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Comparative Study and Possible Phycoremediation of Effluent of JK Pulp and Paper Mill and Select Heavy Metals on Cyanobacteria

Tripathy A.P., Uma Mahesh A., Padhi N. and Panigrahi A.K.*

Environmental Toxicology and Biotechnology Laboratory, Department of Botany, Berhampur University, Berhampur 760 007, Odisha, India

**Corresponding Author*

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Abstract: Pulp and Paper Mill effluent contains toxic heavy metals like Hg, Cd and Pb. These effluent are deadly toxic to the aquatic organisms as these effluents are discharged into River Nagavalli without biological treatment. The main objective of the present study was to assess the Paper mill effluent: its impact on blue green algae (BGA) and whether BGA can be used for detoxification of the effluent waste water. The contaminated river water contained significant amount of heavy metals. An attempt was made to study the impact of heavy metals like Hg, Cd and Pb present in effluent on BGA/cyanobacterium under laboratory controlled conditions and a strategy was planned to study these heavy metals in combination as mixture. All the heavy metals tested showed stimulation and better growth at sub-lethal concentrations (MAC value) but at higher concentrations heavy metals were deadly toxic. The heavy metals when combined produced higher toxicity. No stimulation in the exposed alga was observed in heavy metal combinations. The algae could remove heavy metals from the effluent of the paper mill. The paper mill effluent though highly toxic but showed better behavior at sub-lethal concentrations towards the cyanobacterium. From the observed data it is evident that *Westiellopsis* was more tolerant than other BGA tested. This alga has potency to grow under stressful conditions and can be safely used for phycoremediation of paper mill effluent after dilution of the effluent. Along with the tested BGA other aquatic macrophytes in combination can be tried for total biological treatment of paper mill effluent before being discharged into the environment.

Keywords: Effluent, Heavy metals, Cyanobacteria, Pigment, Phycoremediation, Paper mill effluent, Pulp mill effluent, *Westiellopsis*

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Introduction

Heavy metal pollution caused by industries is a direct threat to the very existence of plant and animal life in nature. Pulp and Paper mills are the major players to contribute heavy metals like mercury, cadmium and lead, polluting nearby water bodies and affecting aquatic flora and fauna. The paper mills use huge amount of fresh water drawn from water bodies in the manufacturing process and discharge significant amount of effluent as waste. Dey *et al*. (2018) indicated that in India, more than 55% of the mills do not have adequate effluent treatment facilities and also do not adopt modern treatment technologies. The paper mill effluents are discharged from the industry into the aquatic environment and significantly affects the water bodies (Iqbal *et al*., 2013) after simple physical and chemical treatments (Tripathy *et al*., 2022) indicating the need of a biological treatment. Jin *et al*. (2018) reported that bacteria can be used for microbial remediation of heavy metals in soil and reduce heavy metal pollution. These authors also reported that biosorption which was dependent on the special structure of the cell wall and was reported to be the primary mechanism of accumulation of heavy metals.

The effluent can be treated primarily by physical and chemical treatment and followed by biological treatment using potential and promising microphytes and macrophytes. Most of the industries do not follow these biological treatment methods. JK Paper Mills at Jaykaypur subjects the effluent to chemical treatment and discharges its effluent without any biological treatment. International Paper Mill at Rajmundry discharges its effluent in to a tank containing *Eichhornia*, *Pistia* and *Azolla* but the retention time of the effluent in treatment pond was very low (Dixit *et al*., 2018) and the so called biologically treated effluent was actually toxic for fish. Dixit *et al*. (2018) reported presence of mercury, cadmium and lead in the final discharged International Paper mill effluent after biological treatment. Waste generation in any industry and the quality of waste depends on the technology and used treatment technology of any Pulp and Paper industry. It is not possible to eliminate waste generation by the system (Kaur *et al.,* 2021) but cleaner and environment friendly technology can be used and periodically positive modifications in the technology or alterations in the treatment technology should be adopted. Kaur *et al.* (2021) reported maximum flocculation at 0.3 g/l of chitosan concentration, 81% of total suspended solids could be removed and COD depleted by 78%. The same author suggested that flocculent chitosan (naturally biodegradable) and alum (coagulant) can be used for better treatment of Paper mill effluent but the authors did not mention anything about heavy metal presence, its toxicity and possible removal of heavy metals from the effluent of paper mills.

Wang *et al.* (2020) reported a novel method of microbial immobilization which is eco-friendly to decrease the heavy metal enrichment in plants. Nwaehiri *et al*. (2019) reported that bacteria isolated from soil and effluent samples were capable of remediation and reduce the concentration of heavy metals tested. On site observation revealed that micro- and macro-algae were growing profusely at the meeting point where Paper mill effluent joins the river water. It seems those algae can resist the available toxicants of the effluent and grow in the effluent canal. The possible way of resistance and growth of algae at the meeting point could be the dilution factor. At the meeting point the river water diluted the effluent and the diluted effluent favored the growth of the algae. It was also observed that blue-green scum was seen in the crop fields of the nearby crop fields where paper mill effluent contaminated river water was used for irrigation. Observation of the blue-green/yellowish green scum indicated the presence of *Westiellopsis* sp., *Anabaena* sp., *Nosto*c species along with other species. These three selected algae were farmer friendly heterocystous blue-green algae and can fix atmospheric nitrogen. These algae were eco-friendly and act as biofertilizer in the crop fields. The dominant species *Westiellopsis* was selected to study the impact of paper mill effluent on the survivability and growth of algae. An attempt was also made to assess the potentiality of this alga to be used for possible phytoremediation along with other potential aquatic macrophytes and microphytes. It was also planned to analyze the impact of heavy metals like mercury, cadmium and lead individually and in different combinations and the effluent containing these heavy metals on the growth and physiology of Blue-green algae (cyanobacteria).

Materials and Methods

JK Pulp and Paper Mill, Jaykaypur is located at latitude 19.247ºN and longitude 83.409 º E, the ET (effluent treatment) plant is located at latitude 19.248ºN and longitude 83.413º E and the effluent canal originating from the industry travels around 825.4 m and joins the River Nagavalli (Fig. 1) at 19.252ºN and longitude 83.420º E. The industry utilizes Nagavalli river water collected from the upstream of the river for all its day to day requirements and discharges its effluent in to the river at downstream. The effluent sample was collected from the mid effluent canal (S) (Figs. 2, 3) in air tight plastic containers and brought in cold containers to the laboratory for analysis.

