IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Eco-physiological Effects Of Sumicidin On The Growth & Pigment Content Of A Blue-Green Alga And Its Toxicological Significance.

Bijay Kumar Nayak* and A. K. Panigrahi,

Environmental Toxicology & Biotechnology Laboratory, Department of Botany & Environmental Science, Berhampur University, BERHAMPUR-760 007, Odisha, India.

*Panposh-769 004, Sundargarh Odisha. India.

Highlights:

- Excessive and careless use of large varieties of pesticides in agriculture in the name of crop protection needs attention.
- The present study was planned to find out the impact of Sumicidin, a contact cum fumigant pesticide on a blue-green alga (BGA) residing in the crop fields.
- The pesticide sprayed in the crop fields, ultimate entry of pesticide into the crop field affecting the survival, growth and pigment content of BGA inhabiting the crop fields.
- The pesticide Sumicidin significantly affected the growth and survival of the alga tested.
- The pigments like- Total chlorophyll, total pheophytin and carotenoid content significantly decreased in Sumicidin exposed alga compared to control alga.
- The exposed alga could not recover during recovery period even after prolonged recovery period indicating permanent damage caused to the exposed system..

Abstract

Sumicidin, the fungicide cum insecticide showed significant difference in action at different concentrations on a blue-green alga, is well evident from the tables and figures described in the result chapter. At higher concentration of the fungicide and higher exposure period, drastic effects on the blue-green alga were observed. At the highest concentration (Conc. Z) of the toxicant, the alga showed typical toxic symptoms, beyond which survival of the alga becomes extremely difficult and at times impossible under laboratory control conditions. In the present investigation, the maximum allowable concentration (MAC) used for this alga for 15 days exposure was 0.31ml l^{-1}. At higher concentration of the fungicide 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, Sumicidin caused permanent damage to the exposed algal system. Hence, it can be safely concluded that the damage caused in exposed system was only due to the toxicant. The total chlorophyll content initially increased from 0.025±0.003 to 0.102±0.016 mg /50 ml culture within 15 days of exposure in the control set and the value increased to 0.134±0.011mg /50 ml culture on 15th day of recovery. The percent decrease increased with the increase in exposure period, where a positive correlation was marked. A maximum of 97.06% decrease was recorded on 15th day of exposure in conc. Z set. The phaeophytin content decreased by 2.6% on 6th day of exposure and than partial recovery up to 12th day of exposure and 3.3% decrease on 15th day of exposure was recorded in conc-X. The phaeophytin content increased by 11.5% on 9th day of exposure and than decreased on 12th day of exposure showing 10.5% increase over control value and with the increase in exposure period, 6.5% decrease on 15th day of exposure was recorded in conc-Y. When the exposed alga was transferred to toxicant free medium, instead of showing any recovery, further significant depletion in the pigment content was recorded and a maximum of 50% decrease was noted when compared to control. At conc-X, the carotenoid content increased at all exposure periods, when compared to

the control value except on 3rd day. A maximum of 4.1% increase over the control value was marked on 12th day of exposure was recorded. In contrast, at conc.-Y, the percent change significantly & linearly decreased showing a maximum of 36.6% decrease on 15th day of exposure and 45.2% decrease on 15th day of recovery. In case of conc-Z, highly significant decrease in the carotenoid level was noted. A maximum of 84.8% decrease was noted on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, 96.9% decrease when compared to control was marked showing the highest damage caused to the exposed system. The pigment ratio value showed an initial increase from 1.39 to 1.89 followed by linear decrease to 1.11 on 15th day of exposure and the ratio value further depleted to 0.73 when the alga was transferred to toxicant free medium during recovery studies. From the data, it was evident that the pigment ratio value can be an indicator of stress in plant systems.

Keywords: Pesticide, Sumicidin, Toxicity, *Anabaena*, Growth, Chlorophyll, Pheophytion, Carotenoid.

Introduction

Modern agriculture with its rapid mechanization and spreading of fertilizes, besides the use of protective treatments such as herbicides, insecticides, and fungicides exerts an ever increasing pressure on the natural environment. Some of these chemicals are non-biodegradable, and hence, persist in the ecosystem. These persistent chemicals can be absorbed and concentrated by the living organisms via any mechanism or pathway. This phenomenon is known as bio-enrichment or bioaccumulation and creates various problems, especially when the pollutants are of high toxic nature with high biological half life time. This is because the level of pollutants in an organism increases with the increase in its distance from he primary producer in the food chain, i.e. with increase in the trophic levels. Thus, as a result of bio-magnification, the organisms placed at higher trophic levels usually accumulate a persistent pollutant in its tissues to a concentration much greater than those present in the surrounding habitat. Industry is the third and the most important source of pollution. Over 75,000 chemicals are in common use to-day and several thousands of new compounds are being added to this figure each year (Miller, 1984). These chemicals are now, an inevitable part of the process of industrialization and mostly they are produced and used by the industry to deliver finished goods. Some of these chemicals, the by-products and the waste discharges of the industry are released inadvertently or accidentally into the environment to such an extent that they really threaten the ecosystem in global scale. The mining operations also contribute significantly to the cause of pollution.

