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Eco-Toxicological Assessment, Impact And Possible Phycoremediation Of Pulp & Paper Mill Effluent By Cyanobacteria Under Experimental Conditions.

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Highlights:

- The effluent of the Paper mill is highly toxic and showed deadly impact on blue-green algae and contained heavy metals like mercury, cadmium and lead.
- At Maximum Allowable Concentration (MAC) of the effluent, stimulatory effects were observed and at lethal concentrations of the effluent inhibitory affects were noticed.
- Experimental study revealed that these algae could absorb and accumulate significant amount of heavy metals from the effluent mixture in the culture flasks.
- Significant decrease in biomass, total chlorophyll, phaeophytin & carotene content was observed in Paper mill effluent exposed algae compared to their respective control values.
- *Nostoc, Oscillatoria and Westiellopsis* individually could remove significant amount of heavy metals from the effluent mixture within 20days of exposure.
- The algal mixture (all the 4 algae in a mixture) could remove maximum amount of heavy metals from the effluent and having highest bioaccumulation factor.

Abstract

Pollution caused by Pulp & Paper mills is a threat to all plant and animal life. The Pulp & Paper Mills discharge huge amount of effluent in to water bodies. The effluents contribute heavy metals like mercury, cadmium and lead, polluting nearby water bodies and affecting aquatic flora and fauna. Significant amount of Cd, Hg and Pb was noted in the algal mat collected from the contaminated site, algal scum collected from the crop fields receiving effluent contaminated river water, crop fields and crop field inhabiting organisms. An attempt was made to study the impact of heavy metals like mercury, cadmium and lead present in effluent on BGA / cyanobacterium under laboratory controlled conditions. All the heavy metals (Hg, Cd, Pb) tested showed stimulation and better growth at sub-lethal concentrations (MAC value) but at higher concentrations, the heavy metals were deadly toxic. The alga could remove heavy metals from the effluent of the paper mill either by way of absorption and accumulation or by way of volatilization.. The paper mill effluent though highly toxic but showed better behavior at sub-lethal concentrations. From the observed data it is evident that *Oscillatoria, Westiellopsis and Anabaena* were more tolerant than other cyanobacteria. These algae have potency to grow under stressful conditions and can be safely used for phycoremediation of paper mill effluent after dilution of the effluent. These tested cyanobacteria with other potent aquatic macrophytes in combination can be tried for total biological treatment of paper mill effluent for cent percent removal of heavy metals from the effluent, before discharge into the aquatic environment.

Keywords: Paper mill effluent, heavy metals, cyanobacteria, phycoremediation

Introduction

Environmental pollution is no more a jargon word in the present day. All environmental segments are now polluted by pollutants originating from a variety of sources. Most of the pollutants are either man created or man made in addition to natural additions by different natural mechanisms operating in nature. Human attempt was mostly to understand the under lying mechanism and self regulation of nature and play with nature and natural events. In the process of understanding the nature and natural processes, we came across many more interesting events occurring in nature. We need progress, good health, development and food safety for the human race. We adopted and opted for industrialization using modern technologies developed by man. We produced our desired products of interest from different industries. Industry is such a complex machine mixture which required raw materials that were collected from the environmental segments (air, water and land). The industries use the raw materials and produce the product of interest and many other chemicals as unwanted chemicals. These unwanted chemicals were designated as process waste and released in to the environment depending on their chemical nature and status. The gaseous wastes were discharged in to air, liquid effluent was released in to water bodies and the solid waste was discharged in to the land mass. These wastes after addition in to the environmental segments changed the chemistry of these environmental segments. All the living organisms love to live in a particular environmental condition. The different environmental segments are the habital place of different types of living organisms and these organisms were adapted to a particular set of conditions. Addition of wastes in to their habital place changes the nature of the habital place which influences the life and survivability of living organisms. In the process, if the organisms can resist and tolerate the additions, can survive if not slowly suffer and disappear from the habital place. The Paper industries play a crucial role to pollute the river water by their treated / untreated effluent waste. One of the most important sources of heavy metal pollution is the industries. Among industries, Paper and Pulp industry plays a significant role to contaminate the fresh water bodies damages the water quality (Kumar *et al*., 2015 and Singh *et al*., 2019) & physico-chemical assessment of paper mill effluent and its heavy metal remediation using aquatic macrophytes (Mishra *et al.*, 2013). Pulp and Paper mills are the major players to contribute heavy metals like mercury, cadmium and lead in the environmental segment, polluting nearby water bodies and affecting aquatic flora and fauna. Medhi *et al*., (2011) reported that the physico-chemical characteristics of a paper mill industry effluent waste were exceedingly high than the stipulated limits.

