, the alga showed a decrease in optical density over the control value. At 3.01 ml l^{-1} (B) of Sevin, the optical density values were less than the control values and were significantly less than the values obtained for concentration A. At 3.25 ml l^{-1} (C) of Sevin, drastic decline in optical density values were observed. After 6th day of exposure, even the values were less than the control values at '0' day of exposure, indicating a total disappearance of the live system. From 9th day exposure period onwards, the data did not show any significant change from 0.010 to 0.015 (OD at 530 nm.). When the exposed algal cultures were transferred to toxicant free nutrient medium, at lower concentration (C), no significant change in data line was marked (Fig. 2). Figure 3 and 4 represented the percent change (increase/decrease) in optical density values in exposed alga, exposed to different sub-lethal concentrations of Sevin at different days of exposure and recovery. At 2.5 ml l^{-1}, on the 15th day 12.5% decrease was observed over the control value, than the rate of decrease in optical density increased with the increase in recovery period. At 3.0 ml l^{-1}, no change in optical density was marked on the 3rd day of exposure. After 6th day of exposure, the percent decrease was steady, significant and showed a positive correlation with the increase in exposure period. The value increased up to 29.16% on 15th day of exposure. No significant change was seen, when the exposed alga of 'B' transferred to toxicant free nutrient medium. The percent decrease in optical density values increased from 15.38% to 29.29% within 15 days of exposure. The values still increased up to 38.46%, when the exposed algal cells were transferred to toxicant free nutrient medium (Fig. 3). At higher concentrations of Sevin exposure, no recovery was recorded. Only at lower concentrations of the toxicant an insignificant increase in the percent decrease was marked. No recovery during the recovery period in exposed algal cells clearly indicates the toxic nature of the toxicant. The correlation coefficient analysis indicated the existence of positive significant correlation between days of exposure and optical density values in

control ($r = 0.994$, $p \leq 0.001$), Conc. A. ($r = 0.968$, $p \leq 0.01$) and Conc. B. ($r = 0.914$, $p \leq 0.05$), however, negative non-significant correlation was observed in Conc. C. ($r = -0.561$; p = NS). The percent change values showed all negative but significant correlations (Table 2). The two way analysis of variance ratio test pertaining to optical density indicated that there exists a significant difference between rows and also between columns. The ANOVA test conducted for percent change in growth pattern by optical density method indicated the existence of significant difference between rows and columns.