Analyses of physico-chemical parameters of effluent and soil samples of nearby crop fields were carried out following the procedure of APHA (1985) and EC (1979). In addition, standardized field analysis kit (Systronics, India) and portable instruments were used to measure pH, conductivity and temperature of river water, effluent and crop field soil samples. Dissolved oxygen was measured by modified Winkler's method (APHA, 1985). Sodium and potassium was measured by flame photometer following the instruction manual supplied by Systronics. Effluent samples were brought in plastic containers and stored in the refrigerator for analysis and for laboratory experimental work. Measurement of mercury of the collected effluent and algal samples were carried out by acid digestion in Bethige's apparatus followed the basic principle of Wanntorp and Dyfverman (1955). Mercury in digested samples were estimated following the process described in Analytical Method for estimation of mercury with Mercury Analyser MA 5800A issued and marketed by ECIL (1981). Samples were taken in a Klein's apparatus (digester) with acid digestion mixture and digested separately till the sample was fully digested (Wanntorp and Dyfverman, 1955). Cadmium and lead content of the digested extract was estimated following the procedure of Yoshida *et al*. (1976).

Westiellopsis prolifica Janet 1941 is a photoautotrophic, branched, filamentous, heterocystous BGA belonging to the family Stigonemataceae, was selected as the test organism. Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) and adopted by Sahu (1987) was most suitable basic culture for the growth of the test organism. The experimental algal cultures were grown under controlled conditions of light and temperature inside a culture room with light intensity of 2400±200 Lux, with 14 h photoperiod and 10 h nyctoperiod, to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and was maintained at 28±2 C. The flasks were regularly shaken periodically to avoid clumping of the cells as well as their adhesion to the inner wall of the conical flasks. Growth was measured by recording the dry weight of the alga in a single pan balance (Dhona). To study the pigments, algal material present in different experimental and control flasks were centrifuged and the algal residue was washed thoroughly with double distilled water and again centrifuged. The pigments were extracted and estimated following the procedure of Vernon (1960). The rate of oxygen evolution by algae due to photosynthesis was measured manometrically at 37 C and 2400 ± 200 Lux light intensity in a Photo-Warburg's apparatus (New Paul, India) following the procedure of Hannan and Patouillet (1972) and Oser (1965) modified by Sahu (1987).

For study of the heavy metal pollutants-mercuric nitrate (A), lead nitrate (B), cadmium chloride (C) of pure grade (Analytical grade), mixture of cadmium chloride + lead nitrate (1:1 ratio=D); mixture of mercuric nitrate + lead nitrate + cadmium chloride(1:1:1 ratio=E) were prepared by dissolving in distilled water and effluent of Pulp and Paper Mill (F). A graded series of concentrations of individual and mixed toxicants ranging from 0.1mg l^{-1} to 2.0mg l^{-1} (w/v) and 1 ml/l to 10 ml/l (v/v) was prepared in different experimental conical flasks and UV sterilized in a Laminar Airflow. Dilution in each

Fig. 1: Google photomaps showing JK Papers Ltd., Effluent canal (Site-SS), effluent soaking open site, effluent joining Nagavalli River and nearby crop fields contaminated by effluent and effluent mixed river water.

Figs. 2, 3: Effluent collection sites (S) of effluent canal leaving the Paper industry and joining the River Nagavalli, at Chandilli and algal sample collection.

concentration was made with culture nutrient medium. One ml of unialgal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100 ml of solution, inside the inoculating chamber. The effluent was filtered and diluted with nutrient solution. A graded range of concentrations from 0.25 ml to 5ml/l was prepared. The filtrate effluent was taken (100 ml each) in conical flasks and UV sterilized under aseptic conditions in a Laminar airflow. All dilutions were carried out by taking the algal culture nutrient medium. The homogenized alga was inoculated and the flasks were kept on culture racks. The inoculated flasks were kept inside the culture room and maintained. The test alga was exposed for a period of 15 days in different test media after which their survival and mortality percentages were calculated by counting the number of cells present in one ml of the test solution after micro-tissue homogenization. After 15days of exposure, the obtained values were plotted and the lethal concentration values and maximum allowable concentration (MAC) were determined. From the toxicity testing as described by Sahu (1987), the marked concentrations of the toxicants were selected for laboratory experiments. The observed data was statistically analyzed and presented as the mean of samples ± standard deviation.

Results

Effluent samples were collected from effluent canal at two years interval from the same spot to compare and assess the quality of the effluent released from the industry and discharged into River Nagavalli located at a distance of 1 km. The effluent color ranged from deep yellowish brown to yellowish brown and odour was pungent with filthy smell. The locality area suffered from this pungent H_2S smell. The effluent was slightly alkaline and no significant variation in pH was noted and the values ranged between 7.5 ± 0.6 – 7.9 ± 0.4. The temperature of the effluent ranged within 31.2 ± 1.2 C to 32.5 ± 0.7 C. The effluent was discharged initially into a soaking cum drying open field located nearby and from there the effluent gets discharged into the river continuously. Significantly higher conductivity value to the tune of 1824.4 ± 18.4 mho/cm was noted in 2015 and conductivity values decreased in 2017 and 2019. In 2019, 1731.5 ± 26.2mho/cm conductivity of the effluent sample was recorded (Table 1). The dissolved oxygen of the effluent was significantly low and ranged between 1.5 ± 0.4 mg l^{-1} to 1.8 ± 0.3 mg l^{-1}. When we compared the BOD status, the BOD value of the effluent sample decreased from 139.7 ± 22.8 mg l^{-1} (in 2015) to 126.8 ± 17.2 mg l^{-1} (in 2019). Significant variation in COD value was noted at all study periods but no increase or decrease trend was observed. The total suspended solids (TSS) content of the effluent was significantly high and the TSS value increased from 806.2±17.6 to 826.8 ± 11.5 mg/l with time period. The total dissolved solids (TDS) depleted with time period but no trend of interest was noted (Table 1). The hardness of the effluent samples collected in different time periods indicated 498.8 ± 45.6 mg/l in 2015 to 586.4 ± 18.4 mg/l in 2019. The increase in hardness might be due to chemical treatment of the effluent by chemical methods. No notable variation was noted in total phosphate content of the effluent samples tested (Table 1). The amount of phosphate available in the effluent samples was low (2.65±0.9 mg/l, 2.45±0.7 mg/l and 1.12 ± 0.4 mg/l in 2015, 2017 and 2019, respectively) but sufficient enough to support algal growth (Table 1). Significant amount of total sulphate was recorded in the effluent samples collected from the effluent canal in different time periods. Interestingly, the sodium and potassium content recorded in the effluent was very high and almost similar quantity was recorded in the past three select years (Table 1).