JK Paper Mills at Jaykaypur subjects the effluent to physical & chemical treatment and discharges its effluent in to the environment. 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 heavy metals in the final discharged International Paper mill effluent after biological treatment. The paper mill effluents are discharged from the industry into the aquatic environment (Iqbal *et al*., 2013) after simple physical and chemical treatments indicating the need of a biological treatment (Tripathy *et al*., 2021a). Tripathy *et al*. (2021b) reported temperature stress on the impact pulp and paper mill effluent on cyanobacteria and indicated that with the increase in temperature the impact becomes severe. Tripathy and Panigrahi (2022b, 2023) reported the impact of Paper mill effluent and select heavy metals on crop field plants and animals particularly fish inhabiting crop fields during rice cultivation. The authors also reported heavy accumulation of heavy metals in crop plants and non crop plants of the crop fields. Dey *et al*. (2018) reported that in India, more than 55% of the paper mills do not have adequate effluent treatment facilities and also do not adopt modern treatment technologies for the treatment of effluent waste. The effluent of JK Paper mills are discharged into the Nagavalli River direct and the effluent contaminated river water is used for drinking purposes and for irrigation of nearby crop fields by the farmers at the down stream. It was reported by cultivating farmers that the effluent mixed river water was toxic and the production of crop was depleting year by year. The effluent can be treated primarily by physical and chemical treatment and followed by biological treatment using potential & 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. Tripathy *et al*., (2022a) reported that the effluent of the paper mill severely affected the flora and fauna in and around the effluent canal and impacted the water quality of the river affecting the flora and fauna. The same author also indicated that usage of this contaminated river water to irrigate the crop fields will have far reaching consequences. The Paper Mill effluent contains heavy metals like cadmium, mercury and lead. The protocol of biological treatment of the effluent by the industry was not complete and efficient as this protocol neither benefits the environment nor the dependent plants and animals. Waste generation by any industry and the quality of waste depends on the technology and adopted treatment protocol of any Pulp and Paper industry. It is not possible to eliminate waste generation by the system (Kaur *et al.*, 2021) but cleaner & environment friendly technology can be used and periodically positive modifications in the technology or alterations in the treatment technology should be adopted.

The present report aims at understanding the impact Paper mill effluent waste on the algae present in the form of a mat at contaminated site and an attempted was made to understand and characterize the potency of cyanobacteria to be used in phycoremediation of paper mill waste.

Materials & Methods

JK Paper Ltd 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 at 19.252ºN and longitude 83.420º E. Analysis of physico-chemical parameters were carried out following the procedure illustrated in APHA (1998). In addition the field analysis kit, portable pH meter and minimum-maximum thermometer was also used to measure temperature of water and effluent samples. Pure culture of *Anabaena cylindrica*, Lemm., *Nostoc muscorum*, C. Agardh and *Westiellopsis prolifica*, Janet was grown in the laboratory for the experimental study. 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 BGA test organisms. The same medium was amended with nitrogen as the basic culture solution for non nitrogen fixing alga. The pigment contents of the algae were estimated and calculated in control and exposed algal samples following the method described by Vernon (1960) and Davies (1976). Measurement of mercury, cadmium and lead in the samples was carried out by acid digestion in Bethige's apparatus followed the basic principle of Wantorp and Dyfverman (1955) which has undergone substantial modification in the light of later developments. Mercury, cadmium and lead in digested samples were estimated by Atomic Absorption Spectrophotometer. Bioaccumulation factor (BAF): The *in situ* phytoremediation capability of the algae was estimated by calculating the bioaccumulation factor (BAF) as suggested by Yoon *et al.* (2006) by using the formula as described below. BAF= Metal concentration in alga / metal concentration in the culture medium containing effluent waste

For phytoremediation studies, four types of algae such as- *Oscillatoria sp.*, *Westiellopsis sps.*, *Anabaena sp.* & *Nostoc* sps. pure culture was taken as observed in the algal mat collected from the contaminated site for the experimental purpose. Four selected aquatic microphytes growing in all environmental conditions were chosen as the study material. All these species of microphytes were used for phytoremediation studies individually to observe the individual species potency of the specimen samples for remediation of effluent waste of Pulp and Paper mill. In the next set, all the four microphytes were taken together to test the possible use of these organisms for phytoremediation of effluent by way of removal of heavy metals either by way biosorption or by any other way yet to be confirmed. In case of algal studies the effluent was filtered through a normal filter paper and the filtered supernatant was used for the experiment to avoid clumping of alga to the suspended particles (organic debris) present in the effluent. Experiments were conducted in 150ml conical flasks and all precautions were taken to avoid contamination and the procedure adopted for algal experimental studies was followed. The effluent was UV irradiated before inoculation in to culture flasks. A standard control was maintained in all sets of experiments without effluent. Obtained data were analyzed statistically following standard statistical protocols.