The history of development of pesticides starts from the very introduction of killer chemicals to control the weeds, started by spraying Bordeaux as a control measure of plant disease and subsequently acid - such as sulfuric acid and salts of copper and iron. A good number of chemicals were reported by different authors as pesticides in due course of time. Since then, many of the chemicals with different groups, different combinations, organo complexes were synthesized and tested for their differential actions (Pandey, 1981). Basing on the modes of action, site of action, types of treatment, pesticides were classified under different headings and circulated in the local market under different attractive brand names. Good number of workers reviewed the different possible modes of action of pesticides on target organisms (Ashton & Crafts, 1973). The contamination of soil, water and living organisms with pesticides has been of great concern to scientists, regulatory agencies and also the general public. The fungicide or the pesticide after application gets adhered on the plant surface and during rains all the adhered fungicides or pesticides are washed and the washed pesticide enter into the crop field and become a part of the crop field chemicals. Many a times it was reported that these killer chemicals after entering into crop fields may adhere to the soil particles or may react with the available soil chemicals to form complexes. As these are persistent chemicals, the chemical remains in the same form getting adhered to soil particles. When a pesticide is sprayed, we always keep the target crop plant in mind and the toxicity was conducted on the crop seeds behavior. Never an attempt was made by the pesticide producing company to conduct a toxicity testing for the non-target organisms like heterocystous blue-green alga inhabiting crop fields, fix atmospheric nitrogen and release extra-cellular nitrogenous products into the crop field and acts as a biofertilizer and responsible for the biofertility of the soil. These tiny beautiful organisms ultimately suffer because of excess fertilizer and the applied pesticides and finally disappear from the crop fields. The present piece of work was attempted to study the impact of Sumicidin a very popular pesticide used by the farmers in large quantities, on the growth and photosynthetic efficiency of a blue-green alga inhabiting crop fields.

Materials & Methods:

Toxicant used: Fungicide cum Insecticide: FENVELRATE 20% EC, SUMICIDIN 20E

An excellent new quick-acting photo-stable contact pyrethroid type fungicide /insecticide for the control of pests on cotton and vegetables.

Test organism: ***Anabaena cylindrica***, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family **Nostocaceae**.

Sahu (1987) found that Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) was most suitable for the organism. It was used as the basic culture solution in all the experiments in the present study. The algal cultures were grown under controlled conditions of light and temperature inside a culture room. Light intensity was maintained at 2400± 200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was maintained at 26 ± 2^0C. The culture flasks were regularly hand shaken twice a day to avoid clumping of the cells as well as their adhesion to the wall of the conical flasks. In all the experiments, axenic culture was used.

To study the pigments (Total Chlorophyll, Phaeophytin and Carotenoid) algal material present in different experimental and control flasks were centrifuged, the supernatant was discarded and the algal mass was washed thoroughly with double distilled water twice and centrifuged again. The pigments from the algal mass was extracted in 5 ml of cold 80% acetone with a pre-chilled micro-tissue homogeniser in dark and then kept in a refrigerator overnight. The extract was finally centrifuged to obtain a clear supernatant of the pigment solution. The total chlorophyll was measured (Vernon, 1960) by recording the optical density of the extract at 649 and 665 nm and the amount of carotenoid was measured (Davies, 1976) by recording the optical density of the extract at 475 nm wavelength. The supernatant thus obtained was taken and a pinch of oxalic acid was added. It was shaken thoroughly and was kept in a refrigerator overnight for estimation of phaeophytin (Vernon, 1960), which was measured by recording the optical density of the extract at 655 and 666 nm.

The formulae to calculate total chlorophyll and phaeophytin given by Vernon (1960) and for carotenoid by Davies (1976) are as follows:

Total chlorophyll (mg /g^{-1}dry weight) = 6.45 x (O.D. at 665)+17.72 (O.D. at 649)

Total phaeophytin (mg /g^{-1}dry weight) =6.75 x (O.D. at 666)+26.03 (O.D. at 655)

Carotenoid (mg / g^{-1}dry weight) = D V K x (2500 x 100)

Where D= Optical density at 475 nm, V = Volume of the extract, K = Dilution factor and 2500 = Specific extinction co-efficient at 475 nm. All the obtained values were statistically analyzed.

Results

The experimental chemical pollutant, Sumicidin was prepared by taking the fungicide and diluting with distilled water. A graded series of concentrations of Sumicidin ranging from 0.1ml l^{-1} to 2.0ml l^{-1} (V/V) was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. One ml of unialglal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100 ml of the prepared solution, inside the inoculating chamber.

The total chlorophyll content of the control and Sumicidin exposed alga, at different days of exposure and recovery were presented in Fig.1. The total chlorophyll content increased from 0.025±0.003 to 0.102±0.016mg /50 ml culture within 15 days of exposure in the control set and the value increased to 0.134±0.011mg /50 ml culture on 15^{th} day of recovery. The total chlorophyll content increased significantly ($p \geq 0.05$) in the exposed set initially (Conc.-X), the value increased from 0.025±0.003 to 0.106±0.01mg /50 ml culture on 15^{th} day of exposure. A maximum of 7.3% increase was recorded on 12^{th} day and on 15^{th} day the percent increase decreased but the value was 3.9% increase over the control value (Fig. 6) in conc. X set. When the exposed alga was transferred to fungicide free medium, the chlorophyll content increased from 0.106±0.01mg /50 ml culture to 0.130±0.008mg /50 ml culture after 15 days of recovery in concentration-X set showing good recovery (Fig. 1). The total chlorophyll content increased from 0.025±0.003 to 0.102±0.016mg /50 ml culture within 15 days of exposure in the control set and the value increased to 0.134±0.011mg /50 ml culture on 15^{th} day of recovery. The total chlorophyll content increased insignificantly ($p\geq0.05$) in the exposed set (Conc.-Y), the value increased from 0.025±0.003 to 0.066±0.009 mg /50 ml culture on 15^{th} day of exposure. A maximum of 25.6% decrease was recorded on 12^{th} day and on 15^{th} day 35.3% decrease over the control value was marked (Fig.1) in conc. Y set. When the exposed alga was transferred to fungicide free medium the chlorophyll content increased from 0.066±0.009mg /50 ml culture to 0.085±0.005mg /50 ml culture after 15 days of recovery (Fig.2). The total chlorophyll content decreased significantly ($p \geq 0.05$) in the exposed set (Conc.-Z), the value decreased from 0.025±0.003 to 0.003±0.001mg /50 ml culture on 15^{th} day of exposure.

