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Eco-toxicity and temperature stress studies of heavy metal contained Pulp and Paper Mill industrial effluent on cyanobacteria under laboratory controlled conditions

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ABSTRACT

An attempt is made to study the toxicity of the discharged Paper and Pulp industrial effluent and impact of temperature stress on two microalgae. The industrial effluent is deadly toxic and contained heavy metal like cadmium, mercury and lead at higher concentrations. The values were much more than the stipulated limit prescribed by PCBs. The year wise comparison of effluent analysis data indicated that the industry is not treating the effluents biologically to eliminate the heavy metals present in the effluent but discharges the effluent straight in to the river. The toxicity study indicated that mercuric nitrate, cadmium chloride and lead nitrate individually is toxic to microalgae at lethal concentrations. The study also indicated that these toxic heavy metals when mixed become deadly toxic and the LC values decreased significantly. The effluent containing all the three heavy metals was less toxic when compared to individual chemicals or chemicals in combination probably due to the presence of other chemicals in the effluent which might have decreased the toxicity of the effluent. Out of the two alga tested *Westiellopsis prolifica* is more tolerant than *Anabaena cylindrica*. Both the alga showed similar behaviour towards different toxicant chemicals tested in the present study. Both *Westiellopsis prolifica* and *Anabaena cylindrica* are nitrogen fixers and farmer friendly, their tolerance towards heavy metal toxicants and heavy metal contained toxicant like effluent of the Paper mill effluent bears significance. These two algae showed potency and can be used as potential detoxifiers of the heavy metal contaminated sites.

Key words: *Toxicity, Paper and pulp mill effluent, temperature stress, Cyanobacteria, Mercury, Cadmium, Lead.*

Introduction

Heavy metal pollution in the environment is a serious threat for all plant and animal life inhabiting in different environmental segments. There were many sources for the heavy metals to enter in to the environment. One of the most important sources of heavy metal pollution is the industries. The Paper industries play a crucial role to pollute the river water by their treated / untreated effluent waste. Medhi *et al*. (2011) reported that the physico-chemical characteristics of a paper mill industry effluent waste were exceedingly high than the stipulated limits. Mishra *et al*. (2013) studied the physico-chemical properties of the effluent water discharged from JK Paper mill situated at Jaykaypur in 2013

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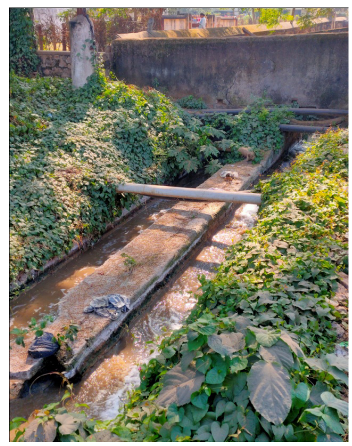
and high values were indicated. The same authors also attempted to suggest the reclamation strategy by some aquatic plants and micro algae. No detailed exhaustive analysis and detailed in depth study was carried out by any other worker on Paper mill effluents. The present work was planned to study the eco-toxicity of heavy metal contained Pulp and Paper Mill effluent and temperature stress on two heterocystous cyanobacteria under laboratory controlled conditions.

Materials and Methods

Location of the industry and collection of samples

JK Pulp and Paper Mills, Jaykaypur was established in the year 1962. The industry 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 825.4mts and joins the River Nagavalli (Photo map-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] (Photograph 2) in air tight plastic containers and brought in cold containers to the laboratory for analysis.

Analysis of select physico-chemical parameters were carried out following the procedure of APHA (1985) and EC (1979). In addition the field analysis kit (Systronics, India), portable instruments were used to measure pH, conductivity and temperature of water and effluent samples. Dissolved oxygen was measured by modified Winkler's method (APHA, 1985).Effluent samples were brought in plastic containers and stored in the refrigerator for analysis and for laboratory experimental work. Measurement of mercury in the samples was carried out by acid digestion in Bethige's apparatus followed the basic principle of Wantorp 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). Known amount of sample was taken in a Klein's apparatus (digester) with acid digestion mixture and digested till the whole sample was fully digested (Wantorp and Dyfverman, 1955). After completion of digestion, the digested extract was



Phot. 2. Effluent collection site (S) at effluent canal leaving the Paper industry and joining the River Nagavalli, at Jaykayour.)

Photo 1. Google map indicating JK Papers Ltd., Effluent treatment plant; Effluent canal, Effluent joining Nagavalli River and site- S (Effluent collection site from Effluent canal.

cooled to room temperature. After cooling, the cadmium and lead content of the digested extract was estimated following the procedure of Yoshida *et al*. (1976).

Toxicity study: For toxicity study of the pollutants, mercuric nitrate, lead nitrate, cadmium chloride of pure grade (Analytical pure grade) was prepared by dissolving in distilled water. A graded series of concentrations of individual and mixed toxicants ranging from 0.1mg.l^{-1} to 2.0 mg.l^{-1} (V/V) was prepared in different experimental conical flasks and UV sterilized in a Laminar Airflow. The dilutions were made with the 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 0.5ml to 5ml / l was prepared. The filtrate was taken (100 ml each) in conical flasks and UV sterilized under aseptic conditions in a Laminar airflow. The effluent was diluted with nutrient medium. The homogenized algae were inoculated and the flasks were kept on culture racks. 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. The inoculated flasks were kept inside the culture room at 28 ± 2 °C and under 14 hours illumination at the intensity of 2400 ± 200 Lux and were shaken periodically daily to avoid clumping of cells. The test algae were 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. From this, different mortality percentage and the lethal concentration values, maximum allowable concentration (MAC) were determined. From the toxicity testing as described by Sahu (1987), the marked X, Y and Z concentrations of the toxicants were selected for future experiments.

