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Toxicity Of Agricultural Pesticide On The Growth Performance of a Blue-Green Alga And Its Ecological Implications.

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Highlights:

- Excessive use of varieties of pesticides in agriculture in the name of crop protection warrants attention.
- Because of illiterate farmers and mass fumigation adopted by farmers led to excessive deposition of pesticides in the crop field. No doubt the crop is protected but the tiny organisms inhabiting in the crop field suffer the most.
- 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 fixing atmospheric nitrogen fixation.
- The pesticide is sprayed at a very high dose (recommended by the company) which severely affects the survival and growth of BGA.

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. The analysis of variance ratio tests and correlation coefficient analysis carried out for all parameters studied indicated clearly that the fungicide cum insecticide, Sumicidin is extremely toxic to blue-green alga. In the present investigation, a graded series of concentrations of the fungicide, Sumicidin was prepared to evaluate the toxic effects of the Sumicidin on the blue-green alga. With the increase in the concentration of the toxicant the percent survival decreased and hundred percent death was noticed at 1.87 $mg.l^{-1}$ of Sumicidin with in a period of 15 days. The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.31ml l^{-1}. The optical density study and the dry weight analysis indicated that the toxicant Sumicidin is deadly toxic. Sumicidin did not show any dichotomous behavior. Complete bleaching of the algal mass inside the test solution was observed. 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 present work was designed to study the effects of the fungicide, Sumicidin, on growth of the blue-green alga, *Anabaena cylindrica*, Lemm.

Keywords: Pesticide, Sumicidin, Toxicity, *Anabaena*, Dry weight, Growth

Introduction

Pollution is intimately related to human activity it stems from and gets augmented by human activities. Pollution is caused due to deliberate or accidental or inadvertent contamination of the environment with man's action and man's waste. Very often the classification based on the origin of the pollutants is generally used, which categorizes the pollution sources into domestic, agricultural, industrial and mining. 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 bioenrichment or bioaccumulation and creates various problems, especially when the pollutants are of high toxic nature with high biological half life time. Pesticides or agro-chemicals are chemicals designed to combat the attacks of various pests on agricultural and horticultural crops. Pesticides may also be divided into two main types, namely contact or non-systemic pesticides and systemic pesticides. Contact or surface pesticides do not appreciably penetrate plant tissues and are consequently not transported, or translocated, within the plant vascular system. The danger of the fungicide causing damage to the host plant is especially formidable in the case of systemic fungicides, since here both host and pest are plants and for success the chemical must show selective toxicity to the fungus. The severity of these problems is reflected in the long time, which elapsed before commercial systemic fungicides appeared on the market. The discovery of several thousands synthetic chemicals and its marketing new style with new slogan forced the biological based killer chemicals to disappear from the market. These newly discovered synthetic chemical are sold in the market in different brand names. The developing countries like India became the dumping ground for these rejected/banned chemicals. Keeping in view; the extensive, non ending use of different types of fungicides and other pesticides in the crop fields for protection of crop and its yield, a huge amount of these pesticides accumulate in the crop fields. The present piece of work was attempted to study the impact of a fungicide/pesticide used by the farmers in large quantities on the toxicity study & effect on the growth on a blue-green alga inhabiting crop fields and acting as a bio-fertilizer fixing atmospheric nitrogen and increasing the fertility of the crop field soil.

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**. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. 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.

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. The culture flasks were kept in series on a culture rack, of glass plate with iron frame. Light was provided by means of white fluorescent tubes, connected at the backside of glass plate of each rack, which illuminates the upper glass surface at the intensity of 2400± 200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and 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. 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 number of individual cells of the algae present in one ml of the culture medium after micro-tissue homogenization was counted under the microscope. Growth measurement was done by recording the dry wt and optical density of the alga per 100 ml culture. Exponentially growing algal suspension of same age, volume and biomass was inoculated initially into the experimental flasks. The optical density was measured by light scattering technique. Optical density (OD) measurement was carried out by withdrawing the culture under aseptic conditions on every 5th day and homogenizing it with micro-tissue homogenizer, and then noting observations in a UV-Visible spectrophotometer (Systronics, PC based, 119) at 530 nm (wave length). The data were expressed as mean of five samples $\pm$ standard deviation. Dry