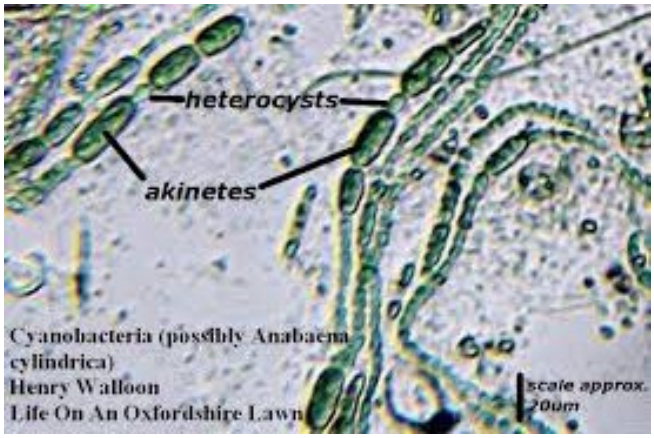
Figure 5 represents the effect of different concentrations of Sevin on the growth of the blue green alga, *Anabaena cylindrica*. The growth pattern of control and exposed algae were analyzed by dry weight analysis. Control set showed the existence of a positive correlation with the exposure period in case of dry weight ($r = 0.996$ $p \leq 0.001$). At 2.54 ml l^{-1} Sevin, the dry weight of the exposed alga increased with the increase in exposure period, showing a significant positive correlation ($r = 0.988$, $p \leq 0.01$). But the values were much less than the control values. At LC_{10}, a non-significant initial increase followed by decrease in dry weight over the control value was marked at 15 d exposure period and an insignificant increase in the recovery periods was marked (Fig. 5 & 6). It showed the highest increase on 9th day of exposure to a tune of 7.5% over the control value. Thereafter, the rate of growth or increase in dry weight depleted with the increase in exposure period and in recovery period, no partial increase was marked, rather further depletion in the values were obtained and a maximum of 8.13% decrease was recorded on 15th day of recovery. However, the data line did not fall below the control values (Fig. 6 & 7). At LC_{50} (B), a gradual increase in dry weight was marked with the increase in exposure period up to 12 days of exposure ($r = 0.947$ $p \leq 0.01$), when compared to the control set, a significant decrease in dry weight was marked (Fig. 6 & 7). 15th day of exposure, 55.12% decrease and on 15th day of recovery, 65.85% decrease over the control value was observed. Interestingly, during recovery period, instead of showing any sign of recovery, higher percentage of decrease in the dry weight value indicated acute poisoning or stress. All the data were statistically significant (Table 1). At LC_{90}, (C) significant decreasing trend was marked (Fig. 6). The dry weight (mg) of exposed alga was significantly less than the control alga, at all exposure periods and recovery periods. Non significant negative correlation exists between exposure period and dry weight of the alga ($r = -0.582$; p = NS), at LC_{90} of Sevin. A maximum of 94.87% decrease on 15th day of exposure and 98.37% decrease on 15th day of recovery over the control value was marked (Fig. 7). The correlation coefficient analysis between days of exposure and dry weight of the control and exposed alga, exposed to different concentrations indicated the existence of a positive significant correlation in control ($r = 0.996$; $p \leq 0.001$), Conc. A ($r = 0.988$, $p \leq 0.01$) and Conc. B ($r = 0.947$; $p \leq 0.01$). A negative but no significant correlation ($r = -0.582$; p = NS) existed between days of exposure and dry weight of the alga in Conc. C (3.25 ml l^{-1}) as observed in tab.-B. The percent change in dry weight showed all negative and highly significant correlation coefficient values (Table 2). The two way analysis of variance ratio test indicated the existence of significant difference between rows and columns.

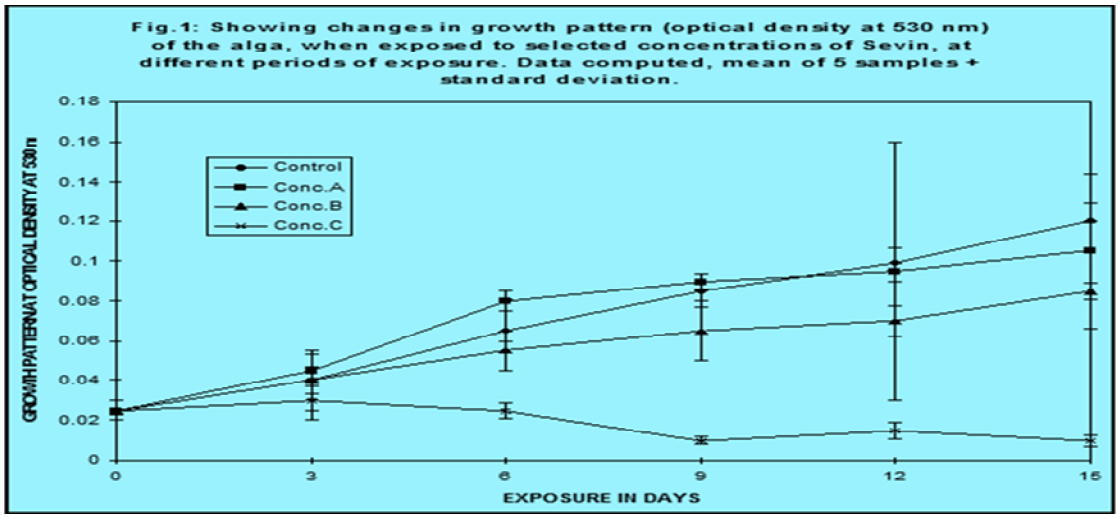
Table.1 Showing deduced lethal concentration values after 15 days of exposure from the toxicity testing data