Table 1: Comparison and analysis of physico-chemical parameters and heavy metals present in the sample collected from the effluent canal before joining the river Nagavalli at Jaykaypur, Odisha in different years; ND = Not detected

Parameters	Present study report in 2019
Colour and Odour	Yellowish brown, Pungent. Filthy smell
pH	7.9±0.4
Temperature (C)	31.2±1.2
Conductivity (mho/cm)	1731.5±26.2
Dissolved oxygen (mg/l)	1.5±0.4
BOD (mg/l)	126.8 ± 17.2
COD (mg/l)	318.5 ± 11.8
TSS (mg/l)	826.8 ± 11.5
TDS (mg/l)	1392.3±11.4
Hardness (mg/l)	586.4±18.4
Total Phosphates (mg/l)	1.12 ± 0.4
Total Sulphate (mg/l)	146.5±11.2
Sodium (mg/l)	61.8 ± 5.2
Potassium (mg/l)	49.5±3.8
Cadmium (mg/l)	0.32±0.05
Mercury (mg/l)	0.46±0.07
Lead (mg/l)	0.21±0.09

Toxic metals like cadmium, mercury and lead were found in the effluent collected from site-S. Significant amount of cadmium was recorded in the collected effluent sample in 2019 to the tune of 0.32 ± 0.05 mg/l. The amount of cadmium in the effluent sample was very small but it is significant and toxic and much more than stipulated guidelines prescribed by Pollution Control Board (PCB). Significant amount of mercury and lead were recorded in the collected effluent sample in 2019 to the tune of 0.46±0.07 mg/l and 0.21±0.09 mg/l, respectively. The amount of mercury in the effluent sample was significant and highly toxic

(Table 1). In few collections at different time periods particularly in rainy season lead was not detectable in the effluent, might be due to heavy dilution.

The pH of the crop field ranged between 7.88 to 7.93 and the soil pH was little higher than the water pH. The soil and water temperature ranged between 23.5 to 24.6 C. The data presented in Table 2, indicated presence of all the three tested heavy metals in the crop field soil and water, residual heavy metals in the algal scum collected from the crop fields. The farmers use Paper mill effluent contaminated Nagavalli river water for irrigation of crop fields at regular intervals. The source of heavy metals in the crop fields being the water used for irrigation from the Nagavalli River. In crop field-A, significant amount of cadmium, lead and mercury was available in the crop field soil to the extent of 81.5 ± 9.8, 12.5± 1.5 and 83.2 ±14.6 µg/g dry weight, respectively. In crop field-A, significant amount of cadmium, lead and mercury was available in the crop field water to the extent of 53.8 ± 11.4, 8.8 ± 2.6 and 48.6 ± 8.8µg/g dry weight, respectively (Table 2). Algal scum collected from the contaminated crop fields showed significant amount of heavy metal accumulation. Algae showed heavy metal accumulation to the tune of 0.28± 0.06 (cadmium), 0.17 ± 0.05 (lead) and 0.76 ± 0.11 (mercury) µg/g dry wt in crop field-A (Table 2). The obtained values in table-2 indicated the pollution status of the nearby crop field contaminated with Paper mill effluent mixed with river water.

Based on the findings of the effluent analysis (Table 1) and crop field analysis (Table 2), it was felt necessary to study the impact of heavy metals like mercury as mercuric nitrate (A), lead as lead nitrate (B), cadmium as cadmium chloride (C) as inorganic chemicals individually and these heavy metals in combination. One mixture was prepared with cadmium chloride and lead nitrate (1:1 ratio) as a toxicant D and the second being the mixture of cadmium chloride, lead nitrate and mercuric nitrate at 1:1:1 ratio as a toxicant E and the effluent containing the these three heavy metals along with other plant debris as toxicant F on cyanobacterium inhabiting the contaminated area crop fields. We wanted to find out the best suitable organism, which can resist, tolerate, survive, support decontamination and help in reclamation of the contaminated environments. Figure 4 illustrates the lethal concentration values of the above 6 types of toxicants on a cyanobacterium, *Westiellopsis prolifica* under laboratory controlled conditions. The maximum allowable concentration (LC_{00}-X) deduced from toxicity test, tested on *Westiellopsis prolifica* were 0.154, 0.261, 0.211, 0.141, 0.124 mg/l and 0.541 ml/l in toxicants A (Mercuric nitrate), B (Cadmium chloride), C (Lead nitrate), D (Cadmium chloride + Lead nitrate), E (Mercuric nitrate + cadmium chloride + lead nitrate) and F (Pulp and Paper Mill effluent), respectively. The LC_{10} deduced from toxicity test on *Westiellopsis prolifica* were 0.161, 0.273, 0.244, 0.159, 0.129 and 0.594 ml/l for toxicants A, B, C, D, E and F, respectively. The LC_{50} (Y) deduced from toxicity test on *Westiellopsis prolifica* were 0.211, 0.363, 0.299, 0.195, 0.137 mg/l and 1.112 ml/l for toxicants A, B, C, D, E and F, respectively). The LC_{90} deduced from toxicity test on *Westiellopsis prolifica* were 0.332, 0.418, 0.328, 0.214, 0.149 mg/l and 1.287 ml/l for toxicants A, B, C, D, E and F, respectively (Fig. 4). The survivability of the alga decreased with the increase in toxicant concentration indicating toxic stress.

Figure 5 depicts changes in dry weight of control and 15 d exposed alga, *Westiellopsis* exposed to different concentrations of the toxicant and different types of heavy metal toxicants. In case of mercuric nitrate (A), the dry weight increased from 16.5±0.3 to 17.9±0.7 mg/l at MAC value showing 8.5% increase over the control value and the dry weight decreased to 16.4±0.4mg/l at LC_{10} showing 0.6% insignificant decrease and to 7.4±0.3 mg/l after 15d exposure in LC_{50} value of the toxicant showing 55.2% decrease as compared to control value. Significant decrease in dry weight from 16.5 ± 0.3 mg/l to 1.8 ± 0.4 mg/l was noted at LC_{90} of the Toxicant-A, showing 89.1% decrease as compared to control value (Fig. 5). In case of lead nitrate (B), the dry weight decreased from 16.7±0.5 to 15.6±0.6 mg/l

Table 2: Heavy metal analysis of water and soil samples of crop fields and algal scum collected from the crop field nearby opposite to the Pulp and Paper mill industry and River Nagavalli at Chandilli, Rayagada district, Odisha, India. Data presented are the mean of samples ± standard deviation.

Parameters studied	Collection and analysis of samples from the crop field-A (Google Map) from the contaminated site opposite to the industry and Nagavalli River at Chandilli		
	Soil	Water	Algal mixture collected from crop fields
pH	7.93	7.88	-
Temperature, C	23.5 ± 0.5	24.6 ± 0.3	-
Heavy metals	µg/g dry wt	µg/l	µg/g dry wt
Cadmium	81.5 ± 9.8	53.8 ± 11.4	0.28 ± 0.06
Lead	12.5 ± 1.5	8.8 ± 2.6	0.17 ± 0.05
Mercury	83.2 ±14.6	48.6 ± 8.8	0.76 ± 0.11