weight of the alga in the culture flasks was estimated centrifuging in a refrigerated centrifuge (High speed centrifuge, Remi) at 8000 rpm for 10 minutes. The algal pellet was transferred to a pre-weighed glass cover slip. It was dried in an oven at 60^0C for 24 hours, cooled in a desiccator and the final weight of the glass cover slip was recorded in a single pan electric balance (Dhona). The data were expressed as mean of 5 samples ± standard deviation in mg / 100 ml culture. The obtained data was statistically analyzed to test the level of significance.

Results

In the present investigation, a graded series of concentrations of the Sumicidin was prepared ranging from 0.1 to 2.0ml l^{-1} (micro-range), to evaluate the toxic effects of the fungicide, Sumicidin on the blue-green alga and to find out maximum allowable concentration of fungicide for the experimental purposes. Below mentioned table (AB) explains the toxicity of the fungicide, Sumicidin to the blue-green alga, *Anabaena cylindrica*. With the increase in the concentration of the toxicant the percent survival decreased and hundred percent deaths were noticed at 1.87 ml.l^{-1} of Sumicidin with in a period of 15 days. With the increase in exposure period the concentration of the fungicide decreased in the toxicity testing at a particular lethal concentration value. The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.31ml l^{-1}. The lethal concentration values for 15 days of exposure periods have been outlined below. The LC_{10} value was 0.45 ml l^{-1}. The LC_{50} value was 0.73 ml l^{-1}. LC_{90} value was 1.21 ml l^{-1} and LC_{100} value was 1.87 ml l^{-1}, for this particular alga, *Anabaena cylindrica.*

TABLE-AB: Determination of lethal concentration values and percent survival values of Sumicidin on a blue-green alga from toxicity test. (Obtained values were subjected to statistical test and from the slope of the regression value ($r= 0.945$; $P \geq 0.01$) the LC values were selected from the statistical toxicity test values).

Lethal concentration percentage (LC)	Concentration of the fungicide, in ml l^{-1}.	Percent survival (PS)
LC_{00} (X)	0.31	PS_{100}
LC_{10}	0.45	PS_{90}
LC_{50} (Y)	0.73	PS_{50}
LC_{90} (Z)	1.21	PS_{10}
LC_{100}	1.87	PS_{00}

X = MAC value mentioned in all table & figures; Y = LC_{50} or PS_{50} value mentioned in all table and figures and Z = LC_{90} or PS_{10} value mentioned in all table and figures. The control set showed 100% survival. The same data can also be interpreted as 10% survival at 1.21ml l^{-1}, 50% survival at 0.73 ml l^{-1}, 90% survival at 0.45 ml l^{-1}, and 100% survival at 0.31 ml $l^{-1}$was marked (Table-AB). Out of the above concentrations, LC_{00} or PS_{100} as safe MAC value of 0.31ml $l^{-1}$was selected as 'X'; LC_{50} or PS_{50} of 0.73 ml $l^{-1}$was selected as 'Y' and LC_{90} or PS_{10} value of 1.21 ml l^{-1} was selected as 'Z' for conducting future experiments.

The growth measured in terms of optical density at 540 nm of the alga exposed to different concentrations of Sumicidin and control set at different days of exposure and recovery was presented in Fig. 1. The optical density value increased from 0.025 $\pm$ 0.005 to 0.121 $\pm$ 0.014 on 15^{th} day of exposure and it increased to 0.205 $\pm$ 0.016 on 15^{th} day of recovery in the control set (Fig.1). The optical density value in case of set 'X' increased from 0.025 $\pm$0.005 to 0.12 $\pm$ 0.01 on 15^{th} day of exposure and increased to 0.215 $\pm$ 0.012 on 15^{th} day of recovery. In optical density study the "X" concentration value was more than the control value on all days of exposure and recovery, and the two values showed a consistent significant increase with the increase in days of exposure up to 15^{th} day (Fig. 1a). In case of "Y" concentration the value increased to 0.075 on 15^{th} day of exposure. All the values were more than the control set. Where as, in case of 'Z', the optical density value initially increased from 0.025 $\pm$0.005 to 0.035 $\pm$0.004 on 9^{th} day and than the value decreased with the increase on exposure period and a minimum of 0.015 $\pm$ 0.0002 value was reached on 15^{th} day of exposure. No change in value was marked in recovery period, rather the OD value further depleted (Fig.1a). A maximum decrease by 0.83%, 38.02% and 87.6% was recorded on 15^{th} day of exposure when compared to the control value in case of concentration X, Y and Z, respectively (Fig.2).