Results

The physico-chemical properties of the effluent changed insignificantly till it reached the river and further the effluent was diluted by river water (Table-1). The pale yellowish brown colour of the effluent changed to light yellow after mixing with river water. The strong pungent smell of the effluent changed to filthy smell after joining river water. The cause of both the parameters change was only due to dilution by river water. Significant amount of mercury and cadmium to the tune of $0.46 \pm 0.07 mg.l^{-1}$ and $0.32 \pm 0.05 mg.l^{-1}$ was available in effluent respectively. The concentration of mercury in the effluent discharge decreased to $0.19 \pm 0.03 mg.l^{-1}$ and $0.08 \pm 0.03 mg.l^{-1}$ after ½ and 1km distance from the meeting point, respectively. The concentration of cadmium in effluent decreased to $0.15 \pm 0.02 mg.l^{-1}$ and $0.07 \pm 0.01 mg.l^{-1}$ at ½ and 1km distance in the river water from the meeting point, respectively. No trace of lead was detected either in the effluent or at any other tested sites in downstream. High conductivity value was noted at all tested sites. The values decreased with the increase in distance from the meeting point. Dissolved oxygen content was significantly low in effluent and with the movement of effluent and dilution by river water, the dissolved oxygen content increased from $2.1 \pm 0.8 mg\ l^{-1}$. Interesting changes were noted in BOD and COD content of effluent in the downstream. All the observed changes were due to dilution of the effluent by the river water. The suspended solid load decreased from $819.3 \pm 19.6 mg.l^{-1}$ to $118.2 \pm 12.4 mg.l^{-1}$ along with river water flow. Similar result was also seen in case of TDS, chloride content and hardness of the samples. The nutrient en1richment in water and its use in crop fields increased the yield of the crops but accumulation of heavy metals in the body of the crop plant and algae present in the crop field needs attention. Another significant chemical like fluoride was present in the effluent, which probably got diluted in the river water and presence of fluoride was not detected at the downstream sites. It was observed that parameters like conductivity, TDS, TSS, BOD, COD, dissolved oxygen, chloride, metal ions and few heavy metals like Hg, Cd & Pb etc were present in the effluent and river water much more than the prescribed limit by Pollution Control Boards.

Table-1: The analysis of physico-chemical parameters of the effluent collected from effluent canal at the meeting point near crop fields. Data represent mean of samples ± standard deviation.

Colour: Pale- yellowish brown; Odour: Pungent with strong H_2S smell; Temperature: 26.8± 2.1°C; pH: 7.8 ± 0.6; Conductivity: 1694.7±17.5mho/cm; Dissolved Oxygen: 2.1± 0.8mg.l^{-1}; Biological Oxygen Demand: 108.3± 11.3mg.l^{-1}; Chemical Oxygen Demand: 276.4± 12.5mg.l^{-1}; Total Suspended Solids: 819.3 ±19.6mg.l^{-1}; Total Dissolved Solids: 1387.9±22.7mg.l^{-1}; Chlorides: 311.5 ±14.2mg.l^{-1}; Hardness: 514.2 ±21.6mg.l^{-1}; Fluoride, (F): 1.89±0.8 mg.l^{-1}; Total Nitrogen: 2.26±0.5mg.l^{-1}; Total Phosphates:1.26±0.3mg.l^{-1}; Total Sulphate: 132.1±9.8mg.l^{-1}; Sodium: 61.8±5.2mg.l^{-1}; Potassium:49.5±3.8mg.l^{-1}; Mercury: 0.46±0.07mg.l^{-1}; Cadmium:0.32±0.05mg.l^{-1}; Lead: 0.26± 0.09mg.l^{-1}.

The algal mat collected from the effluent joining river site indicated presence of significant amount of heavy metals. The contaminated algae contained 3.2±0.7 mg of mercury g^{-1} dry wt, 2.6±0.4 mg of cadmium g^{-1} dry wt and 0.88±0.05 mg of lead g^{-1} dry wt absorbed from the effluent. The total chlorophyll content of the algal mat depleted from 27.9±6.8mg g^{-1} dry wt to 12.8±7.8mg g^{-1} dry wt; the total phaeophytin content depleted from 28.9±9.2mg g^{-1} dry wt to 15.6±11.2mg g^{-1} dry weight and the total carotene content depleted from 18.2±5.4mg g^{-1} dry wt to 10.8±6.2mg g^{-1} dry wt indicating 54.1%, 46.02% and 40.7% decrease in pigment content respectively (Fig. A1 & A2). The decrease in pigment content in effluent contaminated mixed algae was significant compared to control algal pigments.

Fig.A.1: Residual heavy metal in the algal mat collected from the contaminated site.