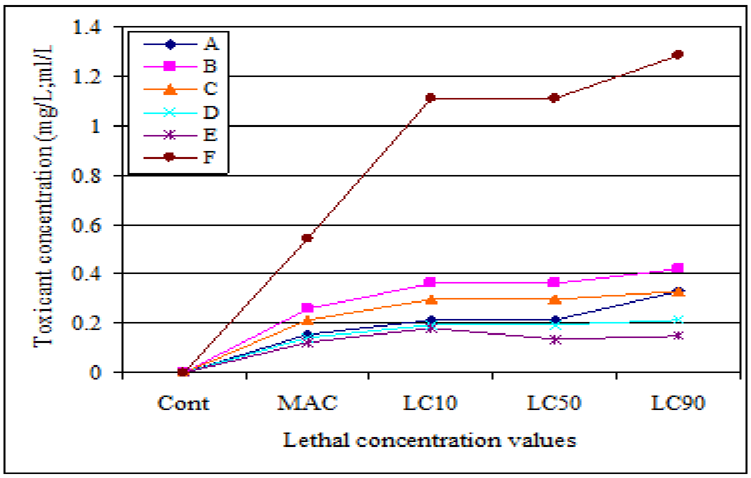

Fig. 4: Deduced lethal concentration (LC) values of Mercuric nitrate (A), Lead nitrate (B), Cadmium chloride (C), Lead nitrate + Cadmium chloride mixture (D) (1:1 ratio), Mercuric nitrate + Lead nitrate + Cadmium chloride mixture(E) (1:1:1 ratio) (mg/l) and Pulp and Paper Mill effluent containing Hg, Pb and Cd (ml/l) (F), for the alga, *Westiellopsis prolifica* after 15 days of exposure under laboratory controlled conditions.

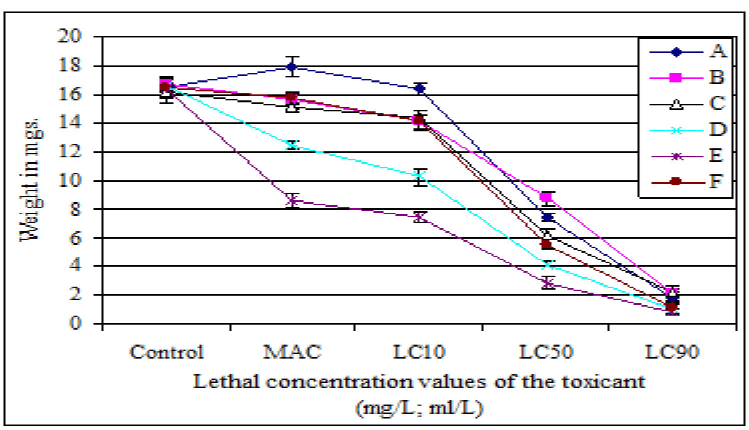

Fig. 5: Changes in dry weight (mg/100ml culture) induced by different toxicants, (A), (B), (C), (D)), (E) and (F) at different lethal concentrations in the algae, *Westiellopsis prolifica* after 15 days of exposure under laboratory controlled conditions.

at MAC value showing 6.6% decrease over the control value and the dry weight decreased to 14.2±0.7 mg/l at LC_{10} showing 14.9% significant decrease and to 8.8±0.5 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 47.3% decrease as compared to control value. Significant decrease in dry weight from 16.7 ± 0.5 mg/l to 2.1 ± 0.6 mg/l was noted at LC_{90} of the Toxicant-B, showing 87.4% decrease compared to control value (Fig. 5). In case of cadmium chloride (C), the dry weight decreased from 16.2±0.8 to 15.1±0.6 mg/l at MAC value showing 6.8% decrease over the control value and the dry weight decreased to 14.4±0.5 mg/l at LC_{10} showing 11.1% insignificant decrease and decreased to 6.2±0.4 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 61.7% decrease as compared to control value. Significant decrease in dry weight from 16.2 ± 0.8 mg/l to 2.2 ± 0.2 mg/l was noted at LC_{90} of the Toxicant-C, showing 86.4% decrease as compared to control value (Fig. 5). In case of cadmium chloride + lead nitrate mixture (D), the dry weight decreased from 16.6±0.4 to 12.5±0.3 mg/l at MAC value showing 24.6% decrease over the control value and the dry weight decreased to 10.3±0.6 mg/l at LC_{10} showing 37.9% significant decrease and further decreased to 4.2±0.2 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 74.7% decrease as compared to control value. Significant decrease in dry weight from 16.6 ± 0.4 mg/l to 1.1 ± 0.5 mg/l was noted at LC_{90} of the Toxicant-D, showing 93.3% decrease compared to control value (Fig. 5). In case of cadmium chloride + lead nitrate + mercuric nitrate mixture (E), the dry weight decreased from 16.4±0.7 to 8.6±0.5 mg/l at MAC value showing 47.5% decrease over the control value and the dry weight further significantly decreased to 7.4±0.4 mg/l at LC_{10} showing 54.9% decrease and further decreased to 2.9±0.4 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 82.3% decrease as compared to control value. Significant decrease in dry weight from 16.4 ± 0.7 mg/l to 0.9 ± 0.2 mg/l was noted at LC_{90} of the Toxicant-E, showing 94.5% decrease as compared to control value (Fig. 5). In case of toxicant-F, where effluent of the Paper mill was taken, no stimulation was observed in dry weight rather decrease in dry weight was marked. The dry weight decreased at all lethal concentration values. The dry weight of the exposed alga decreased from 16.5±0.2 to 15.7±0.4 mg/l at MAC value showing 4.8% insignificant decrease over the control value and the dry weight further decreased to 14.4±0.5 mg/l at LC_{10} showing 14.5% decrease and further decreased to 5.5±0.3 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 66.6% decrease as compared to control value. Significant decrease in dry weight from 16.5±0.2 mg/l to 1.2± 0.6 mg/l was noted at LC_{90} of the toxicant-F, showing 92.7% decrease compared to control value. From the observed data, it can be concluded that all the toxicants tested are deadly toxic and in case of mercury little increment in dry weight was noted at maximum allowable concentration in both the tested algae. From Figure 5, it is clear that toxic heavy metals and their combinations were more toxic compared to the paper mill effluent containing similar heavy metals.

Figure 6 depicts changes in total chlorophyll content of control and 15d exposed alga, *Westiellopsis* exposed to different concentrations of the effluent toxicant and different types of heavy metal toxicants. In case of mercuric nitrate (A), the total chlorophyll content increased from 0.116±0.008 to 0.128±0.011 mg/l at LC_{00} value (MAC) showing 10.3% increase over the control value and the total chlorophyll content decreased to 0.104±0.012 mg/l at LC_{10} and 0.076± 0.009 mg/l after 15 d exposure in LC_{50} value of the toxicant showing 10.3 and 34.5% decrease compared to control value, respectively. The chlorophyll content decreased by 77.6% at LC_{90} compared to control value in case of toxicant-A. In case of toxicants B, C, D, E and F the total chlorophyll content notably decreased in all the select toxicant concentrations, except in case of toxicant A where 10.3% increase over control value was observed at MAC value and at toxicant-F, 10.5% increase was also noted at MAC value (Fig. 6). Maximum depletion in total chlorophyll content by 37.9%, 47.4%, 64.6% and 93.1% was