Fig.1. Changes in optical density in control and Sumicidin exposed BGA at different exposure periods.

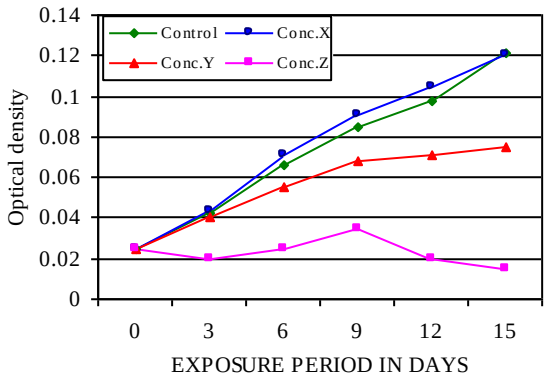


Fig.1a.Changes in optical density of the exposed cultures during recovery periods.

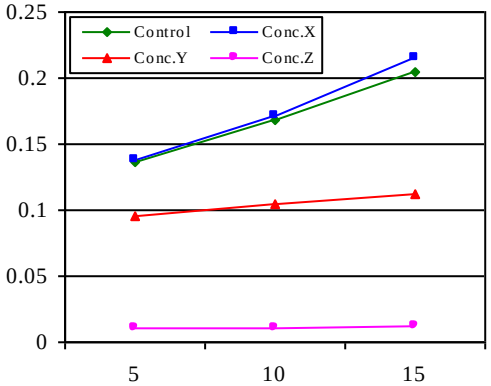


Fig.2. Percent change in optical density at different exposure period in Sumicidin exposed BGA when compared to control. Data calculated from the mean values.

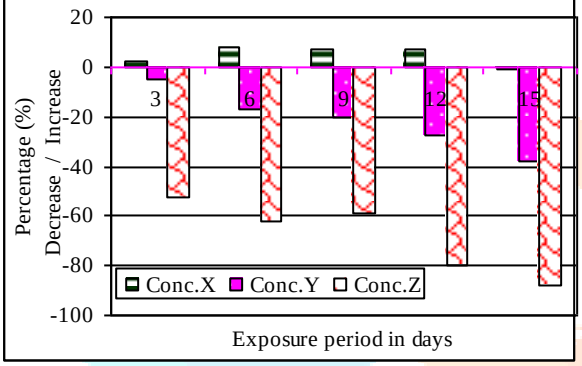
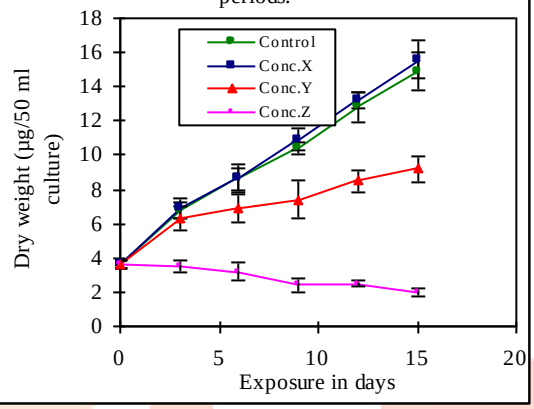


Fig.3. Showing changes in dry weight of control and exposed alga different exposure periods.