Results and Observations

Table 1 represents the physico-chemical properties of the discharged effluent collected from the effluent canal at different time periods. The table also showed the comparison of effluent analysis data at different time periods. All the parameters studied showed significantly higher values much more than the values indicated by PCBs. Periodic and constant monitoring of the discharged effluent revealed minor variation in select parameters studied during 2015 to 2019. The odour of discharged effluent is pungent with rotten egg smell and the colour ranged from deep yellowish brown to yellowish brown during 2015 to 2019. The pH ranged from 7.4±0.4 to 7.9±0.4 and the temperature ranged between 30.8 ± 0.8°C to 32.5 ± 0.7°C. The dissolved oxygen present in the discharged effluent ranged from 1.5 ± 0.5 to 2.8 ± 0.6 mg l^{-1} far less than the dissolved oxygen of normal water of a river. Significant amount of copper, cadmium, lead and mercury was recorded in the year 2015. In 2015, the effluent showed 0.11 ± 0.04 mg of copper.l^{-1}, 0.39 ± 0.11 mg of cadmium l^{-1}, 0.38 ± 0.12 mg mercury l^{-1} and 0.12 ± 0.06 mg of lead l^{-1} in the effluent sample collected. In 2017, the effluent showed 0.09 ± 0.05mg of copper l^{-1}, 0.31 ± 0.05 mg of cadmium l^{-1}, 0.49 ± 0.06 mg mercury l^{-1} and 0.09±0.03 mg of lead l^{-1} in the effluent sample. In 2018, the effluent showed 0.29 ± 0.04 mg of cadmium l^{-1}, 0.39 ± 0.08 mg mercury l^{-1}, 0.11 ± 0.04mg of lead l^{-1} and presence of copper was not detected in the effluent sample collected. In 2019, the effluent sample showed 0.32 ± 0.09 mg of cadmium l^{-1}, 0.46 ± 0.14 mg mercury l^{-1}, and presence of lead and copper was not detected (Table 1). Non detection of heavy metals is a good sign but the very availability of other heavy metals in a significant quantity warrants attention.

To study the alterations of lethal concentration values, experiments were conducted to test the impact of lower and higher temperature stress on toxicity values. One set was designed with 5 °C higher than the normal room temperature and the second set was designed at 5 °C lower than the room temperature for mercuric nitrate. Similarly another set was designed to test the alteration of lethal concentration values experiments were designed to study the impact of temperature on toxicity values. One set was designed with 10 °C higher than the normal room temperature and the second set was designed at 10 °C lower than the room temperature for mercuric nitrate. The MAC value for mercuric nitrate exposed alga after 15 days of exposure was 0.172 mg/ l at 28 °C, normal room temperature. The MAC value for mercuric nitrate exposed alga after 15days of exposure were 0.176 mg/L and 0.164mg/l at 23 °C and 18 °C, where no significant change was noted. It seems the alga was more comfortable at 5 °C lower than the room temperature and same value was also recorded at 10 °C lower than room temperature. But when the temperature was raised by 5 ^{o}C from the normal value, the MAC value was 0.137mg/l and the MAC was 0.104 mg/l at 10 ^{o}C higher than the room temperature {Table-2}. From the MAC value it is well evident that during summer when the temperature was high, higher death rate was marked. The alga could tolerate low temperature under mercuric nitrate stress but not in high temperature stress. In field conditions, the alga dies in summer season and survives in winter season. The LC_{00} values were 0.172 mg/l at 28^{o}C; 0.176mg/l at 23^{o}C (5 ^{o}C lower than room temperature); 0.164 mg/l at 18^{o}C (10 ^{o}C lower than room temperature); 0.137 mg/L at 33^{o}C (5^{o}C higher than room temperature) and 0.104 mg/l at 38 ^{o}C (10 ^{o}C higher than the room temperature) for the alga {Table-2}. The LC_{50} values were 0.231 mg/l at 28^{o}C; 0.242 mg/l at 23^{o}C (5 ^{o}C lower than room temperature); 0.218 mg/l at 18^{o}C (10^{o}C lower than room temperature); 0.174 mg/l at 33 ^{o}C (5 ^{o}C higher than room temperature) and 0.128 mg/l at 38^{o}C (10 ^{o}C higher than the room temperature) (Table 2). The LC_{90} values were 0.268 mg/l at 28 ^{o}C; 0.273 mg/l at 23 ^{o}C (5 ^{o}C lower than room temperature); 0.247 mg/l at 18^{o}C (10 ^{o}C lower than room temperature); 0.178 mg/l at 33 ^{o}C (5 ^{o}C higher than room temperature) and 0.144 mg/l at 38^{o}C (10^{o}C higher than the room temperature)(Table 2). The LC_{100} values were 0.276 mg/l at 28^{o}C; 0.283 mg/l at 23^{o}C (5^{o}C lower than room temperature); 0.254 mg/l at 18 ^{o}C (10 ^{o}C lower than room temperature); 0.183 mg/l at 33^{o}C (5 ^{o}C higher than room temperature) and 0.163 mg/l at 38 ^{o}C (10 ^{o}C higher than the room temperature) (Table 2). From the above result it is clear that at higher temperature the alga could not survive in mercuric nitrate exposure under laboratory controlled condition, whereas, the alga could resist at lower temperatures but not higher temperatures under laboratory controlled conditions. The exposed alga *Anabaena cylindrica* grew well at 0.33 mg of lead nitrate /l after 15 days of exposure where no death was recorded compared to control alga. At LC_{00} (0.361mg of lead nitrate / liter) also no death was recorded but at 0.382 mg of lead nitrate/l dose death started (1.1% death was noticed). Hence, 0.33 mg of lead nitrate / liter was recommended for the toxicant as safe dose. For future experimental purpose 0.361 mg of lead nitrate /l was considered as LC_{00} (Conc. X) value. At 0.764 mg of lead nitrate/l 50% death of the algal population was noticed and was considered as LC_{50} (Conc. Y) value . At 1.143mg of lead nitrate/l 90% death of the algal population was noticed and was considered as LC_{90} (Conc. Z) value. At 1.302 mg of lead nitrate / l 100%death of the algal population was noticed and was considered as LC_{100} value after 15 days of exposure. Death was not marked in the control sets during the experimental periods. Future experiments were conducted taking Conc. X (0.361mg of lead nitrate / l), Conc. Y (0.764 mg of lead nitrate /l) and Conc. Z (1.143 mg of lead nitrate/l) along with a control set for comparison. For toxicity study of the pollutant, cadmium chloride solution was prepared by taking analytical grade cadmium chloride and dissolved in distilled water. With the increase in toxicant concentration, the percent survival decreased and hundred percent