Lethal Concentration (LC)	Pesticide Concentration (ml. l^{-1})	Percent Survival (PS)
LC_0	2.13	PS_{100}
LC_{10} (A)	2.54	PS_{90}
LC_{50} (B)	3.01	PS_{50}
LC_{90} (C)	3.25	PS_{10}
LC_{100}	3.35	PS_0

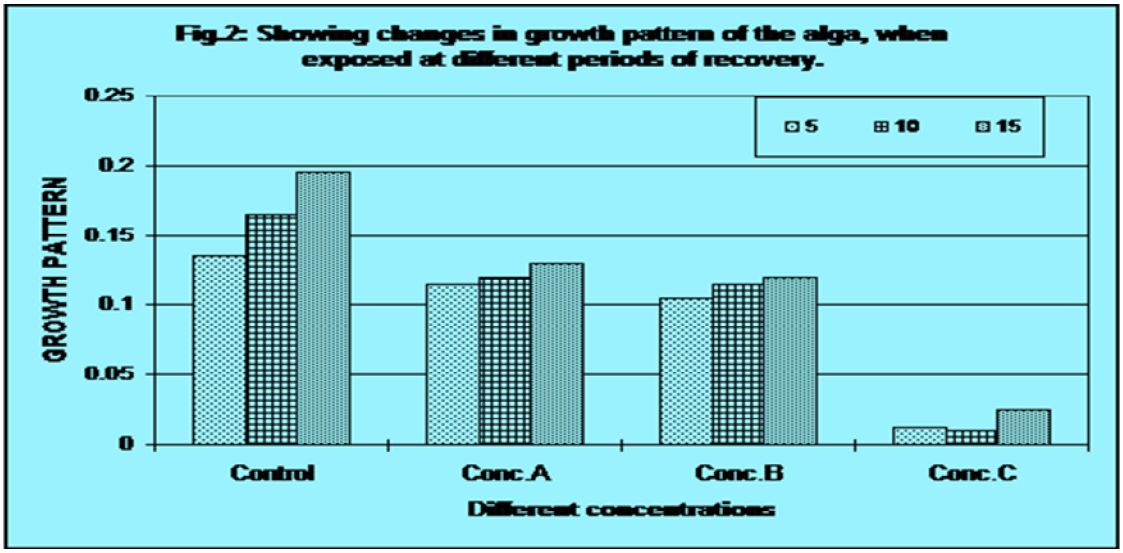
Table.2 Correlation co-efficient (r) between days of exposure and different parameters of study of the algae, exposed to three different concentrations of the pesticide and control (NS = Not significant)

Concentration of the insecticide	Optical density at 530 nm.	Percent change in O.D.	Dry weight (mg)	Percent change in dry weight
Control (0.0) P ≤	0.994 0.001	-----	0.996 0.001	-----
A (2.54ml l^{-1}) P ≤	0.968 0.01	- 0.765 NS	0.988 0.01	- 0.889 0.05
B (3.01ml l^{-1}) P ≤	0.914 0.05	- 0.850 NS	0.947 0.01	- 0.926 0.05
C (3.25ml l^{-1}) P ≤	- 0.561 NS	- 0.992 0.001	- 0.582 NS	- 0.988 0.01





LC_{10}, LC_{50} and LC_{90} were expressed as A, B & C respectively and 'Con.' stands for control