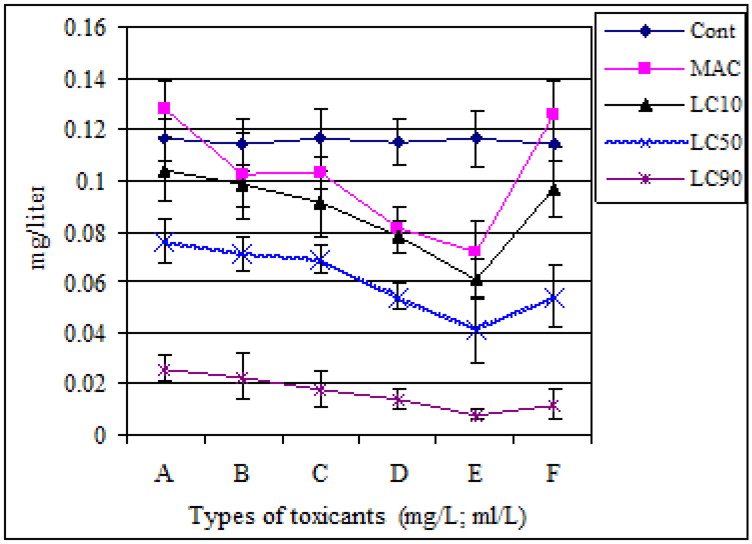

Fig. 6: Changes in total chlorophyll content (mg/l) caused by different toxicants, (A), (B), (C), (D)), (E) and (F) at different lethal concentrations in the algae, *Westiellopsis prolifica*, Janet after 15days of exposure under laboratory controlled conditions.

recorded in toxicant-E at MAC, LC_{10}, LC_{50} and LC_{90}, respectively, when compared to control total chlorophyll content, where three heavy metals like Hg, Cd and Pb was taken as a mixture showing maximum toxicity. In case of toxicant-F, 10.5% increase was noted at MAC value of the toxicant. Maximum depletion in total chlorophyll content by 14.9%, 52.6% and 89.4% was recorded in toxicant-F at LC_{10}, LC_{50} and LC_{90}, respectively, when compared to control total chlorophyll content, where three heavy metals like Hg, Cd and Pb was present in the effluent mixture showing significant toxicity. Little less decrease in total chlorophyll content was noted in toxicant-C when compared to toxicant A, B, D and E where lead nitrate was taken as test toxicant. In case of toxicant- B, C, D and E, no increment in total chlorophyll content was recorded at LC_{00}, LC_{10}, LC_{50} and LC_{90} concentration value (Fig. 6). In case of toxicant-F, where effluent of the Paper mill was taken, increase in total chlorophyll content was noted at MAC concentration in the tested algae and at higher doses of the toxicant at LC_{50} and LC_{90} decrease in total chlorophyll content was observed. From the observed data, it can be concluded that all the toxicants tested are deadly toxic at higher concentrations and in case of mercury and paper mill effluent little increment in total chlorophyll content was noted at maximum allowable concentration (MAC) in the tested algae (Fig. 6). This data gave an indication that both the toxicants (A and F) could be diluted to their respective MAC values for decreasing the toxicity of the toxicants.

Figure 7 illustrates changes in total phaeophytin content of control and 15d exposed alga, *Westiellopsis* exposed to MAC, LC_{10}, LC_{50} and LC_{90} concentrations of the effluent and different heavy metal toxicants. In case of mercuric nitrate (A), the total phaeophytin content increased from 0.092±0.005 to 0.098±0.007 mg/l at MAC value showing 6.5% increase over the control value and the total phaeophytin content decreased to 0.074 ± 0.004 mg/l, 0.068 ± 0.005 mg/l and 0.024 ± 0.007 mg/l after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 19.5%, 26.1%and 73.9% decrease compared to control value, respectively. In case of cadmium chloride (B), the total phaeophytin content increased from 0.089 ± 0.008 to 0.091±0.004 mg/l at MAC value showing 2.2% increase over the control value and the total phaeophytin content decreased to 0.088±0.005 mg/l, 0.084±0.004 mg/l and 0.031±0.005 mg/l after 15 d exposure in LC_{10}, LC_{50}

and LC_{90} value of the toxicant showing 1.1%, 5.6% and 65.1% decrease compared to control value, respectively. In case of lead nitrate (C), the total phaeophytin content decreased from 0.091±0.006 to 0.084±0.002 mg/l at MAC value showing 7.6% decrease over the control value and the total phaeophytin content decreased to 0.079±0.006 mg/l, 0.062±0.007 mg/l and 0.029±0.003 mg/l after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 13.1%, 31.8% & 68.1% decrease, respectively as compared to respective control value (Fig. 7). In case of cadmium chloride and lead nitrate mixture (D), the total phaeophytin content decreased from 0.092 ± 0.005 to 0.072±0.005 mg/l at MAC value showing 21.7% decrease over the control value and the total phaeophytin content decreased to 0.066±0.004 mg/l, 0.049±0.005 mg/l and 0.016±0.002 mg/l after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 28.2%, 46.7% and 82.6% decrease, respectively. In case of cadmium chloride, lead nitrate and mercuric nitrate mixture (E), the total phaeophytin content depleted from 0.092±0.005 to 0.054±0.008 mg/l, further decreased to 0.038±0.002 mg/l, 0.022±0.009 mg/l and 0.011±0.002 mg/l after 15 d exposure in MAC, LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 41.3%, 58.6%, 76.1% and 88.1% decrease, respectively over the control values. In case of effluent of the paper mill (F), the total phaeophytin content decreased from 0.091±0.007 to 0.083±0.005 mg/l, further decreased to 0.071±0.007 mg/l, 0.036± 0.003 mg/l and 0.014±0.004 mg/l after 15 d exposure in MAC, LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 8.8%, 21.9%, 60.4% and 84.6% depletion, respectively as compared to control values. From the observed data, it can be concluded that all the toxicants tested are deadly toxic at higher doses and in case of mercury and cadmium little insignificant increment in total phaeophytin content was noted at maximum allowable concentration (Fig. 7).

Figure 8 depicts changes in photosynthetic rate of control and 15d exposed alga, *Westiellopsis* exposed to MAC, LC_{10}, LC_{50} and LC_{90} concentrations of the effluent and different heavy metal toxicants. In case of mercuric nitrate (A), the photosynthetic rate increased from 234.5±24.6 to 292.6±31.2 µmole of O_2 liberated/g/h at MAC value showing 24.7% increase over the control value and the photosynthetic rate decreased to 228.2±18.4 µmole of O_2 liberated/g/h , 124.4±18.4 µmole of O_2 liberated/g/h and 64.2± 19.2 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 2.7%, 46.9% and 72.6% decrease in photosynthetic rate compared to control value, respectively. In case of cadmium chloride (B), the photosynthetic rate decreased from 234.8±25.2 to 212.4±11.6 µmole of O_2 liberated/g/h at MAC value showing 9.5% decrease over the control value and the photosynthetic rate decreased to 201.4±22.7 µmole of O_2 liberated/g/h, 134.2±28.4 µmole of O_2 liberated/g/h and 69.1±17.6 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 14.2%, 42.8% and 70.5% decrease as compared to control value, respectively. In case of lead nitrate (C), the photosynthetic rate increased from 234.5±28.4 to 236.4±14.8 µmole of O_2 liberated/g/h at MAC value showing 0.8% insignificant increase over the control value and the photosynthetic rate decreased to 196.8±28.6 µmole of O_2 liberated/g/h, 122.9±11.8 µmole of O_2 liberated/g/h and 63.2±12.8 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 16.1%, 47.6% and 73.04% decrease as compared to control value, respectively (Fig. 8).