The highest percent of increase in O. D. value over the control value was seen at "X" concentration on 6th day of exposure (7.58%) and highest percent decrease (38%) was seen on 15th day of exposure in "Y" concentration and a maximum of 87.6% decrease was noted at concentration "Z" on 15th day of exposure (Fig. 2). When Sumicidin exposed algae was transferred to toxicant free medium during recovery studies, on 15th day of recovery insignificant recovery by 4.9% was noted in concentration-X, when compared to control value. Where as, in case of concentration-Y and Z, a maximum of 45.3% and 94.1% decrease was recorded on 15th day of recovery. These two values were much more than the 15th day exposure value. This clearly indicated that the toxicant is deadly toxic and the exposed alga could not recover even during recovery period in toxicant free nutrient medium. No recovery was marked at higher concentrations of the toxicant rather further depletion in the parameters was noted indicating permanent damage caused to the exposed system. Only at sub-lethal or at lowest concentration selected (Con. X), the exposed alga indicated good recovery. On 15th day of exposure 0.8% decrease when compared to control was seen and on 15th day of recovery 4.9% increase over the control value was recorded. This increase in optical density during recovery period over the exposed value indicated partial insignificant recovery (Fig.2). The correlation coefficient analysis indicated the existence of positive significant correlation between days of exposure and optical density values in control ($r = 0.988$, $p \leq 0.01$), Conc. X ($r = 0.994$, $p \leq 0.001$), and Conc. Y ($r = 0.966$, $p \leq 0.05$), however, negative non-significant correlation was observed in Conc. Z ($r = -0.412$; p = NS). The two way analysis of variance ratio test, pertaining to optical density indicated that there exists a significant difference between columns and significant difference between rows.

The data presented in Fig- 3 and 3a depict the change in dry weight of the alga *Anabaena cylindrica*, at different days of exposure and recovery, when exposed to different concentrations of Sumicidin. The dry weight increased from 3.6 ± 0.4mg to 14.9 ± 0.7mg on 15th day of exposure and the dry weight further increased to 23.2 ± 1.4mg on 15th day of recovery in the control set. At concentration-X, the dry weight increased from 3.6 ± 0.4mg to 15.6 ± 1.1mg on 15th day of exposure and the dry weight further increased to 23.8 ± 0.9mg on 15th day of recovery. At concentration-Y, the dry weight increased from 3.6 ± 0.4mg to 9.2 ± 0.5mg on 15th day of exposure and the dry weight further increased to 14.8 ± 0.5mg on 15th day of recovery. At concentration-Z, the dry weight decreased from 3.6 ± 0.4mg to 2.0 ± 0.4mg on 15th day of exposure and the dry weight increased to 2.9 ± 0.2mg on 15th day of recovery (Fig.-3). At concentration "X" the dry matter value was greater than the control value on all days of exposure and recovery. Higher rate of

growth was marked in case of concentration "X" than "Y and Z". Highest increase in percentage of growth (4.8%) was marked on 9th day of exposure over the control value (Fig. 4).

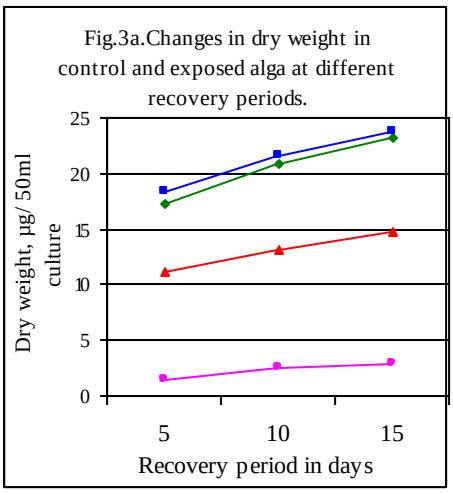


Fig.3a.Changes in dry weight in control and exposed alga at different recovery periods.