Table 1. Comparison and analysis of physico-chemical parameters of the effluent sample collected from the effluent canal before joining the river Nagavalli at Jaykaypur, Odisha. Data presented are the mean ± standard deviation; NA = Not available.

Parameters of study. (mg.l^{-1})	Year wise study by the authors in different years.			
	2015	2017	2018	2019
Colour	Deep Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Odour	Pungent, Strong H_2S smell	Pungent, Strong H_2S smell	Pungent, Rotten egg smell	Pungent, Filthy smell
pH	7.8±0.5	7.5±0.6	7.4±0.4	7.9±0.4
Temperature, °C	32.5±0.7	32.2±0.4	30.8±0.8	31.2±1.2
Dissol. oxygen	1.6±0.5	1.8±0.3	2.8±0.6	1.5±0.4
Copper	0.11±0.04	0.09±0.05	NA	NA
Cadmium	0.39±0.11	0.31±0.05	0.29±0.04	0.32±0.09
Mercury	0.38±0.12	0.49±0.06	0.39±0.08	0.46±0.14
Lead	0.12±0.06	0.09±0.03	0.11±0.04	NA

deaths were noticed at 0.943 mg/l of cadmium chloride with in a period of 15 days. The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.21 mg/l. The lethal concentration values for 15 days of exposure periods have been outlined below. The LC_{10} value was 0.251 mg/l. The LC_{50} value was 0.584mg of cadmium chloride / L. LC_{90} value was 0.841 mg/l and LC_{100} value was 0.943mg/L, for this particular alga, *Anabaena cylindrica.* The control set showed 100% survival through out the experimental periods. The same data can also be interpreted as 50% survival at 0.584 mg/l, 10% survival at 0.841 mg/l, and 100% survival at 0.251 mg/l was marked. Out of the above concentrations, safe MAC value of 0.251 mg/l was selected as 'X'; LC_{50} or PS_{50} of 0.584 $mg.l^{-1}$ was selected as 'Y' and LC_{90} or PS_{10} value of 0.943 $mg.l^{-1}$ was selected as 'Z' for conducting future experiments (Table 2). To test the alteration of lethal concentration values experiments were designed to study the impact of temperature stress on toxicity values. One set was designed with 5 °C higher than the normal room temperature and the second set was designed at 5 °C lower than the room temperature for lead nitrate. Similarly another set was designed to test the alteration of lethal concentration values experiments were designed to study the impact of temperature on toxicity values. Another set was designed with 5 °C higher than the normal room temperature and the second set was designed at 5 °C lower than the room temperature for lead nitrate. The MAC value for lead nitrate exposed alga after 15days of exposure was 0.361 mg/l at 28 °C, normal room temperature. The MAC value for lead nitrate exposed alga after 15days of exposure were 0.381 mg/l and 0.372 mg/l at 23 °C and 18 °C, where no significant change was noted at lower concentrations rather the alga could tolerate little higher concentrations of the toxicant. It seems the alga was more comfortable at 5^0C lower than the room temperature and approximately same value was also recorded at 10 °C lower than room temperature. But when the temperature was raised by 5 °C, the MAC value was 0.352mg/L and the MAC was 0.274 mg/l at 10^0C higher than the room temperature. From the MAC value it is well evident that during summer when the temperature was high, higher death rate was marked. The alga could tolerate low temperature under lead nitrate stress but not in high temperature stress. In field conditions, the alga dies in summer season and survives in winter season. The LC_{00} values were 0.361 mg/l at 28°C; 0.382 mg/l at 23 °C (5 °C lower than room temperature); 0.372 mg/l at 18°C (10 °C lower than room temperature); 0.352 mg/l at 33°C (5°C higher than room temperature) and 0.274mg/l at 38°C (10°C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). The LC_{50} values were 0.764 mg/l at 28°C; 0.811 mg/L at 23°C (5°C lower than room temperature); 0.804 mg/l at 18°C (10°C lower than room temperature); 0.741 mg/l at 33°C (5°C higher than room temperature) and 0.526 mg/l at 38°C (10 °C higher than the room temperature) (Table 2). The LC_{90} values were 1.143 mg/l at 28 °C; 1.212 mg/l at 23 °C (5°C lower than room temperature); 1.151 mg/l at 18°C (10°C lower than room temperature); 1.125 mg/l at 33 °C (5°C higher than room temperature) and 0.642 mg/l at 38°C (10°C higher than the room temperature) (Table 3). The LC_{100} values were 1.302 mg/l at 28°C; 