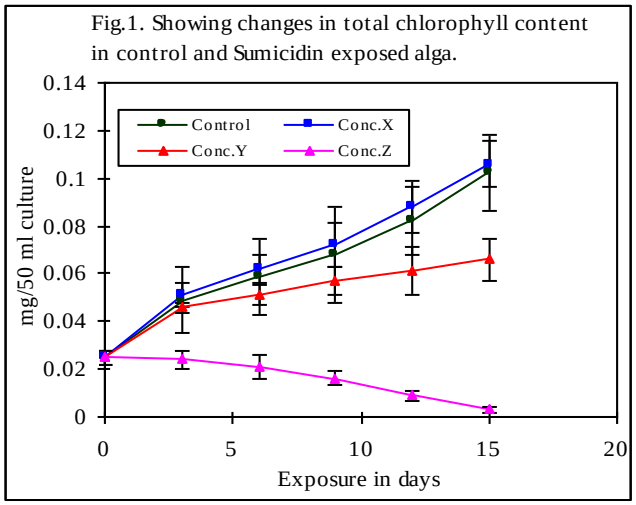


Fig.1. Showing changes in total chlorophyll content in control and Sumicidin exposed alga.

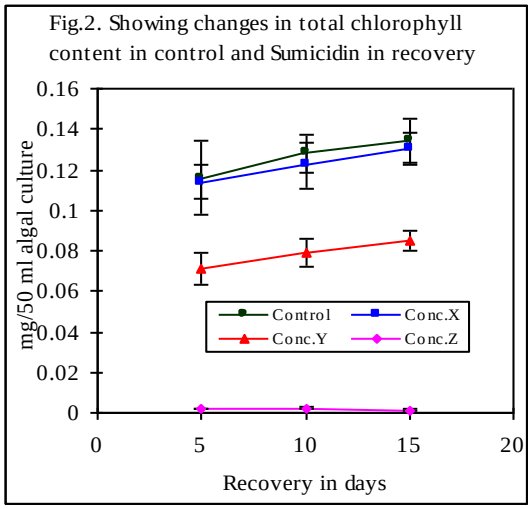


Fig.2. Showing changes in total chlorophyll content in control and Sumicidin in recovery

A maximum of 89% decrease was recorded on 12th day and on 15th day 97.1% decrease over the control value was marked (Fig.2) in conc. Z set. When the exposed alga was transferred to fungicide free medium the chlorophyll content further decreased from 0.003±0.001mg /50 ml culture to 0.001±0.0005mg /50 ml culture after 15 days of recovery (Fig.1, 2 & 3). Conc. X set showed an increase in total chlorophyll content at all exposure periods (Fig.3). In case of conc. Y, the total chlorophyll content though increased on 3rd day onwards, when compared to 0 day value but the chlorophyll amount was far less than control and conc. X and a maximum of 35.3% decrease was recorded, when compared to the control value. The percent decrease increased with the increase in exposure period, where a positive correlation was marked. A maximum of 97.06% decrease was recorded on 15th day of exposure in conc. Z (1.21ml of Sumicidin l^{-1}) set (Fig.3). The correlation coefficient analysis between days of exposure verses total chlorophyll indicated the existence of significant positive correlation in control ($r = 0.993$, $p \geq 0.001$) and Conc. X ($r = 0.991$, $p \geq 0.001$). In case of Conc. Y, a positive correlation was marked between the chlorophyll content and days of exposure ($r = 0.918$, $p \geq 0.01$). A negative correlation ($r = -0.806$, $p \geq 0.05$) was marked in Conc. Z. The two way analysis of variance ratio test based on the data indicated the existence of significant difference between rows and significant difference between columns. The total chlorophyll present in control, exposed and recovered alga was clearly indicated in Fig. 1. The change observed in total chlorophyll content at different days of exposure and at different concentrations was almost same with dry weight change trend. The correlation coefficient value of total chlorophyll with days of exposure was significant at $p \geq 0.001$ levels for control and "X" conc. of the toxicant, i.e. the total chlorophyll amount increased significantly with the increase in days of exposure at control, and "X" conc., whereas at "Z" conc. it was not significant. With the increase in concentration, the total chlorophyll content decreased on all days of exposure but on 6th, 12th and 15th day the values were significant at $p \geq 0.05$ levels. The percent change value of total chlorophyll increased with the increase in days of exposure at "X" conc. but the values were not statistically significant, whereas this value decreased with days of exposure in "Y" conc and significantly decreased in "Z" conc. With the increase in concentration the percent change value of total chlorophyll showed gradual decrease on all days of exposure which was statistically significant at $p \geq 0.001$ levels except on 3rd day where it was significant at $P = 0.01$ level. In recovery studies, it was clearly observed that no recovery was marked in conc. X, Y and Z set. The chlorophyll content was drastically affected in Sumicidin exposed alga.