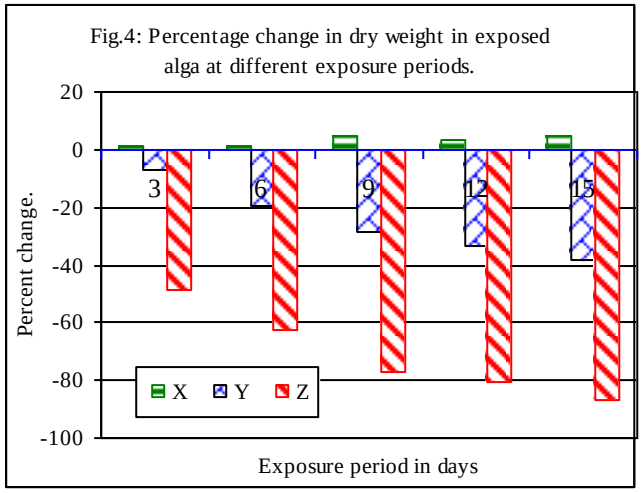


Fig.4: Percentage change in dry weight in exposed alga at different exposure periods.

The "Y" concentration showed a significant decreasing trend in growth when compared to control and concentration "X", in which a maximum decrease of 38.3% was marked on 15th day of exposure. Whereas, an increasing trend was marked up to 15th day of exposure when compared to 0 day value and with the increase in exposure period the dry weight of the alga increased and then the value further increased during recovery period when compared to 15th day exposure value, however all the values were much less when compared to control and concentration-X. In case of concentration-Z, the dry weight value did not show any increase when compared to 0 day value. Rather the dry weight decreased with the increase in exposure period. All the exposed values were significantly less than the control, concentration-X and concentration-Y. A maximum of 86.6% decrease when compared to control value was noted indicating severe damage caused to the exposed system. A significant dual behavior of the toxicant on the exposed alga was clearly evident from the obtained data (Fig. 3, 3a &4).

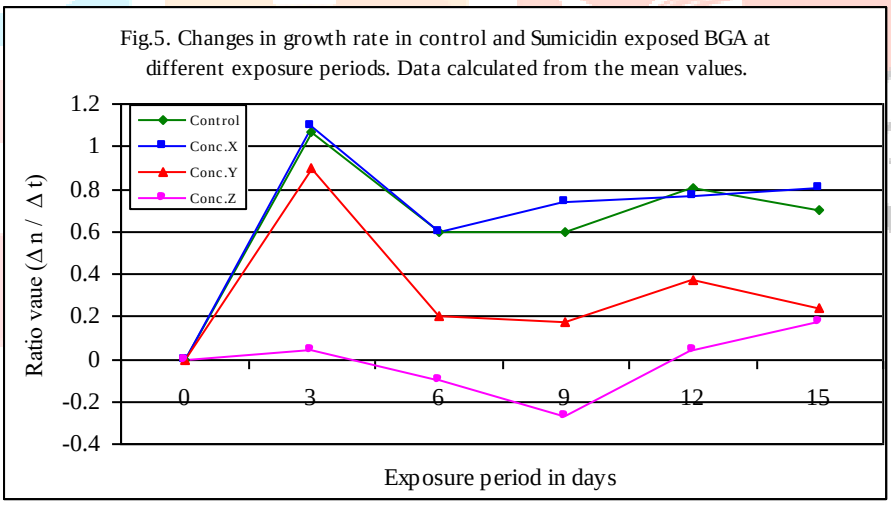


Fig.5. Changes in growth rate in control and Sumicidin exposed BGA at different exposure periods. Data calculated from the mean values.

Complete bleaching of the algal mass inside the test solution (Z) was observed from 3rd day of exposure period onwards. Gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 30 days of recovery period. Unlike of mercury or sumicidin poisoning case where recovery or tiny particles appear from 12th day of exposure itself and become dominating blue-green during recovery period at lower concentration of mercury, where as in sumicidin poisoning such a case was not observed by earlier workers. These particles grew in size with time. It was probably due to the appearance of photosynthetic pigments which disappeared due to the fungicide stress on the alga. Slowly the entire white mass got converted into a blue-green mass with the increase in recovery period (75 days of recovery). At "Z" (1.21mg/l) concentration (LC_{90}) a drastic decrease in dry weight value was noticed on 9th day of exposure and afterwards it showed continuous decrease up to 15th day of exposure (Fig. 4), however, the values were far less than control and concentration- "X & Y". The dry wt of control, "X" and "Y" concentration increased significantly with the increase in days of exposure, which were significant. No recovery was marked. Rather the values were much less than the exposure value showing drastic depletion in the parameter. The dry weight values decreased in conc. Z. significantly at all exposure days at $p \geq 0.05$ level except on 3rd day with the increase in concentration of the toxicant. The percent change value of the dry weight over its control also increased insignificantly with increase in days of exposure at