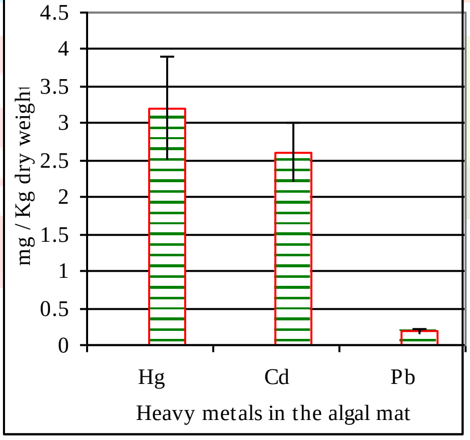
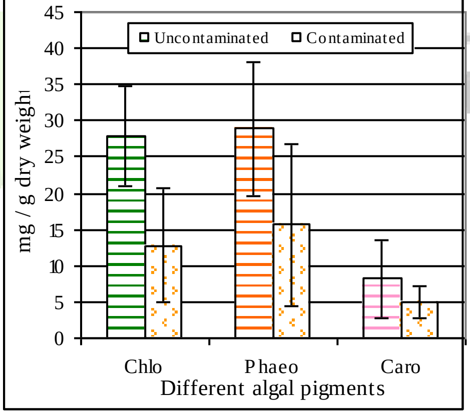


Fig. A.2: Changes in pigment content the algal mat collected from the contaminated site compared to control site.



It was observed that these algae even if they were exposed to paper mill effluent were surviving in the contaminated environment due to dilution by the river water. From this data, it is inferred that algae can tolerate, resist and survive the toxic effluent diluted by river water. This mat was only seen at the joining point of effluent to the river water but not in the effluent canal. Algae in the algal scum collected from the crop fields contained *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Scytonema* sp., *Calothrix* sp., *Spirogyra* sp., *Scenedesmus* sp., *Phormidium* sp. and diatom) showed total heavy metal accumulation to the tune of 0.28± 0.06, 0.17 ± 0.05 and 0.76 ± 0.11µg/g dry wt in crop field-A and 0.25± 0.05, 0.18 ± 0.07 and 0.74 ± 0.09µg/g dry wt in crop field-B receiving effluent contaminated river water used for irrigation of the crop fields. The values in indicated the pollution status of the nearby crop fields contaminated with Paper mill effluent mixed with river water. The effluent mixed river water was filtered through filter paper to remove the organic debris and the filtrate was used for the experiments. Basing ion the data indicated above, it was planned to test the possible use of farmer friendly BGA, fixing atmospheric nitrogen increasing the biofertility of the crop fields for the experimental purpose to test the possibility of these algae for phycoremediation of paper mill effluent waste.

Graded series of concentrations of the Paper mill effluent was prepared for toxicity studies with nutrient medium as the diluents. Pure algal cultures were inoculated in to control and exposed flasks in a Laminar air flow to maintain aseptic conditions. The effluent mixed water was initially UV irradiated. After 20days of exposure, the toxicity values were deduced for all the three algae tested. Approximately 3.85% effluent concentration was selected (sub-lethal dose, MAC (Maximum allowable concentration value deduced from toxicity test) for the experimental study purpose. All the four selected algae were inoculated and allowed to grow for 20days and after 20days; the algae were harvested and taken for heavy metal analysis. The culture solution charged with effluent at 0 day of exposure and culture solution after 20days of exposure were also tested for heavy metal availability for calculating the heavy metal removal from the medium. The obtained data from toxicity testing was statistically analyzed and different lethal concentration values were calculated from the regression curve drawn for different algae. The maximum allowable concentration of the effluent in all the four tested algae and the algal mixtures ranged between 3.84 to 3.86ml/100ml culture and 3.85ml/100ml culture solution was taken as the MAC value for the experiments. No significant difference was noted at LC_{10} and LC_{50}, the values ranged between 3.89-3.91ml/100ml culture and 4.19-4.35ml/100ml culture, respectively. The lethal concentration values indicated significant difference at LC_{90} and LC_{100} compared to lower lethal concentration values. *Oscillatoria* sp. was more tolerant to the effluent than *Anabaena* sp., *Westiellopsis* sp and *Nostoc* sp in the decreasing order (Fig.1).

Fig.1: Lethal concentration values of Paper mill effluent on different algae after 20days of exposure under laboratory controlled conditions.

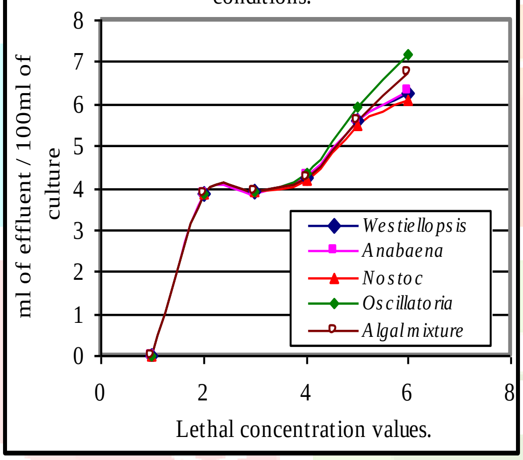


Fig.1A. Residual accumulation of mercury, cadmium & lead in exposed algae exposed to sub-lethal concentration of the Paper mill effluent at different days of exposure. (C-Control; E-Exposed)