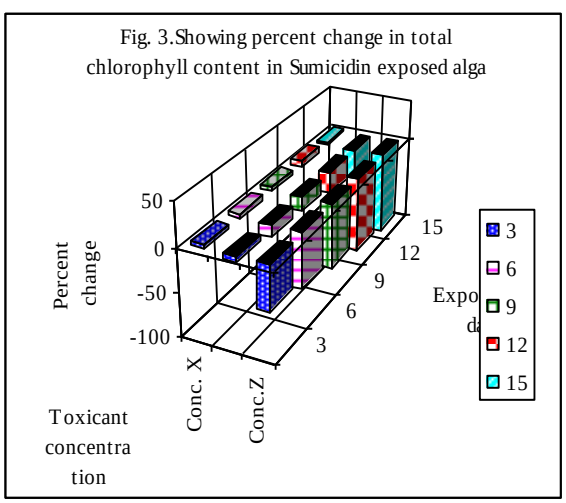


Fig. 3.Showing percent change in total chlorophyll content in Sumicidin exposed alga

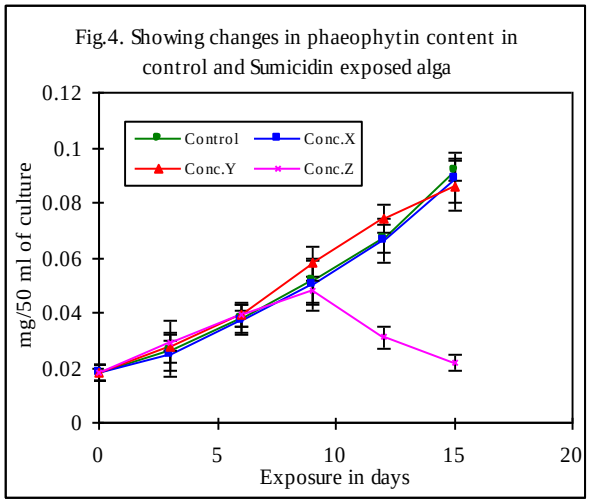


Fig.4. Showing changes in phaeophytin content in control and Sumicidin exposed alga

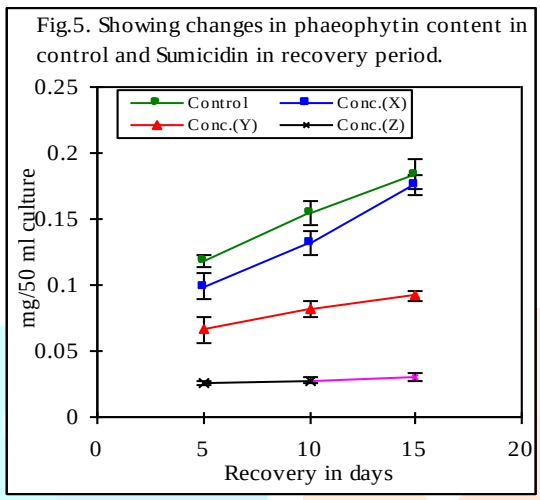


Fig.5. Showing changes in phaeophytin content in control and Sumicidin in recovery period.

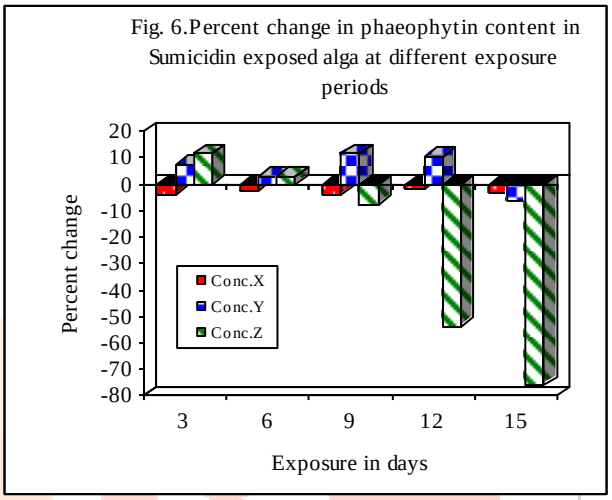


Fig. 6.Percent change in phaeophytin content in Sumicidin exposed alga at different exposure periods

Fig.4 indicated the changes in total phaeophytin content in control and Sumicidin exposed blue-green alga at different exposure and recovery periods. The total phaeophytin content increased from 0.018±0.003 to 0.092±0.004mg /50 ml culture in a period of 15 days. The value further increased from 0.092±0.004 to 0.184±0.011mg /50 ml culture in 15 days of recovery. The phaeophytin content interestingly declined from control value at all exposure and recovery periods in conc-X. No doubt, the phaeophytin content increased from 0.018± 0.003 to 0.089±0.009mg /50 ml culture on 15th day of exposure and the same value increased from 0.089±0.009 to 0.176±0.008mg/50 ml culture, showing a positive correlation after 15 days of recovery ($p \geq 0.01$), with the increase in recovery period, like the control set but the values were interestingly less than the respective control value for each exposure and recovery period (Fig.8). In conc-'Y', the phaeophytin content increased from 0.018±0.003 to 0.074±0.005mg/50 ml culture on 12th day of exposure showing more than the control and conc. X set and than the value significantly declined with the increase in exposure period. The value declined from 0.074±0.005 to 0.086±0.009mg /50 ml culture on 15th day of exposure. When the exposed alga of conc. 'Y' was transferred to Sumicidin free nutrient medium, no recovery was altogether marked. Rather the values further depleted to 0.066± 0.01mg /50 ml culture, on 5th day of recovery, indicating total damage and destruction of the pigment (Fig.5). At higher recovery periods, the phaeophytin content increased from 0.066± 0.01 to 0.092±0.004mg /50ml culture. In conc. 'Z', the phaeophytin content increased from 0.018±0.003 to 0.048±0.005mg/50 ml culture on 9th day of exposure showing less than the control and conc. X and Y sets and than the value significantly declined with the increase in exposure period. The value declined from 0.048±0.005 to 0.022±0.003mg/50 ml culture on 15th day of exposure. When the exposed alga of conc. 'Z' was transferred to toxicant free nutrient medium, partial insignificant recovery was marked. The values increased to 0.031± 0.003 mg/50 ml culture, on 15th day of recovery, indicating total damage and destruction of the pigment. Fig.6indicated the percent change in phaeophytin content in the exposed alga at different exposure periods when compared to the control set. The phaeophytin content decreased by 2.6% on 6th day of exposure and than partial recovery up to 12th day of exposure and 3.3% decrease on 15th day of exposure was recorded in conc. X (Fig.6). The phaeophytin content increased by 11.5% on 9th day of exposure and than decreased on 12th day of exposure showing 10.5% increase over control value and with the increase in exposure period, 6.5% decrease on 15th day of exposure was recorded in conc.-Y. When the exposed alga was transferred to toxicant free medium, instead of showing any recovery, further significant depletion in the pigment content was recorded and a maximum