1.541 mg/l at 23°C (5°C lower than room temperature); 1.283 mg/l at 18 °C (10 °C lower than room temperature); 1.277 mg/l at 33°C (5°C higher than room temperature) and 0.734 mg/l at 38°C (10°C higher than the room temperature)(Table 2). The LC_{100} values were 1.3 mg/l at 28°C; 1.541 mg/L at 23 °C (5°C lower than room temperature); 1.283 mg/l at 18 °C (10°C lower than room temperature); 1.277 mg/l at 33°C (5 °C higher than room temperature) and 0.734 mg/l at 38 °C (10°C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). From the above result it is clear that at higher temperature the alga could not survive in lead nitrate exposure under laboratory controlled condition, whereas, the alga could sustain at lower temperatures under laboratory controlled conditions. To study the alterations of lethal concentration values experiments were designed to study the impact of temperature stress on toxicity values. One set was designed with 10 °C higher than the normal room temperature and the second set was designed at 10 °C lower than the room temperature for lead nitrate. Similarly another set was designed to test the alteration of lethal concentration values experiments were designed to study the impact of temperature on toxicity values. One set was designed with 10 °C higher than the normal room temperature and the second set was designed at 10^0C lower than the room temperature for cadmium chloride. The MAC value for cadmium chloride exposed alga after 15days of exposure was 0.251 mg/l at 28 °C, normal room temperature. The MAC value for cadmium chloride exposed alga after 15days of exposure were 0.272 mg/l and 0.261 mg/l at 23 °C and 18 °C, where insignificant change was noted. It seems the alga was more comfortable at 5 °C lower than the room temperature and same value was also recorded at 10 °C lower than room temperature. But when the temperature was raised by 5 °C, the MAC value was 0.213 mg/L and the MAC was 0.165 mg/l at 10 °C higher than the room temperature (Table 2). From the MAC value it is well evident that during summer when the temperature was high, higher death rate was marked. The alga could tolerate low temperature under cadmium chloride stress but not in high temperature stress. When we compared the cadmium data with lead nitrate data, it is a fact that

Table 2. Lethal concentration 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), at low & high temperatures for the alga, *Anabaena cylindrica*, Lemm. after 15 days of exposure under laboratory controlled conditions.

Lethal concentration (LC) values	A. Mercuric nitrate, mg/liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.164	0.176	0.172	0.137	0.104
LC_{50} (Y)	0.218	0.242	0.231	0.174	0.128
LC_{90} (Z)	0.247	0.273	0.268	0.178	0.144
LC_{100}	0.254	0.283	0.276	0.183	0.163
Lethal concentration (LC) values	B. Lead nitrate, mg/liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.372	0.382	0.361	0.352	0.274
LC_{50} (Y)	0.804	0.811	0.764	0.741	0.526
LC_{90} (Z)	1.151	1.212	1.143	1.125	0.642
LC_{100}	1.283	1.541	1.302	1.277	0.734
Lethal concentration (LC) values	C. Cadmium chloride, mg/liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.261	0.272	0.251	0.213	0.165
LC_{50} (Y)	0.593	0.611	0.584	0.542	0.361
LC_{90} (Z)	0.822	0.882	0.841	0.821	0.544
LC_{100}	0.895	0.961	0.943	0.892	0.623
Lethal concentration (LC) values	D. Lead nitrate + Cadmium chloride, (1:1 ratio) mg/liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.166	0.212	0.184	0.165	0.094
LC_{50} (Y)	0.281	0.351	0.325	0.254	0.133
LC_{90} (Z)	0.492	0.532	0.542	0.412	0.162
LC_{100}	0.581	0.634	0.626	0.444	0.193
Lethal concentration (LC) values	E. Mercuric nitrate + Lead nitrate + Cadmium chloride mixture solution, (1:1:1 ratio) mg/liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.124	0.143	0.122	0.121	0.072
LC_{50} (Y)	0.184	0.258	0.225	0.193	0.095
LC_{90} (Z)	0.248	0.431	0.321	0.272	0.143
LC_{100}	0.256	0.463	0.363	0.337	0.156
Lethal concentration (LC) values	F. Paper Mill effluent (containing Hg, Pb and Cd) ml / liter				
	18^0C(-10^0C)	23^0C(-5^0C)	28^0CNormal	33^0C(+5^0C)	38^0C(+10^0C)
LC_{00} (X)	0.714	0.782	0.728	0.663	0.342
LC_{50} (Y)	1.183	1.265	1.145	0.962	0.581
LC_{90} (Z)	2.283	2.544	2.361	1.954	1.462
LC_{100}	2.354	2.783	2.477	2.183	1.753