concentration "X" and the increase was not consistent. At concentration "Y" the dry weight value increased with the increase in exposure period and the marked values were less than the control and concentration-X values at all exposure and recovery periods. The percent change in dry weight in concentration 'Y' showed an increasing trend up to 15th day of exposure and then declined during recovery period. However, the dry weight values in conc. X were significantly higher than the control value. However, in case of concentration Y, no increase in the dry weight value indicated stress but consistent decrease in value was noted. Than with the increase in recovery period the dry weight increased and a maximum of 38.3% decrease was recorded on 15th day of exposure and 36.2% decrease was noted on 15th day of recovery (Fig. 4). The dry weight significantly decreased and showed a maximum decrease by 86.5% on 15th day of exposure and when the exposed alga was transferred to Sumicidin free medium further depletion in dry weight was noted at initial recovery period. With the increase in recovery period, partial recovery was noted and the percent recovery was not significant (Fig.4). The recovery values of different days of exposure were quite prominent from Fig. 3, 3a & 4. In "X" concentration highest percent of recovery was observed in exposed alga when exposed to toxicant free culture media on 10th day of recovery. With the increase in days of recovery the percent recovery value became less and it attained the lowest value after 15th day of recovery. In case of concentration-Y, a maximum of 2% recovery was noted. But in case of "Z" concentration, zero percent recovery was seen and the dry weight gradually decreased up to 5th day of recovery and after 10th day of recovery little insignificant increment was noted. The correlation coefficient analysis between days of exposure and dry weight of the control and exposed alga, exposed to different concentrations of Sumicidin indicated the existence of a positive significant correlation in control ($r = 0.991$; $p \leq 0.001$) and Conc. X ($r = 0.997$, $p \leq 0.001$). In case of concentration-Y, a positive correlation was noted ($r = 0.976$, $p \leq 0.01$). A negative and significant correlation ($r = -0.829$; $p \leq 0.05$) existed between days of exposure and dry weight of the alga in Conc. Z as observed. Table- 5 indicates the changes in growth rate pattern ($\Delta N/\Delta t$) based on dry weight data. In the control set, the growth rate showed an initial increasing trend and static growth rate with the increase in exposure period up to 15th day of exposure was marked. During recovery period, significant variation in growth rate was observed. In conc. X, the growth rate values were more than the growth rate values of the control set except 12th day of exposure, where a slight decrease in growth rate was marked and on the 15th day of exposure increase was noted. Higher growth rate in conc. X. was marked during recovery period, when compared to control set except on 15th day of recovery. In conc. Y, an initial increase on 3rd day of exposure and afterwards with the increase in exposure period decrease in growth rate was marked. When the exposed alga was transferred to toxicant free medium, the growth rate significantly declined when compared to control and conc. X set. With the increase in exposure period, the growth rate showed all negative values indicating death of the cells. No recovery was marked in conc. Y. In case of concentration-Z, negative values were obtained at initial periods of exposure and an insignificant value at higher exposure periods. When the exposed alga was transferred to toxicant free medium, an initial negative value followed by significant fall in the growth rate was marked (Fig.5). The growth rate pattern ($\Delta N/\Delta t$) computed from optical density values at different days of exposure showed, no change in case of control up to 15th day of exposure and "X" concentration showed a constant increase up to 15th day by exposure. In "Z" concentration it showed an increase up to 3rd day of exposure and then declined up to 15th day of exposure. With the increase in toxicant concentration, the optical density values decreased non-significantly at different days of exposure. The ANOVA test indicated the existence of a non-significant difference between rows and a significant difference between columns. From the obtained data, it was clear that the fungicide, Sumicidin is deadly toxic and the availability of this fungicide in the environment is dangerous. Hence, this fungicide cum pesticide should not be allowed for use in agricultural practices, as this fungicide will kill all these tiny blue-greens which are responsible for natural nitrogen fixation.