In case of cadmium chloride and lead nitrate mixture (D), the photosynthetic rate decreased from 234.6±18.4 to 194.5±18.2 µmole of O_2 liberated/g/h at MAC value showing 17.1% significant decrease over the control value and the photosynthetic rate decreased to 164.2±12.9 µmole of O_2 liberated/g/h, 98.2±22.4 µmole of O_2 liberated/g/h and 39.6±15.3 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 30%, 58.1% and 83.1% decrease compared to control value, respectively (Fig. 8). In case of cadmium chloride,

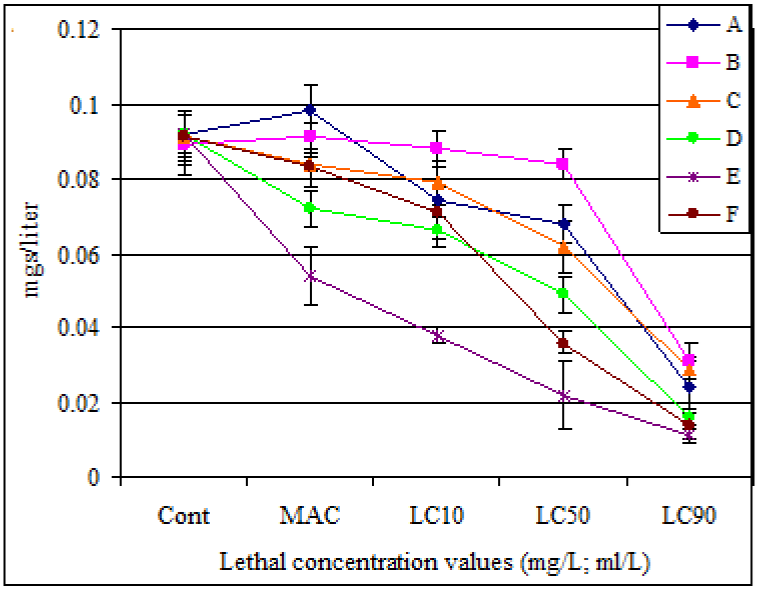

Fig. 7: Changes in total phaeophytin content (mg/l) induced by different toxicants, (A), (B), (C), (D)), (E) and (F) at different lethal concentrations in the algae, *Westiellopsis prolifica* after 15 days of exposure under laboratory controlled conditions.

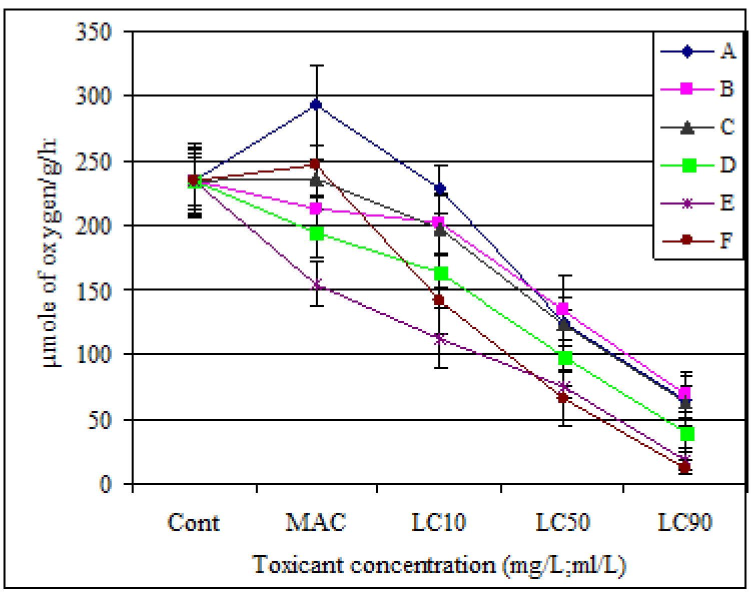

Fig. 8: Changes in photosynthetic rate (µmole of O_2 liberated/g/h) induced by different toxicants, (A), (B), (C), (D)), (E) and (F) at different lethal concentrations in the algae, *Westiellopsis prolifica*, Janet after 15 days of exposure under laboratory controlled conditions.

lead nitrate and mercuric nitrate mixture (E), the photosynthetic rate decreased from 234.5±21.2 to 154.5±17.6 µmole of O_2 liberated/g/h at MAC value showing 34.1% significant decrease over the control value and the photosynthetic rate decreased to 112.5±22.79 µmole of O_2 liberated/g/h, 76.2±9.8 µmole of O_2 liberated/g/h and 19.2±8.4 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 52%, 67.5% and 91.8% decrease when compared to control value, respectively (Fig. 8). In case of effluent of the paper mill (F), the photosynthetic rate increased from 234.6±26.4 to 246.2±15.4 µmole of O_2 liberated/g/h at MAC

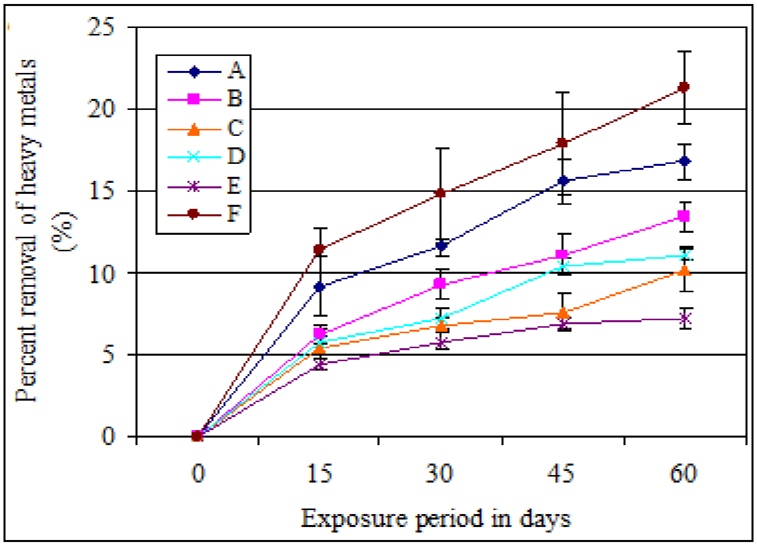

Fig. 9: Showing percent removal of different heavy metal toxicants individually and in combination and effluent containing heavy metals by the alga, *Westiellopsis prolifica*, Janet at different days of exposure. Data are the mean of samples ± standard deviation.

value showing 4.9% increase over the control value and the photosynthetic rate decreased to 141.3±26.2 µmole of O_2 liberated/g/h , 66.4±21.2 µmole of O_2 liberated/g/h and 12.8±5.6 µmole of O_2 liberated/g/h after 15 d exposure in LC_{10}, LC_{50} and LC_{90} value of the toxicant showing 39.7%, 71.7% and 94.5% decrease in photosynthetic rate compared to control value, respectively. In case of toxicant-F, where effluent was taken, increase in photosynthetic rate content was noted MAC in the tested alga and at high doses of toxicant, decrease in photosynthetic rate content was observed.