cadmium chloride is more toxic than lead nitrate for this particular alga. In field conditions, the alga dies in summer season and survives in winter season. The LC_{00} values were 0.251 mg/l at 28 °C; 0.272 mg/l at 23 °C (5°C lower than room temperature); 0.261 mg/l at 18 °C (10 °C lower than room temperature); 0.213 mg/l at 33 °C (5 °C higher than room temperature) and 0.165 mg/L at 38 °C (10 °C higher than the room temperature) (Table 4). The LC_{50} values were 0.584 mg/l at 28 °C; 0.611 mg/l at 23 °C (5°C lower than room temperature); 0.593 mg/l at 18°C (10 °C lower than room temperature); 0.542 mg/l at 33°C (5 °C higher than room temperature) and 0.361 mg/l at 38 °C (10 °C higher than the room temperature) (Table 4). The LC_{90} values were 0.841 mg/l at 28 °C; 0.882 mg/l at 23°C (5 °C lower than room temperature); 0.822 mg/l at 18°C (10°C lower than room temperature); 0.821 mg/l at 33°C (5 °C higher than room temperature) and 0.544 mg/l at 38°C (10°C higher than the room temperature) (Table 2). The LC_{100} values were 0.943 mg/l at 28°C; 0.961 mg/l at 23°C (5 °C lower than room temperature); 0.895 mg/L at 18 °C (10°C lower than room temperature); 0.892 mg/l at 33 °C (5 °C higher than room temperature) and 0.623 mg/l at 38 °C (10 °C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). From the above result it is clear that at higher temperature the alga could not survive in cadmium chloride exposure under laboratory controlled condition, whereas, the alga could sustain at lower temperatures under laboratory controlled conditions.

To study the alterations of lethal concentration values experiments were designed to study the impact of temperature on toxicity values. One set was designed with 10 °C higher than the normal room temperature and the second set was designed at 10 °C lower than the room temperature for lead nitrate + cadmium chloride mixture at 1:1 ratio. Similarly another set was designed to test the alteration of lethal concentration values, experiments were designed to study the impact of temperature on toxicity values. One set was designed with 10 °C higher than the normal room temperature and the second set was designed at 10 °C lower than the room temperature for lead nitrate + cadmium chloride mixture at 1:1 ratio. When we have taken a mixture of two heavy metals like lead nitrate + cadmium chloride at 1:1 ratio as the test chemical, very interesting results were obtained. From the data it can be well said that the mixture of heavy metals were more toxic than the individual toxicants as such type of combinations were seen in field conditions of the contaminated sites. The MAC or LC_{00} values were 0.184 mg/l at 28 °C; 0.212 mg/l at 23 °C (5 °C lower than room temperature); 0.166 mg/l at 18 °C (10°C lower than room temperature); 0.165 mg/l at 33 °C (5 °C higher than room temperature) and 0.094 mg/L at 38 °C (10°C higher than the room temperature)(Table 2). The LC_{50} values were 0.325 mg/l at 28 °C; 0.351 mg/l at 23 °C (5 °C lower than room temperature); 0.281 mg/l at 18 °C (10 °C lower than room temperature); 0.254 mg/l at 33 °C (5°C higher than room temperature) and 0.133 mg/l at 38 °C (10 °C higher than the room temperature) (Table 2). The LC_{90} values were 0.542 mg/l at 28 °C; 0.532 mg/l at 23 °C (5°C lower than room temperature); 0.492 mg/l at 18 °C (10 °C lower than room temperature); 0.412 mg/l at 33 °C (5°C higher than room temperature) and 0.162 mg/l at 38 °C (10 °C higher than the room temperature) (Table 2). The LC_{100} values were 0.626 mg/l at 28 °C; 0.634 mg/L at 23 °C (5 °C lower than room temperature); 0.581 mg/L at 18 °C (10°C lower than room temperature); 0.444 mg/l at 33 °C (5 °C higher than room temperature) and 0.193 mg/l at 38 °C (10°C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). From the above result it is clear that at higher temperature the alga could not survive in lead nitrate + cadmium chloride mixture exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any waste containing these two heavy metals in combination can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. When we have taken a mixture of three heavy metals like mercuric nitrate + lead nitrate + cadmium chloride at 1:1:1 ratio as the test chemical, very interesting results were obtained. From the data it can be well said that the mixture of heavy metals were more toxic than the individual toxicants as such type of combinations were seen in field conditions of the contaminated sites. The MAC or LC_{00} values were 0.122 mg/l at 28 °C; 0.143 mg/l at 23°C (5 °C lower than room temperature); 0.124 mg/l at 18 °C (10 °C lower than room temperature); 0.121 mg/l at 33 °C (5°C higher than room temperature) and 0.072 mg/l at 38 °C (10°C higher than the room temperature){Table-2}. The LC_{50} values were 0.225 mg/l at 28°C; 0.258 mg/l at 23°C (5°C lower than room temperature); 0.184 mg/L at 18°C (10°C lower than room temperature); 0.193 mg/l at 33 °C (5°C higher than room temperature) and 0.095 mg/l at 38 °C (10 °C higher than the room temperature) (Table 2). The LC_{90} values were 0.321 mg/l at 28 °C; 0.431 mg/l at 23 °C (5°C lower than room temperature); 0.248 mg/l at 18°C (10 °C lower than room temperature); 0.272 mg/l at 33 °C (5°C higher than room temperature) and 0.143mg/L at 38°C (10°C higher than the room temperature)(Table 2). The LC_{100} values were 0.363 mg/l at 28 °C; 0.463 mg/l at 23°C (5°C lower than room temperature); 0.2561 mg/l at 18 °C (10 °C lower than room temperature); 0.337 mg/l at 33 °C (5°C higher than room temperature) and 0.156 mg/l at 38°C (10°C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). From the above result it is clear that at higher temperature the alga could not survive in mercuric nitrate + lead nitrate + cadmium chloride mixture exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any waste containing these three heavy metals in combination can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals.