Discussion

Generally, algae are more sensitive than animals to complex wastes such as industrial and municipal effluents (Walsh *et al.*, 1982). Their use in bioassays is of ecological significance, since algae are the dominant primary producers in aquatic environments. A wide range of toxicity tests has been developed in the recent decades to predict the probable effects of new chemicals and industrial wastes in aquatic ecosystems utilizing different organisms such as algae, crustaceans, mollusks and fish (Miller *et al.*, 1978). The single most important factor in determining whether a specific chemical / compound is damaging or not, the factor is otherwise known as dose / concentration. The very concept leads to the conclusion that no chemical compound is completely safe and that none is entirely harmful. It is only the dose/concentration of a chemical which makes a thing poison or decides toxicity. Algae, the most important primary producers of the aquatic environments have received least attention. Very few references are available particularly on the toxicity effects and physiological changes induced by heavy metals on algae. The review made by Whitton

(1970), Gadd & Griffiths (1978) and Sorentino (1979) on impact and effect of heavy metals on algae added a lot of information to the literature of algal toxicology. Algae have been shown to concentrate heavy metals to a larger extent (Mclean & Jones, 1975; Jannett & Wixson, 1975; Trollope & Evans, 1976; and Say *et al*., I & II, 1977). Information's are available pertaining to the toxicity of mercury in the form of metal, mercury based pesticides, industrial wastes containing mercury etc. on fresh water blue-green algae (Rai *et al*., 1981 b; De Filippis and Pallaghy (1976), Shaw, 1987; Sahu, 1987; Rath, 1984; Rath, 1991). Agarwal & Kumar (1978) showed decrease in growth of *Chlorella* sp when exposed to mercurial effluent and solid wastes indicating toxic nature of mercury on the organism. A liquid industrial waste may affect the algal growth in any of three ways: stimulation, inhibition and stimulation at lower concentrations but inhibition at higher concentrations (Walsh & Alexander, 1980; Sahu, 1987 and Rath, 1991). The enhancements of growth, heterocyst frequency and nitrogen fixation at lower doses of furadon (0.75 µg / ml of carbofuran) have also been reported. The present investigation did not agree with the above conclusions. But such stimulation in the growth cannot be easily explained at this stage of the study. Hufford (1971) attributed the reason for stimulation, for the presence of some growth regulating compounds and/or trace elements in the crude oil. Some suggested uptake and metabolisation of the constituent as the probable mechanism for growth stimulation. Prasad & Prasad (1982) observed stimulation in the algal growth at low concentrations of Cd, Pb and Ni. Neither Cd, Pb and Ni have been reported to be essential micronutrients for algae (O'Kelley, 1974) nor the pure solution of these heavy metals are expected to contain any growth regulator. Thus, an ideal explanation for stimulation of growth at lower concentrations of the toxicant is yet to be ascertained. The solid waste extract under present investigation contains huge amount of mercury. Compounds based on mercury are toxic to algal organisms (Rath *et al*., 1983, 1985). Further inorganic forms are active only when these are present in free ionic state (Jardim & Pearson, 1985). Say *et al*., (1977) reported that the toxicity of zinc to green and blue-green algae could be reduced by supplementing the culture medium with phosphate, calcium and magnesium. Possible formation of complex of heavy metals with calcium and phosphate ions might be responsible for reduction in the toxicity, because of the complex formation they might not be getting an entry into the cell either through membrane transport or by surface adsorption which are the two known mechanisms for uptake of the substances (Rai *et al*., 1981). Further, different heavy meals interact among themselves either to reduce or to increase the toxicity of each other. In the present study, however, it was difficult to assign the definite role being played by mercury in affecting the growth of the blue-green alga. The solid waste was heterogeneous in nature, as it contained many ions at a concentration beyond tolerance level of the plant system. Rath *et al*. (1983) reported a maximum tolerable concentration of 2 µg Hg / 50 ml culture in inorganic form of *Westiellopsis prolifica*, Janet. Shaw (1987) reported 1.1 µg Hg / 50 ml culture in the form of effluent as tolerable to the same alga. Shaw (1987) reported that *Westiellopsis prolifica* could tolerate up to 2.0% SWE containing 13.65 µg of Hg/100 ml culture. But in this study, the alga could tolerate 0.2% of the solid waste extract concentration, the mercury concentration of which was 3.065 µg / 100 ml culture. It was expected that the presence of Ca, Mg, Na and PO_4^{3-} etc. would have reduced the toxicity of the extract. Shaw (1987) showed the antagonistic effects of different concentrations of PO_4^{3-}, NO_3^-, Cl^- and SiO_3^{2-}, at different levels of pH in relation to the mercury toxicity and demonstrated that the effluent of a caustic chlorine industry was toxic to the BGA, *Westiellopsis prolifica*, Janet, but because of the presence of other residues, its toxicity was reduced to a great extent and that the high amount of mercury present was in no way related to the toxicity observed. Several authors have found that heavy metals cause prolongation of lag phase more or less in proportion to doses, followed by normal growth (Zingmark & Miller, 1975) but in the present study, the situation was altogether different. It is possible that the algal filaments were present in the medium inside the gelatinous sheath but in greatly reduced and bleached form which made their detection impossible (by naked eye). However, it can be said with some degree of rationality that the reappearance of the alga was not directly from those filaments. From the granular structure it appears that the alga had made its reappearance through spores which have germinated and developed, might be after creating some sort of barrier which was impermeable for the toxicants. Formation of spores with hard cysts under unfavorable conditions is a well known phenomenon in blue-green algae. The alga had adopted to avoid the stress, immediately, when it came in contact with toxicants by formation of spores. Pradhan *et al* (2005) and Hannan & Patouillet (1972) pointed out that if enough mercury was present to inhibit the growth severely at the beginning of the test, the cells do not recover, which was not necessarily the case with the other pollutants. Dry weight measurement and optical density measurements were considered as the parameter of growth. Previous authors of the laboratory reported that algal systems behaved very differently towards light scattering in presence of different stresses. Optical density of the homogenised medium of the culture has been considered as a growth parameter in normal studies but in pollution studies, in presence of pollutants a consistent data in optical density was never obtained. Rath