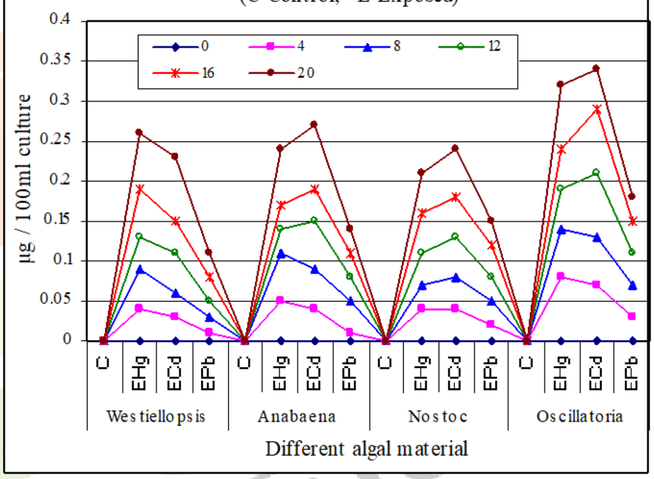


Fig.2a: Changes in biomass in control & effluent exposed algae at different days of exposure (W-*Westiellopsis;* A-*Anabaena*).

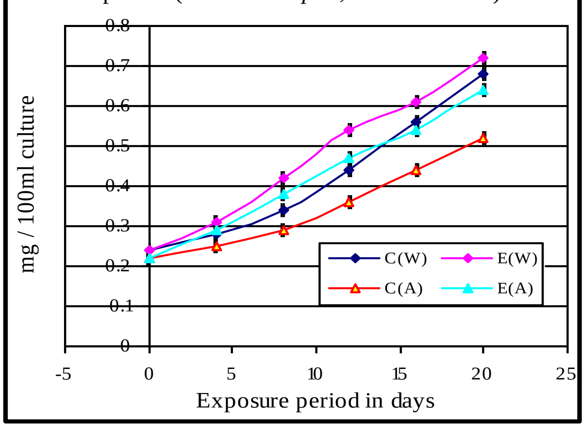


Fig. 2b: Changes in biomass in control and effluent exposed algae at different days of exposure (N-*Nostoc* , O-*Oscillatoria*)

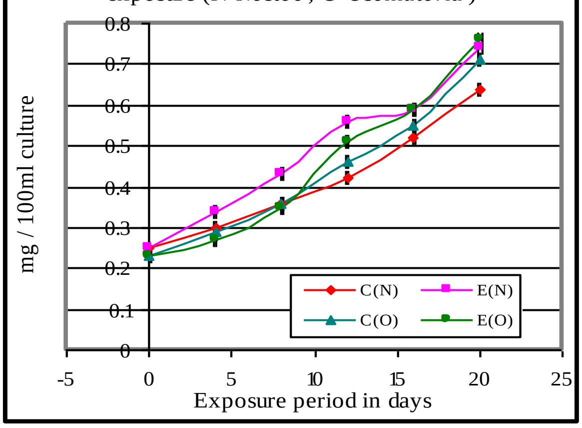


Fig.3: Changes in total chlorophyll content in control and effluent exposed algae at different exposure periods.

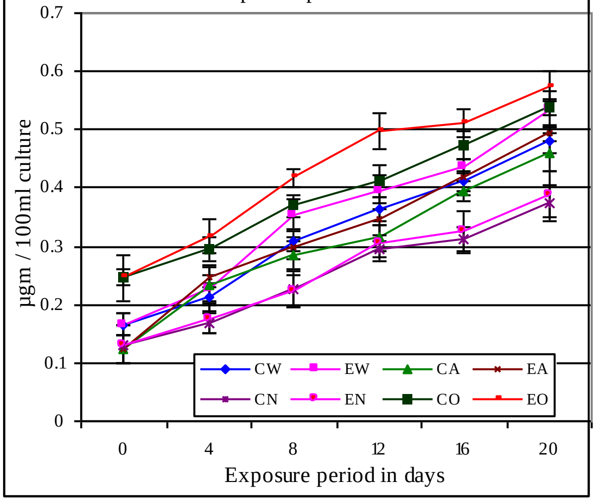
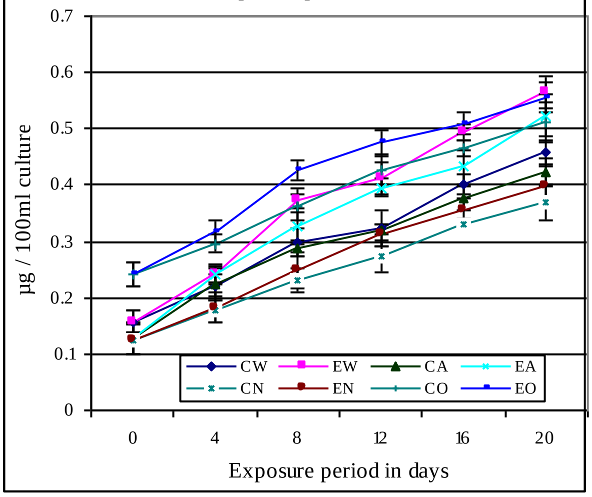


Fig. 4: Changes in total phaeophytin content of control and effluent exposed algae at different exposure periods.