The effluent containing the above cited three heavy metals like Hg, Cd and Pb as the test chemical, interesting results were obtained. From the data it can be well said that the effluent containing heavy metals were less toxic than the individual toxicants or toxicants in combination, as such type of combinations were seen in field conditions of the contaminated sites. The MAC or LC_{00} values were 0.728ml/L at 28 °C; 0.782 ml/l at 23 °C (5 °C lower than room temperature); 0.714 mg/l at 18 °C (10 °C lower than room temperature); 0.663 mg/l at 33 °C (5°C higher than room temperature) and 0.342 mg/l at 38 °C (10°C higher than the room temperature) for the alga (Table 2). The LC_{50} values were 1.145 mg/l at 28°C; 1.265mg/L at 23°C (5°C lower than room temperature); 1.183 mg/l at 18 °C (10 °C lower than room temperature); 0.962 mg/l at 33 °C (5°C higher than room temperature) and 0.581 mg/l at 38°C (10°C higher than the room temperature) (Table 2). The LC_{90} values were 2.361 mg/l at 28 °C; 2.544 mg/l at 23°C (5 °C lower than room temperature); 2.283 mg/L at 18 °C (10°C lower than room temperature); 1.954 mg/l at 33 °C (5 °C higher than room temperature) and 1.462 mg/l at 38 °C (10°C higher than the room temperature)(Table 2). The LC_{100} values were 2.477 mg/l at 28 °C; 2.783 mg/l at 23 °C (5°C lower than room temperature); 2.354 mg/l at 18 °C (10°C lower than room temperature); 2.183 mg/l at 33 °C (5°C higher than room temperature) and 1.753 mg/l at 38°C (10°C higher than the room temperature) for the alga, *Anabaena cylindrica* (Table 2). From the above result it is clear that at higher temperature the alga could not survive in the Paper mill effluent exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any liquid waste containing these three heavy metals in combination with other organic chemicals like effluent can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals.

The second set was planned to test the toxicity of different heavy metal chemicals and the paper mill effluent on *Westiellopsis prolifica* under similar set of conditions. Table 3 indicated the toxicity study the Lethal concentration 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/liter) (F), at low and high temperatures for the alga, *Westiellopsis prolifica*, Janet after 15days of exposure under laboratory controlled conditions (Table 3). Table-3 gives a clear picture of toxicity values of different toxicants on the alga, *Westiellopsis prolifuca* after 15 days of exposure. When the data of Table 2 and 3 were compared, we found that *Westiellopsis prolifica* is more tolerant and resistant than *Anabaena cylindrica*. In both the tables, we find almost similar data except minor changes at third digit level. Hence detailed writing of the data was avoided. The collected effluent containing Hg, Cd and Pb showed interesting results. From the data it can be well said that the effluent containing heavy metals were less toxic than the individual toxicants or toxicants in combination to the tested alga, as such type of combinations were seen in field conditions of the contaminated sites. The MAC or LC_{00} values were 0.72ml/l at 28 °C; 0.78 ml/l at 23 °C (5°C lower than room temperature); 0.71 mg/l at 18°C (10 °C lower than room temperature); 0.66 mg/l at 33 °C (5°C higher than room temperature) and 0.34 mg/l at 38°C (10°C higher than the room temperature)

(Table 3). The LC_{50} values were 1.14 mg/l at 28 °C; 1.26 mg/l at 23°C (5°C lower than room temperature); 1.18 mg/l at 18 °C (10 °C lower than room temperature); 0.96 mg/l at 33 °C (5°C higher than room temperature) and 0.58 mg/l at 38 °C (10°C higher than the room temperature) (Table 3). The LC_{90} values were 2.36 mg/l at 28 °C; 2.54 mg/l at 23 °C (5°C lower than room temperature); 2.28 mg/l at 18°C (10°C lower than room temperature); 1.95 mg/l at 33°C (5°C higher than room temperature) and 1.46 mg/l at 38 °C (10 °C higher than the room temperature)(Table 3). The LC_{100} values were

Table 3. Lethal concentration 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 & Paper Mill effluent containing Hg, Pb and Cd (ml/l) (F), at low and high temperatures for the alga, *Westiellopsis prolifica*, Janet after 15 days of exposure under laboratory controlled conditions.