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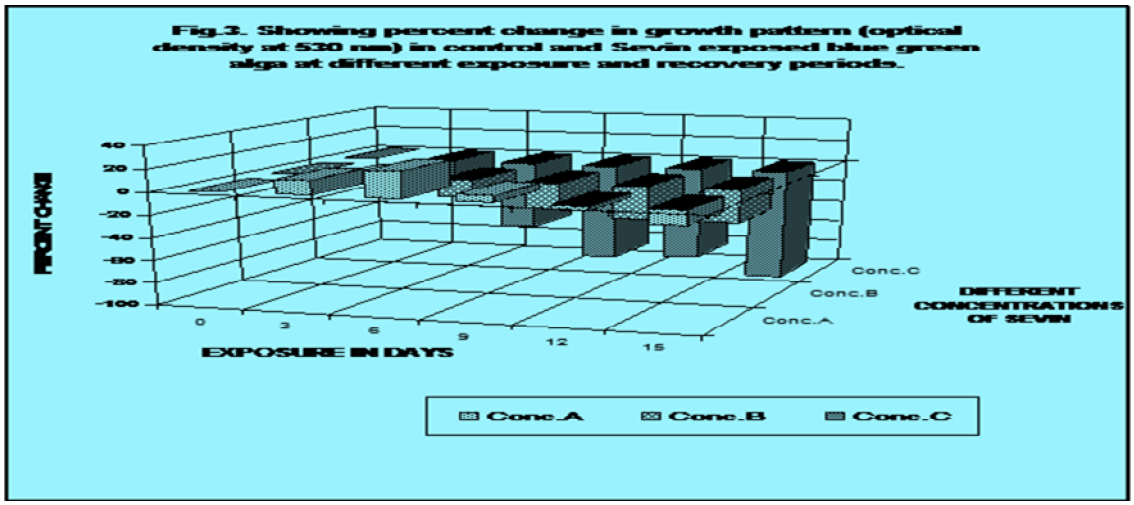

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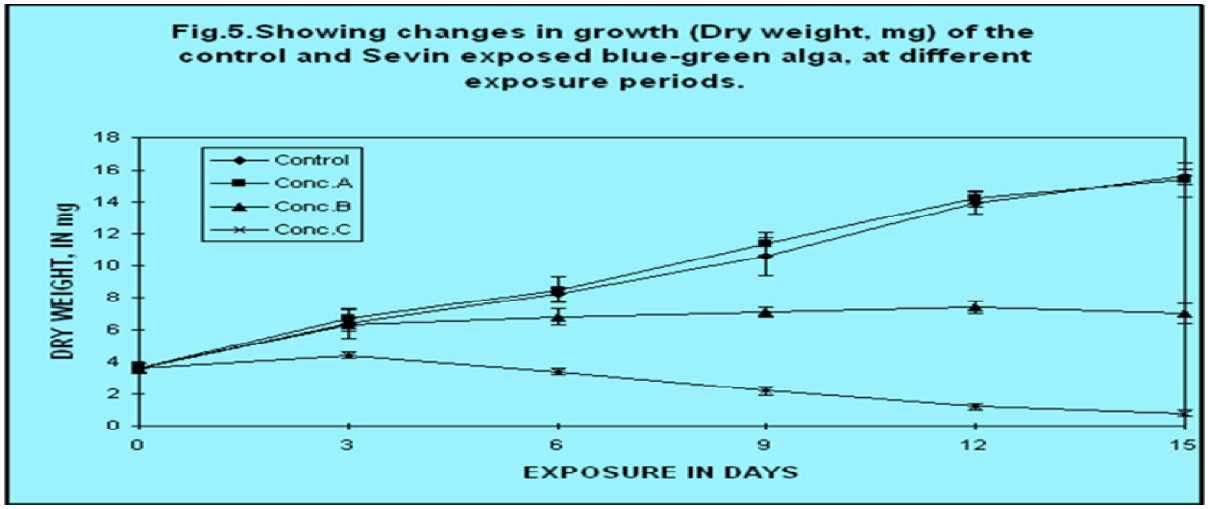