of 50% decrease was noted when compared to control. In conc.- Z, 11.5% increase over the control value was recorded on 3rd day of exposure and the phaeophytin content significantly declined with the increase in exposure period and a maximum of 76.09% decrease was recorded on 15th day of exposure. No significant recovery was recorded in conc.-Y set and no recovery was marked in conc.-Z set, when the exposed alga was transferred to toxicant free nutrient medium, rather higher depletion was noted (Fig.6). The correlation coefficient analysis between phaeophytin content and days of exposure indicated the existence of positive and significant ($p \geq 0.001$) correlation in control ($r = 0.986$); Conc. X ($r = 0.984$) and in conc. Y ($r=0.991$). But in case of Conc. Z a non-significant correlation was marked ($r = -0.924$, $p = NS$). The ANOVA test indicated the existence of non-significant difference between rows and non-significant difference between columns.

Changes in carotenoid content of control and Sumicidin exposed blue-green alga was demonstrated in Fig.7, at different exposure and recovery periods. The carotenoid content of the control alga increased from 0.0024±0.0009 to 0.0112±0.0014mg /50ml culture within a period of 15 days and the value further increased to 0.0166±0.0013 mg/50 ml culture at 15 days of recovery. The carotenoid content significantly increased at conc.-X at all exposure and recovery periods. The carotenoid content increased from 0.0024±0.0009 to 0.0116± 0.0011mg /50 ml culture within 15 days of exposure and the value increased from 0.0116± 0.0011 to 0.0174±0.0014mg /50 ml culture on 15th day of recovery, when the exposed alga was transferred to toxicant free medium (Fig. 8). The carotenoid content increased from 0.0024±0.0009 to 0.0071±0.0009mg/50 ml culture within 15 days of exposure and the value increased from 0.0071±0.0009 to 0.0091±0.0008mg/50 ml culture on 15th day of recovery, when the exposed alga was transferred to toxicant free medium (Fig. 8). Where as, in conc.-Z, the carotenoid content increased insignificantly up to 6th day of exposure and then decreased linearly with the increase in exposure period, showing a significant negative correlation.

Fig.7. Showing changes in carotenoid content in control and sumicidin exposed alga.

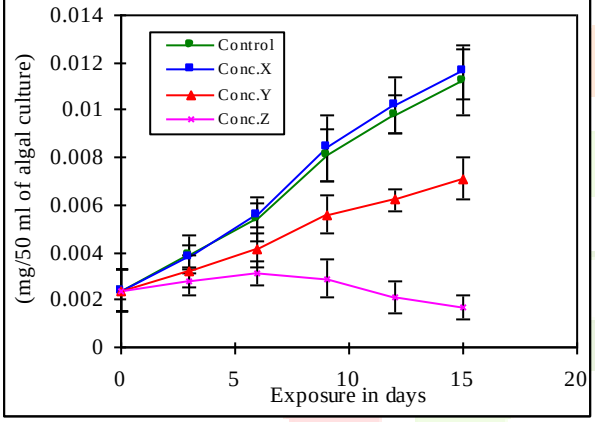


Fig. 8. Showing changes in carotenoid content in control and sumicidin exposed recovery alga.