Lethal concentration (LC) values	A. Mercuric nitrate, mg/l				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C (+10⁰C)
LC_{00} (X)	0.17	0.18	0.18	0.13	0.10
LC_{50} (Y)	0.21	0.24	0.24	0.16	0.12
LC_{90} (Z)	0.24	0.27	0.26	0.17	0.14
LC_{100}	0.25	0.28	0.27	0.18	0.16
Lethal concentration (LC) values	**B. Lead nitrate, mg/l**				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C (+10⁰C)
LC_{00} (X)	0.37	0.38	0.36	0.35	0.27
LC_{50} (Y)	0.80	0.81	0.76	0.74	0.52
LC_{90} (Z)	1.15	1.21	1.14	1.12	0.64
LC_{100}	1.28	1.54	1.30	1.27	0.73
Lethal concentration (LC) values	**C. Cadmium chloride, mg/l**				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C (+10⁰C)
LC_{00} (X)	0.26	0.27	0.25	0.21	0.16
LC_{50} (Y)	0.59	0.61	0.58	0.54	0.36
LC_{90} (Z)	0.82	0.88	0.84	0.82	0.54
LC_{100}	0.89	0.96	0.94	0.89	0.62
Lethal concentration (LC) values	**D. Lead nitrate + Cadmium chloride, (1:1 ratio) mg/l**				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C(+10⁰C)
LC_{00} (X)	0.16	0.21	0.18	0.16	0.09
LC_{50} (Y)	0.28	0.35	0.32	0.25	0.13
LC_{90} (Z)	0.49	0.53	0.54	0.41	0.16
LC_{100}	0.58	0.63	0.62	0.44	0.19
Lethal concentration (LC) values	**E. Mercuric nitrate + Lead nitrate + Cadmium chloride mixture solution, (1:1:1 ratio) mg/l**				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C (+10⁰C)
LC_{00} (X)	0.12	0.14	0.12	0.12	0.07
LC_{50} (Y)	0.18	0.25	0.22	0.19	0.09
LC_{90} (Z)	0.24	0.43	0.32	0.272	0.14
LC_{100}	0.25	0.46	0.36	0.33	0.15
Lethal concentration (LC) values	**F. Paper Mill effluent (containing Hg, Pb and Cd) ml/l**				
	18⁰C (-10⁰C)	23⁰C (-5⁰C)	28⁰C Normal	33⁰C (+5⁰C)	38⁰C (+10⁰C)
LC_{00} (X)	0.71	0.78	0.72	0.66	0.34
LC_{50} (Y)	1.18	1.26	1.14	0.96	0.58
LC_{90} (Z)	2.28	2.54	2.36	1.95	1.46
LC_{100}	2.35	2.78	2.47	2.18	1.75

2.47mg/l at 28 °C; 2.78 mg/l at 23 °C (5°C lower than room temperature); 2.35 mg/l at 18 °C (10 °C lower than room temperature); 2.18 mg/l at 33 °C (5°C higher than room temperature) and 1.75 mg/l at 38°C (10°C higher than the room temperature) for the alga, *Westiellopsis prolifica* (Table 3). From the above result it is clear that at higher temperature the alga could not survive in the Paper mill effluent exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any liquid waste containing these three heavy metals in combination with other organic chemicals like effluent can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. Both the algae are heterocystous blue-green alga and atmospheric nitrogen fixers, inhabitants of crop fields and are eco-friendly and farmer friendly. Mostly these two alga show similar behavior and almost show the similar growth pattern probably behave and react in a similar way with the toxicants in the environment.