Figure 9 illustrates the per cent removal of heavy metals from the exposed medium after different days of exposure. The per cent removal of heavy metals increased with the increase in exposure period showing a positive correlation. Maximum heavy metal removal was observed in paper mill effluent exposure (21.3±2.2% - F) followed by mercuric nitrate exposure (16.8±1.8% - A) Least removal of heavy metal (7.2±0.6% - E) was noted in toxicants where mercuric nitrate + cadmium chloride + lead nitrate was taken as a mixture (Fig. 9). From the observed data, it can be concluded that all the toxicants tested are deadly toxic at higher concentrations and in case of mercury, lead and paper mill effluent little increment in photosynthetic rate content was noted at maximum allowable concentration in the tested algae. The data also indicated that *Westiellopsis* sp., showed promise as a potential agent for reclamation, at sub-lethal or maximum allowable concentration of the Paper mill effluent, as a toxicant.

Discussion

Pulp and Paper mill waste (effluent) is generally treated to remove the contaminants which can cause serious pollution problem and a threat to the aquatic ecosystem and organisms living therein. The idea of treatment is to remove all toxicants from the waste and convert into an eco-friendly effluent which can be discharged into natural environment and can be returned to water cycle. All the industries adopt either physical methods or chemical methods or both, for the treatment of waste effluent. It was observed that the Pulp and Paper mill located at Jaykaypur is discharging their treated waste effluent into River Nagavalli, which is deadly toxic as evinced from the reports of Mishra *et al.* (2013) and Tripathy *et al.* (2022). One most important point to note was the presence of heavy metals like cadmium, mercury and lead. Higher values of COD and BOD were due to higher amount of organic matter in the effluent. Heavy metals like cadmium, mercury and lead were found in the effluent of the paper mills collected from site-S. Significant amount cadmium was recorded in the collected effluent sample in 2019. The amount of cadmium in the effluent sample was very small but significant and toxic and much more than stipulated guidelines prescribed by Pollution Control Boards (PCBs). Significant amount of mercury was recorded in the collected effluent sample in 2019. Screening of the literature clearly divulges that the effluent analyzed by different authors from different paper industries indicated the availability of significant amount of heavy metals in the discharged effluent. All the industries in India including JK Paper mills physico-chemically treat the effluent before discharging into natural aquatic ecosystems. Most of the treatment processes are either physical or chemical or both as suggested by recovery technology adopted by industries. None of the industries made any significant attempt to treat the treated effluent biologically before discharge to ensure heavy metal free effluent for discharge into the aquatic ecosystems. Waste generation and the quality of wastes depend on the technology and used treatment technology by Pulp and Paper industries. It is not possible to eliminate waste generation by the industrial system (Kaur *et al.,* 2021) but cleaner and environment friendly technology can be used and periodically positive modifications in the technology or alterations in the treatment technology should be adopted. Kaur *et al.* (2021) reported maximum flocculation at 0.3 g/l of chitosan concentration, 81% of total suspended solids could be removed and COD depleted by 78%. These authors suggested that flocculent chitosan (naturally biodegradable) and alum (coagulant) can be used for better treatment of Paper mill effluent but they did not mention anything about presence of heavy metal, its toxicity and possible removal from the effluent of pulp and paper mills.

The toxicity study clearly indicated that mercuric nitrate (A) was more toxic than cadmium chloride (B) and lead nitrate (C) as independent single chemical. The combination of lead and cadmium (D) showed higher toxicity when compared to toxicants A, B and C. All three heavy metals like Hg, Cd and lead in combination (E) was proved to be the most toxic when compared to toxicants A, B, C, D and F. Interestingly Pulp and paper mill effluent (as toxicant F) showed positive values and the alga *Westiellopsis* sp. could survive and resist higher concentrations of the effluent even in presence of three heavy metals in the effluent along with other chemicals. Less impact of the effluent was due to low concentrations of Hg, Cd and Pb in the effluent, presence of other chemicals in the effluent supported growth and dilution of the effluent was an important factor as observed during experiment. The Paper mill effluent contained heavy metals like Hg, Cd and lead, needs attention of all ecologists and the government. Mishra *et al*. (2013) reported reduction of Cu and Hg when the paper mill effluent was treated biologically by using macrophytes. Nwaehiri *et al*. (2019) reported that heavy metals of paper mill effluent can be reduced significantly by bacterial remediation. These heavy metals were absorbed by the aquatic flora and fauna and prolonged exposure to these toxicants lead to bioconcentration of heavy metals. Higher levels of TSS, TDS, Hardness and lower level of DO in the effluent, indicate toxic nature of the effluent and needs biological treatment. Higher BOD values of the effluent depletes the dissolved oxygen of the effluent and also the flowing water when effluent was added (Nwaehiri *et al*., 2019). These authors also indicated that high TSS and TDS will increase turbidity and higher turbidity levels may not support diversity of aquatic organisms. High turbidity will reduce penetration of sunlight and restrict the photosynthetic zone only to surface layer.