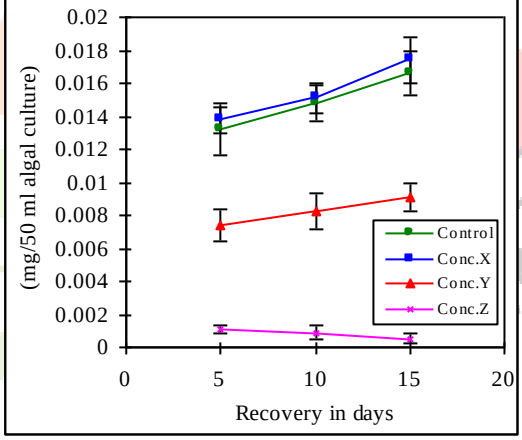


Fig.9. Percent change in carotenoid content in sumicidin exposed alga

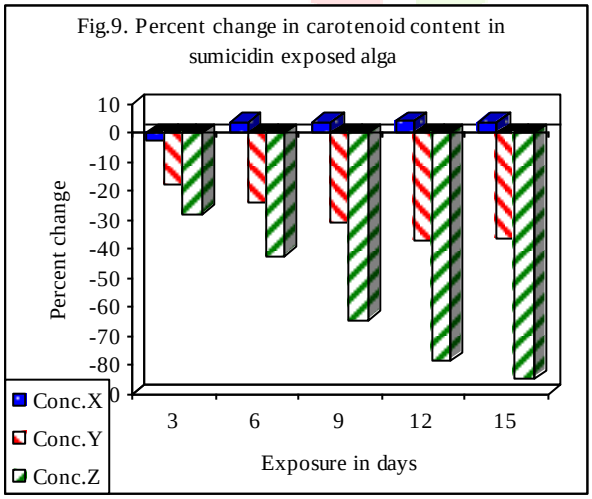
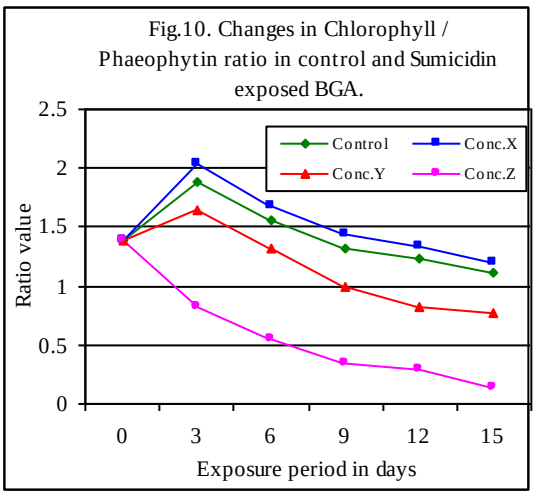


Fig.10. Changes in Chlorophyll / Phaeophytin ratio in control and Sumicidin exposed BGA.



The carotenoid content depleted from 0.0031±0.0005 to 0.0017±0.0005mg /50 ml culture on 15th day of exposure. When the exposed alga was transferred to toxicant free medium, instead of showing any recovery, the value further depleted to 0.0005 ± 0.0003 mg /50 ml culture on 15th day of recovery (Fig.8). Fig.9 shows the percent change in carotenoid content, where, dichotomous behavior of the toxicant was clearly evinced. At conc.-X, the carotenoid content increased at all exposure periods, when compared to the control value except on 3rd day. A maximum of 4.1% increase over the control value was seen on 12th day of exposure and

3.6% increase was recorded on 15th day of exposure. In contrast, at conc.-Y, the percent change significantly & linearly decreased showing a maximum of 36.6% decrease on 15th day of exposure and 45.2% decrease on 15th day of recovery (Fig.9). Insignificant partial recovery was recorded in concentration 'X' and no recovery was marked in concentration 'Y', when the exposed alga was transferred to toxicant free, nutrient medium. In case of concentration-Z, highly significant decrease in the carotenoid level was noted. A maximum of 84.8% decrease was noted on 15th day of exposure and when the exposed alga was transferred to sumicidin free medium, 96.9% decrease when compared to control was marked showing the highest damage caused to the exposed system (Fig.9). The correlation coefficient analysis between carotenoid content of control and exposed alga versus days of exposure indicated the existence of a positive correlation in the control set ($r = 0.991$, $p \geq 0.01$) and the value was significant. In Conc. X positive correlation ($r = 0.994$, $p \geq 0.001$) was marked. In conc.-Y, positive correlation ($r= 0.889$, $p \geq 0.05$) was noted and in conc.-Z, a non-significant correlation was marked. The two way of analysis of variance ratio test indicated the existence of significant difference between rows and columns. The pigment ratio value showed an initial increase from 1.39 to 1.89 followed by linear decrease to 1.11 on 15th day of exposure and the ratio value further depleted to 0.73 when the alga was transferred to Sumicidin free medium during recovery studies. In conc. X, the ratio value was significantly high when compared to control value at all exposure periods and recovery periods. Interestingly, the ratio value decreased in conc. Y at all exposure and recovery periods when compared to control and concentration-X. In case of conc-Z, further depletion of pigment ratio value was marked at all exposure and recovery periods. From the data, it was evident that the ratio value can be an indicator of stress in plant systems.

Discussion

The use of toxic chemicals as killer chemicals together with fertilizers and improved hybrid crop varieties has a greater contribution to higher yield in agriculture. With the fast growing population of the world, a drastic increase in food production is the immediate need, where the use of pesticides to check the destruction of food seems to play an important role. However, the extensive use of pesticides created a wide range dilemma pertaining to its mode of action and behavior pattern on all other biosystems, present in the exposed/contaminated ecosystem. It was reported that the wide use of certain pesticides has more serious and permanent drastic effect on microorganisms. It is a well established fact that nitrogen-fixing organisms, particularly blue-green algae, are known to play a key role in increasing soil fertility, especially in paddy fields under water logged condition (Singh, 1961 and Patnaik, 1966). Thus, the pesticides which enter into the paddy field might be affecting the growth and nitrogen fixing capacity of the blue-green algal systems. The universal use of pesticides in agriculture creates a necessity to study the effects of these chemicals on soil micro-organisms. There are very few reports on the tolerance of blue-green algae towards aldrin, dieldrin, endrin and other metabolites (Venkataraman & Rajyalakshmi, 1972). However, it was reported that 2,4-D levels equivalent to field application rates (i.e. 1.1×10^{-2} M) prevented nitrogen-fixation by BGA *Nostoc punctiformii*, *Nostoc muscorum* and *Cylindrospermum* sp. In contrast, it was observed that nitrogen fixing capacity and growth of *Anabaenopsis raciborskii* were almost unaffected at 100 mg/ml of 2,4-D. Diuron was also known to suppress the growth of nitrogen fixing algae, *Tolypothrix tenuis* and *Aulosira fertilissima* (Venkataraman & Rajyalakshmi, 1971). Even at 1mg l^{-1} of Diuron the growth of both green and blue-green algae was inhibited. It was reported that *Anabaena cylindrica* was more sensitive to higher doses of phenyl carbamate under nitrogen-fixing conditions while its lower doses were stimulative to algal growth. It was reported that the pigment extract of *Tolypothrix tonuis* and found a depleted level of phycoerythrin and cholorophyll-a, with the relative increase in phycocyanin in the presence of chloro-prophan. Similar changes in pigmentation have also been observed in *Anacystis nidulans*. Photosynthetic pigments are known to participate in generation of energy and CO_2 fixation (Kashyap & Gupta, 1981). The chlorophylls have long been recognized as the primary light acceptors, a small portion of which acts as the primary reaction centre where light conversion occurs. Carotenoids, not only help in photosynthesis, by transferring light energy but also protect the other photosynthetic pigments, preventing photo-oxidation and providing light shielding (Krinsky, 1966). Decrease in the level of chlorophyll in algae and other plants exposed to different toxicants have been reported. Geike (1977) reported decrease in the chlorophyll level in algae exposed in mercury. De Filippis and Pallaghy (1976 a) observed reduction in the chlorophyll content in *Chlorella*, treated with zinc and mercury. Rai *et al*. (1981 a) reported a reduction in chlorophyll content of *Chlorella vulgaris*, when exposed to $HgCl_2$ between 100-1000µg/lt concentration, for 3 weeks. De *et al*. (1985) suggested that 20 mg/lt concentration of $HgCl_2$ decreased chlorophyll content of *Pistia stratiotes*, when exposed for 2 days. The decrease in chlorophyll level was a result of increase in the chlorophyllase activity. Decrease in pigment content of algae, cultured in solid waste from a chlor-alkali industry, with a crop plant, with the increase in concentration of the waste soil and exposure period which was also significantly correlated with mercury