(1984) indicated the idea that in presence of a pollutant, the deflection in optical density may not be exactly due to the algal growth or increase, it may also be due to the pollutant and dead cells present, as it deflects the light from the original path of penetration. The changes observed in optical density study exactly do not reflect the real changes induced by the pollutant, but an approximation can be made out of this data. Growth is a summation of all cellular metabolisms. So, any inhibition of growth reflects toxic effects on a number of metabolic processes. It was also confirmed that mercury appears to be less toxic in media with a high concentration of dissolved salts. To confirm the effect of mercury in a combined form on the growth of the alga, the dry weight and optical density measurements are not enough and basing on these data, no clear cut presumption can be made. The agricultural wastes have caused havoc for all living organisms. The present fungicide which also acts as a pesticide is toxic and should be used carefully in agricultural fields. Instant action of the killer chemical is not a boon but it can be also hazardous and can kill all the microflora and fauna and destroying the natural ecosystem of crop fields. The vital microflora responsible for biofertility of the crop field soil should be protected at all cost. One such microorganism is blue-green algae inhabiting crop fields whose importance as biofertilizer in rice cultivation is undisputed and well documented. A rice field with a healthy growth of BGA would require no input of nitrogenous fertilizer, India being predominantly an agriculture based country and the agriculture is totally based on the nitrogen economy, any effect on the blue-green algae will affect the nitrogen budget of the paddy fields. The eco-toxicological assay of such chemicals is a complex task involving the identification of the consequences to individuals in the natural environment, comparing with effects generally demonstrated in the laboratory studies and the ecological significance of these effects experienced by an individual organism.

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Author Contribution statement

Prof. A.K. Panigrahi: Conceptualization, planning, supervision, field visit, script preparation, reviewing and editing. B. K. Behera- Experimental planning and execution of the project, field visit, original draft preparation, supervision, editing. Sri Behera contributed reagents, glassware, field related work, manuscript preparation, calculation and finalization of data.

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