Algal scum collected from the contaminated crop fields showed significant amount of heavy metal accumulation. Algae showed heavy metal accumulation to the tune of 0.28± 0.06, 0.17 ± 0.05 and 0.76 ± 0.11 μg/g dry wt in crop field-A. The obtained values indicated the pollution status of the nearby crop fields contaminated with Paper mill effluent mixed with river water. The crop fields situated near the bank of River Nagavalli are irrigated by pumping water from the contaminated river. The algal scum contained 0.76±0.11 μg of Hg/g dry weight, 0.28±0.06 μg of Cd/g dry weight and 0.17±0.05 μg of Pb/g dry weight. Mercury, cadmium and lead are the major abiotic stresses. These metals significantly affect the growth, development and gross production of the crop field inhabiting algal species. Accumulation of cadmium leads to disturbance of chloroplast function and inhibits the enzymes required for chlorophyll biosynthesis and the enzymes necessary for CO_2 fixation (Noor *et al*., 2018). Toxicity study indicated that Hg, Cd, Pb individually and in combination was deadly toxic at higher concentrations. Non-availability of lead in the effluent sample in the year 2019 (in rainy season) is a good sign from environmental stand point. It was observed that the effluent samples collected from the effluent canal in different years indicated almost the same status except few insignificant variations. At LC_{00} (MAC) value of $Hg(NO_3)_2$, Lead nitrate and Paper mill effluent showed better results with two BGA tested and can be used for phycoremediation of Paper mill effluent. At higher concentrations of 6 varieties of toxicants tested showed significant impact like decrease in dry weight of the culture, decrease in chlorophyll and phaeophytin pigment and depletion in photosynthetic rate. Combined heavy metal chemicals were more toxic than individual heavy metal chemicals. From this study, it is evident that the Paper mill effluent is highly toxic. It was observed that the alga, *Westiellopsis prolifica* is more tolerant than *Anabaena cylindrica* towards heavy metals and heavy metal contained pollutants. At higher level of heavy metal toxicants (beyond MAC value) both the algae die. Hence, the effluent should diluted to MAC level and then these two tested algae can inoculated for better phycoremediation of paper mill effluent. In our planned next segment, tests will be conducted to study heavy metal enrichment in the tested algae and per cent removal of heavy metals from the effluent contained algal cultures. Sahu (2017a) reported that *Nostoc, Westiellopsis prolifica*, *Anabaena cylindrica,* and *Oscillatoria* sps could remove significant amount of mercury from the inoculated cultures containing mercury and effluent containing mercury by way of absorption and bioaccumulation of mercury and probable volatilization of mercury in culture flasks. Sahu (2017b) reported that mercury and mercury contained effluent at lower concentration of mercury stimulated growth of blue-green alga (BGA) and showed increase in dry weight, chlorophyll and phaeophytin pigment in exposed cultures compared to control algal cultures. However, at higher concentration of mercury, depletion in dry weight and algal pigments were observed. The same author also indicated that significant amount of residual mercury in BGA was responsible for the damage caused to the exposed BGA system. It was observed that the tested BGA could remove significant amount of heavy metals from the effluent and this alga can be used for biological treatment in treatment protocol along with potential macrophytes as suggested by Mishra *et al*. (2013). Verma *et al*. (2018) reported that mercury contained effluent can be reclamated by cyanobacterium in presence of other essential environmental chemicals, which either can support growth or can react with mercury to form a complex and make mercury non-functional. The availability of sodium, potassium, phosphate in the effluent supported the growth of blue-green algae as evident from the observed results. The "high levels of nitrogen and phosphorus support robust growth of many different species of fresh-water microalgae and thereby represent a very effective remediation strategy at the same time" (Mohsenpour *et al.*, 2021). Bhatti *et al.* (2021) indicated that "biological treatment methods are environmentally friendly and cost effective compared to physicochemical methods of treating wastewater. The treatment of pulp and paper mill wastewater effluents and recycling of water from pulp and paper mills by microalgae is particularly interesting from the point of view of environmental sustainability". These authors also indicated that "treatment of pulp and paper mill wastewater effluents and recycling of water from pulp and paper mills by microalgae was particularly interesting from the point of view of environmental sustainability". "Biodegradation and toxicity reduction of pulp paper mill waste water could be achieved by treating the waste water by inducting isolated lactase producing *Bacillus cereus* AKRC03" (Kumar and Chandra, 2021). The same author reported 70% reduction of toxicity of waste water with *Phaseolus mungo* L. seeds and recommended the bacteria for use in bio-augmentation process for detoxification and degradation of paper mill waste water. In this study we tried to indicate that BGA/cyanobacteria can reduce heavy metal content significantly and these heterocystous Blue Green algae can also fix atmospheric nitrogen and the BG Algal exudates (extra-cellular products) can be used as a biofertilizer if used in crop fields. The amount of cadmium in the effluent sample was very small but significant and toxic and much more than stipulated guidelines prescribed by Pollution Control Board (PCB). From the toxicity study, it can be inferred that heavy metals at low concentrations (sub-lethal/maximum allowable concentration (MAC)) stimulate growth as evident from dry weight, chlorophyll content and photosynthetic rate analysis. The MAC value can be used for diluting the effluent or any other heavy metal for safe discharge into the environment. In case of toxicant-F, where effluent of the Paper mill was taken, increase in photosynthetic rate was noted at LC_{00} in both the tested algae and at high doses of toxicant at LC_{50} decrease in photosynthetic rate was observed. Li *et al*. (2021) indicated that "high levels of heavy metal stress may cause irreversible damage to micro-algal chloroplasts and prevent photosynthesis, thereby leading to cell death" (Yu *et al*, 2019; Alho *et al*., 2019). Studies of Hussain *et al.* (2021) working on cadmium stress in paddy fields support of our observation and conclusion. From the observed data, it can be concluded that all the toxicants tested are deadly toxic at higher doses and in case of mercury, lead and paper mill effluent little increment in photosynthetic rate content was noted at maximum allowable concentration in both the tested algae. The observed data also indicated that *Westiellopsis* sp. *w*as more tolerant and resistant. This alga showed promise as a potential reclamator at sub-lethal or maximum allowable concentration of the heavy metal toxicants and Paper mill effluent containing heavy metals as a toxicant and *Westiellopsis* sp. can be used for phycoremediation of paper mill effluent. Al-Jabri *et al*. (2021) reported that "microalgal bioremediation could be integrated with existing treatment methods or adopted as the single biological method for efficiently treating wastewater. Other drawbacks of conventional wastewater treatment processes are high energy consumption, greenhouse gas emissions, recyclable resource wastage and excessive solid land filling. Several technologies such as hydrothermal carbonization, anaerobic digestion and pyrolysis were developed and applied to maximize energy recovery from waste bio-solids to phase out from excessive solid land filling. Monitoring, analyzing and characterizing all types of toxicants including heavy metals in aquatic environments indicates the water resource condition, status, usability of water for drinking purposes and agricultural purposes and above all protection of plants, animals, environmental health and human health (Stahl *et al*., 2021). These authors also indicated that aquatic organisms may accumulate contaminants to significant levels much more than the stipulated USEPA (2000) guidelines. This type of bioaccumulation of non-degradable heavy metals in different trophic levels might lead to biomagnification in the ecosystem and finally human health suffers.

Conclusion

Pulp and Paper Mill effluent collected from Jaykaypur in different years was found to be highly toxic. The effluent waste is discharged into River Nagavalli after physicochemical treatment inside the premises of the industry. No proper biological treatment of the effluent is made prior to discharge into the natural environment. Effluent contained highly toxic organic debris and inorganic chemicals including toxic metals like Hg, Cd and Pb. The effluent is loaded with organic debris with high BOD and COD value with significant amount of TDA and TSS. Toxicity study indicated the safe level (MAC) concentrations of different heavy metals individually and in combination. Combined heavy metals were deadly toxic even at sub-lethal concentrations. Significant residual accumulation of mercury, cadmium and lead in economically important plants in crop fields and significant impact on pigment content indicated the impact of the effluent at down stream of the river and needs attention to avoid a future man made disaster. This study indicated that the effluent once diluted to its MAC value showed interesting results. *Westiellopsis prolifica* (BGA / Cyanobacterium) showed potency for reclamation studies and can be used in phycoremediation purposes. This alga along with potential bacteria and aquatic macrophytes can be used for biological treatment of Pulp and Paper industry effluent.

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