The algal mixture showed interesting results, where the toxicity values were probably counterbalanced and most usable concentrations for the effluent can be predicted for the experiments. Fig.2 showed the impact of effluent at sub-lethal concentration on 4 selected algae independently compared to their respective control values. After 20days of exposure the biomass of the effluent exposed algae was more than the control values. *Westiellopsis* sp, *Anabaena* sp., *Nostoc* sp and *Oscillatoria* sp. showed 5.9%, 23.1%, 15.6% and 7.04% increase in dry weight compared to their respective control values after 20days of exposure without showing any signs of toxicity. It can be inferred that all these 4 algae can be used for phycoremediation studies for removal of heavy metals from the Paper Mill effluent.

Fig.Q1 and table- Q1 & Q1A showed fate of heavy metal (HM = Hg, Cd, Pb) in the culture flask and impact on 04 types of the exposed algae (*Oscillatoria princeps*, Vaucher ex Gomont., F: Oscillatoriaceae; *Westiellopsis prolifica*, Janet.; F: Cyanophyceae; *Anabaena cylindrica*, Lemm., F: Cyanophyceae and *Nostoc* sps., F: Cyanophyceae) in the culture medium containing effluent at MAC value after 20days of exposure. It was estimated that the culture medium contained 1.34 µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture. After 20days of exposure, *Oscillatoria sps.* could remove 0.48µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture indicating 35.8% heavy metal removal. Out of which 0.41 µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture was absorbed by the alga and the bioaccumulation factor was 0.31 (Fig.Q1 and Q1A). *Westiellopsis sp.* could remove 0.46µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture indicating 34.3% heavy metal removal. Out of which 0.37µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture was absorbed by the alga and the bioaccumulation factor was 0.28 (Fig.Q1 and Q1A). *Anabaena sp.* could remove 0.43µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture indicating 32.1% heavy metal removal. Out of which 0.396µgm of total heavy metals / 50ml culture was absorbed by the alga and the bioaccumulation factor was 0.29 (Fig.Q1 and Q1A). *Nostoc* sps. could remove 0.48µgm of total heavy metals / 50ml culture indicating 35.8% heavy metal removal. Out of which 0.37µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture was absorbed by the alga and the bioaccumulation factor was 0.28 (Fig.Q1).

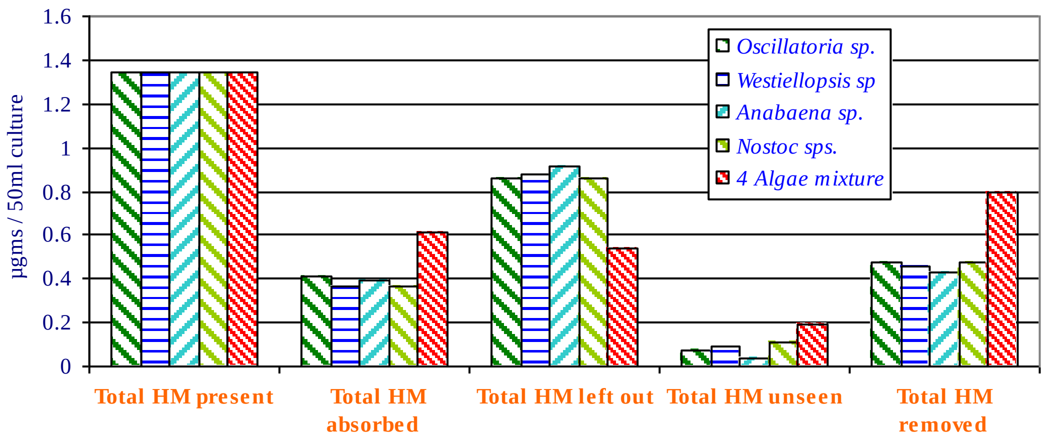
Table-Q1: Fate of heavy metal (HM = Hg, Cd, Pb) in the culture flask and impact by the exposed algae (*Oscillatoria princeps*, Vaucher ex Gomont., F: Oscillatoriaceae; *Westiellopsis prolifica*, Janet.; F: Cyanophyceae; *Anabaena cylindrica*, Lemm., F: Cyanophyceae and *Nostoc* sps., F: Cyanophyceae) in the culture medium containing effluent at MAC value after 20days of exposure. Data are the mean of three replications.