Discussion

The discharge of effluent along with the heavy metals from the industries in to fresh water bodies (river) resulted wide spread environmental problems. The problems become more acute when the heavy metal containing effluent mixed river water is used for irrigation in the agricultural crop fields. Microalgae are known to sequester heavy metal ions by absorption and adsorption on the mucilaginous sheath of the filaments. Heavy metals adhere to the mucilage sheath till absorbed. Effluent containing heavy metals can be treated physically and chemically by different methods before discharge into the environmental segments. The PCBs (Pollution Control Board) of state and centre insist on treatment technology and clear guidelines were published from time to time. No industry is allowed to discharge any constituent toxic wastes more than the stipulated level but in reality most of the industries discharge huge amounts of different types of wastes beyond the stipulated limits set by PCBs. From the present study it is clear that at higher temperature the alga could not survive in the Paper mill effluent exposure under laboratory controlled condition, whereas, the alga could sustain little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any liquid waste containing these three heavy metals in combination with other organic chemicals like effluent can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. Both the algae are heterocystous blue-green alga having similar requirements, nitrogen fixers and inhabitants of crop fields. They are eco-friendly & farmer friendly having physiological and biochemical compatibility. Mostly these two alga show similar behavior and almost show the similar growth pattern probably behave and react in a similar way with the toxicants in the environment. Both the algae are capable of removing heavy metals like Hg, Cd and Pb from the contaminated environments. Chopra *et al.*, (2011) indicated that the effluent is fortified with various toxic chemicals and volatile organic solvents. Singh *et al.*, (2019) reported the seasonal changes in water quality of the river by paper industry. Nagarajan *et al.*, (2020) clearly indicated that the discharge of huge amounts of heavy metals in to the different environmental segments and their bioavailability is a potential threat to human, animal and plant life. Borah *et al* (2018) indicated "high mobility factor values represented relatively higher biological activity of cadmium and copper in paper mill effluent exposed soil samples and hinted that the industry could pose serious environmental threats in the surrounding areas of the Paper mill". Periodic and constant monitoring of the discharged effluent revealed minor variations in select parameters studied during 2015 to 2019. Tripathy *et al.*, (2021) studied the untreated effluent of JK Paper mills Ltd and indicated that the effluent is deadly toxic. The same authors also reported that the plants available surrounding the effluent canal accumulated these heavy metals significantly. The accumulated heavy metals in plant leaves significantly affected the photosynthetic efficiency of the plant by reducing the chlorophyll content of the leaves. All plant pigments were significantly affected by the accumulated heavy metals. Tripathy *et al.*, (2021) also suggested use of modern technology to treat the effluent of the Paper industry before discharge into natural environments. The odour of the discharged effluent was pungent with rotten egg smell and the colour ranged from deep yellowish brown to yellowish brown during 2015 to 2019 study period. The pH of the effluent was slightly alkaline and the effluent temperature was equivalent to air temperature and little more than river water temperature. Significant reduction in dissolved oxygen in the effluent was due to high temperature of the effluent, organic debris, high suspended solids and soluble chemicals present in the effluent. Hence, plant and animal life is difficult for survival. Significant amount of mercury, copper, cadmium and lead was recorded in the year 2015. In 2017, the effluent showed 0.09 ± 0.05 mg of copper l^{-1}, 0.31±0.05 mg of cadmium l^{-1}, 0.49 ± 0.06 mg mercury l^{-1} and 0.09±0.03 mg of lead l^{-1} in the effluent sample. In 2018, the effluent showed 0.29±0.04 mg of cadmium l^{-1}, 0.39±0.08 mg mercury l^{-1}, 0.11 ± 0.04 mg of lead l^{-1} and presence of copper was not detected in the effluent sample collected. In 2019, the effluent sample showed 0.32±0.09 mg of cadmium l^{-1}, 0.46±0.14 mg mercury l^{-1}, and presence of lead and copper was not detected. Non detection of heavy metals was a good sign but the very presence of other heavy metals in a significant quantity warrants attention. The toxicity data of heavy metals individually, in combination and effluent with heavy metals on algae along with the impact of temperature stress (both lower and higher) can open up new direction to plan one eco-friendly protocol for decontaminating the effluent wastes of paper mills. The tested algae can be used for adopting a new protocol for decontamination of contaminated environments. Reports of Tripathy *et al*., (2021) is worth reading for a developing a strategy to purify the effluent before discharge. The same authors also indicated for a biological treatment strategy for screening the heavy metals from the effluent in the light of the paper published by Mishra *et al*., (2013) related to phytoremediation by aquatic micro and macrophytes.

The alga could tolerate low temperature stress in presence of mercuric nitrate but not in high temperature stress. In field conditions, the alga dies in summer season and survives in winter season. The exposed alga *Anabaena cylindrica* grew well at 0.33 mg of lead nitrate/l after 15 days of exposure where no death was recorded compared to control alga. At 1.302 mg of lead nitrate/l, 100% death of the algal population was noticed and was considered as LC_{100} value after 15 days of exposure. From the obtained result it is clear that at higher temperature the alga could not survive in lead nitrate + cadmium chloride mixture exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any waste containing these two heavy metals in combination can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. From the data it can be well said that the mixture of heavy metals were more toxic than the individual toxicants as such type of combinations were seen in field conditions of the contaminated sites. From the observed result it is clear that at higher temperature the alga could not survive in mercuric nitrate + lead nitrate + cadmium chloride mixture exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any waste containing these three heavy metals in combination can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. The effluent containing the above cited three heavy metals like Hg, Cd & Pb as the test chemical, interesting results were obtained. From the data it can be well said that the effluent containing heavy metals were less toxic than the individual toxicants or toxicants in combination, as such type of combinations were seen in field conditions of the contaminated sites. From the above result it is clear that at higher temperature the alga could not survive in the Paper mill effluent exposure, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any liquid waste containing these three heavy metals in combination with other organic chemicals like effluent can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals.

The second set was planned to test the toxcity of different heavy metal chemicals and the paper mill effluent on *Westiellopsis prolifica* under similar set of conditions. Table 2 & 3 indicated the toxicity study and the lethal concentration 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 & Paper Mill effluent containing Hg, Pb and Cd (ml/l) (F), at low and high temperatures for the alga, *Westiellopsis prolifica*, Janet after 15 days of exposure under laboratory controlled conditions. When table-

2 and 3 were compared, we found that *Westiellopsis prolifica* is more tolerant and resistant than *Anabaena cylindrica*. In both the tables, we find almost similar data except minor changes at third digit level. Hence detailed writing of the data was avoided. However, the Paper mill effluent showed interesting results related toxicity and temperature tolerance by both the algae. From the data it can be well said that the effluent containing heavy metals were less toxic than the individual toxicants or toxicants in combination to the tested alga, as such type of combinations were seen in field conditions of the contaminated sites. From the above result it is clear that at higher temperature the alga could not survive in the Paper mill effluent exposure under laboratory controlled condition, whereas, the alga could sustain very little at lower temperatures under laboratory controlled conditions. The above data gave a clear picture that any liquid waste containing these three heavy metals in combination with other organic chemicals like effluent can cause serious damage to the aquatic flora and fauna. Seasonal variation has a greater impact of the toxicants on the plants and animals. Both the algae are heterocystous blue-green alga nitrogen fixers, inhabitants of crop fields and are eco-friendly and farmer friendly. Mostly these two alga show similar behavior and almost show the similar growth pattern probably behave and react in a similar way with the toxicants in the environment.

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