uptake by algae. The wide spread occurrence, as well as certain chemical properties of chlorophyll pigments *in vivo* suggested that these pigments play an active role in photosynthesis functioning as photo-enzymes (Rabinowitch & Govindjee, 1973) and the mercurial compounds were toxic for the biosynthesis of chlorophyll pigments. Results obtained here are peculiar. In toxicological studies, involving algae, estimation of phaeophytin content serves as an important tool, since any unfavorable change in the environment or the effect of the toxicant is reflected by the change in its level. Chlorophylls are known to be converted to pheophytins as a consequence of exposure to weak acids by replacement of Mg^{2+} with two atoms of hydrogen and thereby changing the spectral properties (Singh & Singh, 1984). Degradation to phaeophytin might be the first step towards the breakdown of chlorophyll, which was evident from the increased levels of phaeophytin in the treated cultures. In this investigation at higher concentrations of the toxicants phaeophytin content increased confirming the above presumption. Carotenoids play a vital role as a protector of photosynthetic tissues against photosensitised oxidation. The decrease in carotenoid content in algal cells exposed to heavy metal stress lead to a decrease in protection from the stress to the photosynthetic tissue. The ratio of chlorophyll to carotenoid has long been identified as a valuable parameter for defining environmental conditions unfavorable for algal growth. When the nutrient in the medium are exhausted or a toxicant was introduced into the medium the ratio increased due to decrease in the chlorophyll content (Rai *et al*., 1981 b). Increased ratio indicated inhibition of chlorophyll biosynthesis, inactivation of enzyme systems and disruption of many physiological and biochemical processes (Sorentino, 1979). Rath (1984), Sahu (1987) and Shaw (1987) indicated stimulation of growth, increase in pigment content, photosynthesis rate, respiration rate, and enzyme activity at lower concentrations of mercurial compounds on *W. prolifica*, Janet. Mercury at relatively low concentrations also affects the energy transfer by selectively affecting the phycocyanin in the phycobilisomes of intact cells of *Spirulina*, which was reported by Murty and Mohanty (1991). Murty and Mohanty (1991) reported that mercury at a low concentration (3 µm) caused an enhancement in the intensity of room temperature fluorescence, emitted by phycocyanin and induced a blue-shift in the emission peak of *Spirulina* cells indicating the alterations in the energy transfer within the phycobilisomes, whereas this phenomenon was not seen in *Anacystis*, *in vitro*. It is a common place observation that toxicity of metals showed great variations under field and laboratory conditions (Whitton, 1970). A given concentration of a metal may be more toxic to algae in the field than under laboratory conditions and vice-versa (Rai *et al*., 1981 b). Hence, it becomes explicit that laboratory based information cannot solely be used to stimulate field conditions, because many environmental and nutritional factors operate to bring about metal toxicity in field conditions (Gadd & Griffiths, 1978). By measuring the photosynthetic rate, the growth can be computed and predicted indirectly as growth and photosynthesis are two intimately related terms. Eley *et al*. (1983) suggested that inhibition of photosynthesis might be responsible for growth retardation. Photosynthetic inhibition is mainly due to disturbances in the light energy trapping mechanism. It was reported that the inhibition of photosystem II by oxidation of cytochrome f in the electron transport was caused by $HgCl_2$ in isolated chloroplast. The inhibition of photosynthesis and respiration in plant systems by mercurial compounds was reported by Sahu *et al*. (1988). Gould (1975) reported that mercury influences the photosynthetic capacity of the algae by inhibiting the electron inhibitor. There were also a few reports regarding the stimulating nature of some toxicants at lower concentrations reported earlier. From the observed data it is very clear that the used pesticide is toxic. The pesticide should be used carefully in the field to save the lives of these tiny organisms inhabiting the crop fields.

Acknowledgement

The authors wish to thank the Head, Department of Botany and authorities of Berhampur University, Berhampur, Odisha, India for providing the laboratory and library facilities.

Author Contribution statement

Prof. A.K. Panigrahi: Conceptualization, supervision, script preparation, reviewing and editing. B. K. Nayak-Experiment plans and execution of the project, field visit, original draft preparation, supervision and editing.

Funding statement: Authors have not received any fund from any source. All the expenses were borne by Sri Nayak, Research Fellow.

Conflict of interest statement: The authors declare that they have no conflicts of interest.