Organism	Total HM **present** in the medium on 0day.	Total HM **absorbed** by the alga.	Total HM **left out** in the medium	Total HM **unseen** but removed from the culture	Total HM **removed** from the culture system.
	µgm / 50ml culture				
Control	00	00	00	00	00
Oscillatoria sp.	1.34	0.41	0.86	0.07	0.48
Westiellopsis sp	1.34	0.37	0.88	0.09	0.46
Anabaena sp.	1.34	0.39	0.91	0.04	0.43
Nostoc sps.	1.34	0.37	0.86	0.11	0.48
Above 4 Algae in a mixture	1.34	0.61	0.54	0.19	0.80

Table-Q1A: Removal of heavy metals (HM=Hg, Cd, Pb) by the exposed algae (*Oscillatoria princeps*, Vaucher ex Gomont., F: Oscillatoriaceae; *Westiellopsis prolifica*, Janet.; F: Cyanophyceae; *Anabaena cylindrica*, Lemm., F: Cyanophyceae and *Nostoc* sps., F: Cyanophyceae) individually and as a mixture from the medium after 20days of exposure. Data are the mean of three replications.

Status / Organism	Total HM **present** in the medium on 0day. (X)	Total HM **absorbed** by the alga. (Y)	**Percent removal** of HM from the medium. (%)	Bioaccumulation factor (**BAF**)
	µgm / 50ml culture			(Y / X)
Oscillatoria sp.	1.34	0.41	35.8	0.31
Westiellopsis sp	1.34	0.37	34.3	0.28
Anabaena sp.	1.34	0.39	32.1	0.29
Nostoc sps.	1.34	0.37	35.8	0.28
Algae mixture	1.34	0.61	59.7	0.46

Fig.Q1: Showing fate of total heavy metal present in the effluent in algal culture after 20days exposure. Data are the mean of samples.



For phytoremediation studies, four types of algae such as- *Oscillatoria sp.*, *Westiellopsis sps.*, *Anabaena sp.* & *Nostoc* sps. pure culture was taken as observed in the algal scum collected from the contaminated crop fields for the experimental purpose. All the four algae mixed together and inoculated into culture flask as mixed culture could remove 0.80µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture indicating 59.7% heavy metal removal. Out of which 0.61µgm of total heavy metals (Hg, Cd, Pb) / 50ml culture was absorbed by the alga and the bioaccumulation factor was 0.46. All the obtained values were less than 0.5 bioaccumulation factor. From the data it can be concluded that all the four BG algae can be used in a mixture for effective phycoremediation purposed to treat the effluent by biological treatment. However the use of these algae is not enough for 100% removal of heavy metals from the effluent released by the Paper industry (Fig.Q1 and table-Q1, Q1A). Trial experiments should be conducted to test the role of aquatic macrophytes having potency to remove heavy metals from the contaminated aquatic environments. Second, the microphytes tested in this study can used along with potential macrophytes for

100% removal of heavy metals from the effluent and this protocol could be used for phytoremediation and along with potential bacteria, fungi and other organisms for bioremediation of effluent waste.

Discussion:

The Pulp and Paper industry was considered as one of the best 20 polluting industries of India declared by Department of Environment and Forest, Government of India as reported by Singh *et al* (2016). Most of the industries have Environmental Quality check units to monitor and assess the wastes generated by the industries. They are supposed to be responsible for the quality of the wastes and to maintain the prescribed limits as per stipulation. It was observed that most of the industries do not follow the guidelines and carelessly discharge the wastes in to the abiotic environment contaminating all the environmental segments. The biotic component ultimately becomes the victim of all wastes and suffers. Significant decrease in pigment was observed in the algal mat collected from the contaminated site. The pigment contents like chlorophyll, phaeophytin and carotene significantly depleted in effluent contaminated algal mats. It was also observed that the algae in the algal mats accumulated significant amount of mercury, cadmium and lead in their bodies. It can be inferred from our data that the depletion in pigment content in the exposed algae was due to heavy metals accumulation. This residual accumulation led to depletion in pigment content like chlorophyll and phaeophytin. The decrease in chlorophyll content reduced the photosynthesis rate, ultimately leading to decrease in production. Sahu (2017a) reported stimulation of growth and increase in chlorophyll content and increase in photosynthetic rate at sub-lethal concentrations of the heavy metal exposure. It was observed from phycoremediation studies that these exposed algae exposed to sub-lethal concentration of the effluent could remove these heavy metals from the culture medium by way of absorption and consequent residual accumulation. In addition, some amount of heavy metals was lost from the culture vessel. This loss was probably due to evaporation or volatilization caused by the exposed algae. It was reported by Sahu (2017a, b) and Shaw *et al*., (1989) that mercury can be volatilized by *Westiellopsis* and *Anabaena* from the culture vessels. In absence of any other possibilities, it can be concluded that these 4 algae in combination and also individually can remove heavy metals from the heavy metal contained effluent and can use the organic debris of the paper mill effluent after decomposition for their growth and development. The depletion in pigment contents at higher concentrations of effluent because of residual Hg, Pb and Cd accumulation warrants attention and future studies should be focused to reduce the contamination of heavy metals by physical, chemical (Kausar *et al*., 2020 & Rasheed *et al*., 2020a,b) and by biological treatment (phytoremediation) of the effluent before discharge into water bodies. This residual accumulation led to depletion in pigment content like chlorophyll and phaeophytin. The decrease in chlorophyll content reduces the photosynthesis rate, ultimately leading to decrease in grain production. The depletion in pigment contents because of residual Hg and Cd accumulation warrants attention and future studies should be focused to reduce the contamination of heavy metals by physical, chemical ((Kausar *et al*., 2020; Rashid *et al*., 2020a & Rasheed *et al*., 2020b) and biological treatment (phytoremediation) of the effluent before discharge into water bodies. Muhammad *et al*., (2015) reported mercury as a highly toxic chemical and mercury reduces seed germination, seedling growth and depletion in growth and biomass yield in case of mung bean (*Vigna radiata*). Mercury induced oxidative stresses in *Suaeda salsa* and also mercury at a concentration of 20µg.l^{-1} disturbed protein degradation and energy metabolism in *Suaeda salsa* (Wu *et al*., 2012). The mercury uptake produced serious damage to plants by impairment of the chlorophyll synthesis and reduction of photosynthesis as a result of substitution of Mg by Hg (Lavado *et al*., 2007). Radha *et al*., (2002) reported significant decrease in pigment contents, photosynthetic rate and respiration rate in rice seedlings exposed to mercury contained solid waste of a chlor-alkali industry and indicated suppression in growth. Interestingly accumulation of lead in plants exposed to effluent of the paper mill was not observed. Plants growing in heavy metal contaminated sites probably developed some degree of tolerance and resistance to metal toxicity in order to survive (Patra *et al*., 2004). Plants contain at least some metals in their tissues and are capable of completely excluding potentially toxic elements, but simply by restricting their uptake and / or translocation. If the toxic metal ions are overloaded, oxidative stress defense mechanisms are activated in plants (Patra *et al*., 2004), hence heavy metal exposed plants probably tolerate the metals stress. Lots of literature is available pertaining to the impact of treated effluent of the paper mills on agricultural crops. Li *et al* (2021a) indicated that “high levels of heavy metal stress may cause irreversible damage to microalgal chloroplasts and prevent photosynthesis, thereby leading to cell death” (Yu *et al*, 2019 and Alho *et al*., 2019). Li *et al*., (2021b) reported cadmium contamination in crop fields as a serious human health concern because of its extreme toxicity and wide spread pollution. We do agree with their concern basing on our findings. 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 & 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". The same authors also indicated that "treatment of pulp and paper mill wastewater effluents and recycling of water from pulp & 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 laccase producing *Bacillus cereus* AKRC03" (Kumar and Chandra, 2021). The present study related to phytoremediation by aquatic plants, microphytes capable of removing heavy metals from the environment and plants capable of biosorption and retention coupled with the work of Sharma *et al* (2021,2020a,b,c,d), Hu *et al* (2020) and Chandra *et al*, (2018,2021) can be recommended for biological treatment of paper mill effluent with a strategic protocol.

Conclusion

It is high time to think about the reclamation of Pulp and Paper Mill effluent wastes before its discharge into natural environments particularly on fresh water bodies. These fresh water bodies are the life line of most of the villages, towns and cities located in the banks of these rivers. All the farmers depend on this river water for irrigation of crop fields. Heavy metal accumulation in crop fields and in crops warrants attention. Many physical methods of treatments of the effluent wastes are available and they are in use by most of the industries but proper biological treatment was not followed by many industries. The present piece of work can be a probable protocol for phycological treatment and phytoremediation along with the potential reported macrophytes and microflora for 100% biological treatment of the Pulp and Paper Mill effluent waste. If the biological treatment protocols are properly followed, heavy metal free effluent can be discharged in to water bodies which may not cause any danger to human population or plants and animals on which human population is dependent for their survival.

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Declarations

Author contribution statement

Prof. A.K. Panigrahi: Conceptualization, planning and execution of the project, Original draft preparation, supervision, reviewing and editing; Research work conducted by scholars - A. P. Tripathy paper mill effluent collection of samples, analysis and related field work. Supriya Devi- laboratory experimental work, preparation of first draft and editing Both Supriya & Tripathy contributed reagents & glassware for laboratory experiment related work and other expenses.

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