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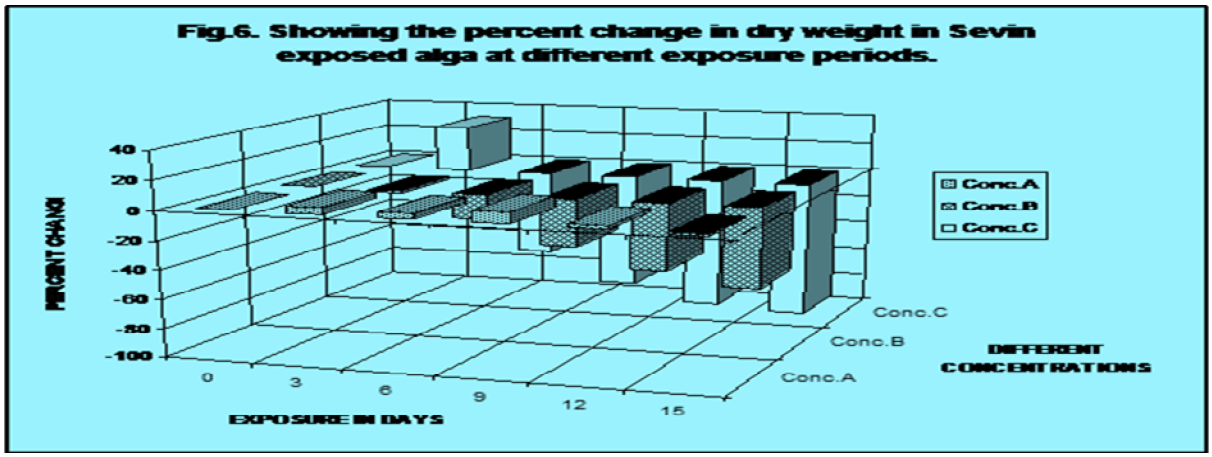

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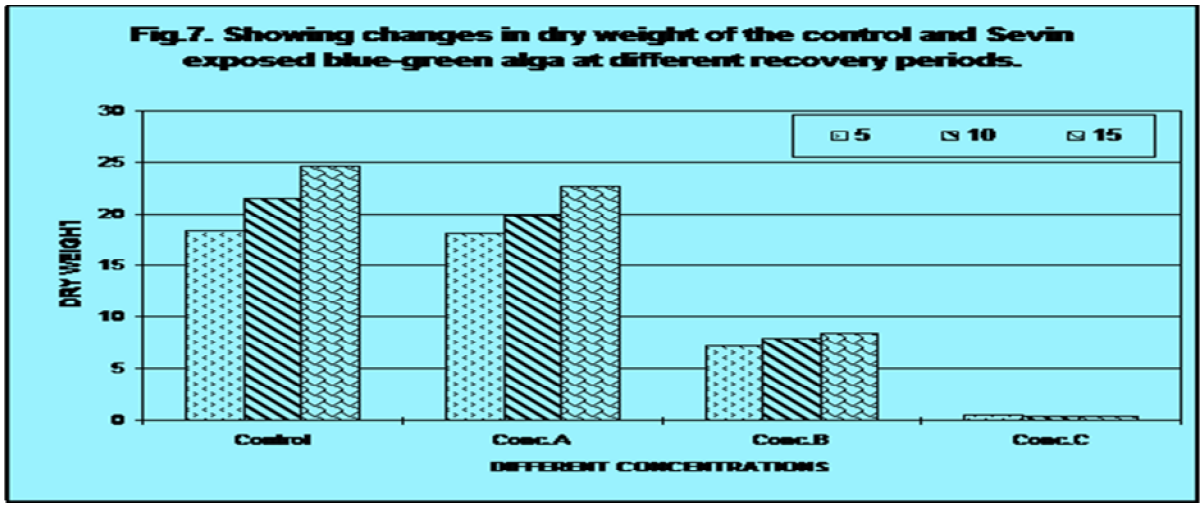

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