References

Allen, M. B. and Arnon, D. I. (1955): Studies on nitrogen fixing blue-green algae, growth and nitrogen fixation by *Anabaena cylindrica*,Lemm. Pl. Physiol., Lancaster.,30, 366-372.

Ashton, F.M. and A.S. Crafts (1973): "Modes of action of Herbicides", John Wiley & Sons, New York.

Cairns, J. Jr.; K. L. Dickson and A. W. Maki (eds.) (1978): Estimating the hazard of chemical substances to aquatic life. ASTM, Philadelphia.

Davies, B. H. (1976): In: Chemistry and Biochemistry of plant Pigments. Vol. 2 (Ed. T. W. Goodwin), Academic Press Inc. London p. 38.

De, A. K., A. K. Sen, D. P. Modak and S. Jana (1985): Studies of toxic effects of Hg (II) on *Pistia stratiotes*. Water, Air & Soil Pollution, 24:351.

De Filippis, L. F. and C. K. Pallaghy (1976): The effects of sub-lethal concentrations of mercury and zinc on *Chlorella*. 1. Growth characteristics and uptake of metals. Z. Pflanzen-physiol. Bd., 78:197-207.

Eley, J. H., J. F. Mc Connell and R. H. Catlett (1983): Inhibition of metribuzin on growth and Photosynthesis of the blue-green alga *Anacystis nidulans*. Environ. Exp. Bot., 23:365-368.

Fogg, G. E. (1949): Growth and heterocyst production in *Anabaena cylindrica*, Lemm. II. in relation to carbon and nitrogen metabolism. Ann. Bot. N. S., 13:241-259.

Gadd, G. M. and A. J. Griffith (1978): Microorganism and metal toxicity. Microbial Ecol., 4:303-317.

Geike (1977) Geike, F. (1977): Effect of some mercury compounds on freshwater algae. J. Plant. Diseases & Protection, 84, 84-94.

Gould, J. M. (1975): Inhibition of photosystem II-dependent phosphorylation in chloroplasts by mercurials. Biochem. Biophys. Res. Commun., 64:673-680.

Kashyap, A. K. and S. L. Gupta (1981): Effects of dichlone, macromolecular synthesis and photosynthetic pigments in blue-green algae. Acta Bota. Indica, 9:265-271.

Krinsky, N. I. (1966): In: "Biochemistry of Chloroplasts" (T. W. Goodwin) (ed.). Academic Press, London and New York, 1, 423-430.

Miller, D.R. (1984): Chemical in the Environment. In: Effects of Pollutants at the Ecosystem level. Sheehan, P. J., Miller, D. R., Butler, G. C. and Bourdeau, P., Eds., John Wiley and Sons, Chichester, p.7.

Murthy, S. D. S. and P. Mohanty (1991): Plant cell Physiol., 32(2):231.

Pandey, A. K. (1981): Heavy metal toxicity in the blue-green algae, *Nostoc calcicola.* Ph. D. Thesis, Banaras Hindu Univ., Varanasi, India.

Pattnaik, H. (1964): Studies on nitrogen fixation by *Westiellopsis prolifica*, Janet, Ph. D. Thesis, University of London, UK.

Rabinowitch, E. and Govindjee (1973): "Photosynthesis", Wiley Eastern Pvt. Ltd., New Delhi.

Rai, L. C.; J. P. Gaur and H. D. Kumar (1981, a): Phycology and heavy metal pollution. Biol. Rev. 56:99-151

Rai, L. C.; J. P. Gaur and H. D. Kumar (1981, b): Protective effects of certain environmental factors on the toxicity of zinc, mercury and methyl mercury to *Chlorella vulgaris.* Environmental Research, 25: 250-259.

Rath, P. (1984): Toxicological effects of pesticides on a blue-green algae, *Westiellopsis prolifica* Janet. Ph. D. thesis, Berhampur University, India.

Sahu, A. (1987): Toxicological effects of a pesticide on a blue-green alga : III. Effect of PMA on a blue-green alga, *Westiellopsis prolifica* Janet. and its ecological implications. Ph. D. Thesis, Berhampur University, India.

Sahu, A.; B. P. Shaw; A. K. Panigrahi and B. N. Misra (1988): Effects of phenyl mercuric acetate on oxygen evolution in a nitrogen fixing blue-green alga, *Westiellopsis prolifica*, Janet. Microb. Lett., 37, 119-123.

Shaw, B. P. (1987): Eco-physiological studies of industrial effluent of a chlor-alkali factory on biosystems. Ph. D. thesis, Berhampur University, Orissa, India.

Singh, R.N. (1961): Role of blue-green algae in nitrogen economy of Indian agriculture. ICAR, New Delhi.

Singh, P.K. (1974): Aligicidal effects of 2,4-Dichlorophenoxy acetic acid on blue-green alga, *Cylindrospermum* sp. Arch. Microbiol.,97,69-72.

Singh, S. P. and R. P. Singh (1984): Bisulphite induced pigment damage in the blue-green algae, *Nostoc calicicola*, Breb.New Phycol., 98:277-283.

Sorentino, C. (1979): The effect of heavy metals on phytoplankton. A review. Phykos, 18: 149-161.

Venkataraman, G. S. and Rajyalakhmi, B. (1972): Relative tolerance of nitrogen fixing blue-green algae to pesticides. Ind. J. Agric. Sci 42 : 119-121

Vernon (1960) Vernon, L. P. (1960): Spectrophotometric determination of chlorophylls and phaeophytins in plant extracts. Anal. Chem., 32:1144-1150.

Whitton, B. A. (1970): Toxicity of heavy metal to fresh water algae: A review. Phykos, 9:116-125.