International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685
www.pharmacyresearchjournal.com
Volume 1; Issue 2; February 2016; Page No. 01-03

Studies on the impact of thermal ash on the productivity of algae (Cyanobacterium): *Spirulina platensis*

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Abstract
In the present investigation various concentrations of ash produced from thermal power stations by burning coal were used to study the impact on the growth and productivity of cyanobacterium, *Spirulina platensis* by measuring the photosynthetic activity. The results clearly indicated the decrease in the growth and productivity. The electron transport measurements studies done with the cells grown in the presence of different ash concentration reveled the inhibition of photosystem II catalyzed transport activity by 50% and 10% the Photosystem I. The ash caused the inhibition in the whole chain electron transport activity at very low concentrations. Between the two photosystems I and II the photosystem II seems to be more susceptible towards the ash concentrations. The possible reasons for the decrease in the electron transport activity in the investigated algae, cyanobacterium, *Spirulina platensis* is due to the presence of heavy metals in the ash content coming out from the thermal power plants and may be changes in the phycobili proteins.

Keywords: Algae, Ash, Cyanobacterium, Photosystem, *Spirulina platensis.*

1. Introduction

Now-a-days fly ash disposal by the thermal power plants into the environment is one of the major concerns throughout the globe. Ash, a by-product of coal combustion, is produced in coal based thermal power plants and industries. Presently India produces 120 million tons per annum and it will cross 150 million tons in the coming next year's [1]. The main instances fly ash consists of plant macronutrients Na, K, P, and Fe and micronutrients Co, B, Zn, Cu, and Mn. Fly ash also contains heavy metals like Pb, Ni, Cr, Cd, etc. [2-4]. Usually, fly ash is disposed of either by dry or wet methods. In dry disposal, fly ash is dumped in landfills and basins. In wet method, fly ash is washed out with water into artificial lagoons and is called ash pond. Both methods ultimately lead to dumping fly ash on open land, which affects the quality and quantity of surface water, ground water, soil, and vegetation of the area [5]. Fly ash contains heavy metals such as Fe, Cu, Ni, Cr, Pb, Cd, etc., that exhibit metal toxicity in plants leads to reduced growth impaired metabolism and decreased in productivity. Thus continuous accumulation of heavy metals ions results in metal toxicity of the environment.

Heavy metal pollution resulting from industrialization and excess use of fertilization, herbicides and pesticides are reasons for heavy metal toxicity in both aquatic and terrestrial environment. The level of toxic heavy metals in water bodies affects mostly the photosynthetic process of plants and cyanobacteria. Therefore, in the present study an attempt was made to find out the impact of thermal ash on the photosynthetic activity (both light and dark reaction) of cyanobacterium; *Spirulina platensis.*

2. Materials and Methods

2.1. Samples Collection: The fly ash was collected (5 Kg) from the ash pond located near the thermal power station near Ibrahimpatnam, Krishna District, Andhra Pradesh, India. The fly ash collected was dried in sun for 10 days before the study. For the purpose of treatment the ash was mixed with sterile distilled water and supplemented with cultured media of 0.25, 0.50, 2.50 and 5.00% concentrations (crude).

2.2. Algae culture: *Spirulina platensis* which belongs to the cyanobacteria was grown axenically in the medium of Zarrouk [6] at 25±2 ^{0}C. Both control and treated cells are cultured separately under continuous irradiance of 40 µ mol (photon) m^{-2} S^{-1}. Cells from the late log grown cultures were harvested by centrifuging at 6,000 Xg for 10 min. The collected cells were suspended in 25 mM HEPES-NaOH buffer (pH 7.5) at a Chl concentration of 200 µg ml^{-1}. The reaction mixure used for the assay of whole chain electron transfer (H_2O→methyl vilogen(MV) contained reaction buffer 25 mM HEPES-NaOH buffer, (pH 7.5), 0.5 mM MV and 1 mM Na-azide [7]. The reaction mixture for PS II catalysed electron transfer (H_2O → *p*-benzoquinone (pBQ) contained the above mentioned reaction buffer and 0.5 mM pBQ [8-9]. Thylakoid membranes were prepared according to the method of [10]. The reaction mixture of PS I catalysed electron transfer ($DCPIPH_2$ → MV) contained reaction buffer, 0.1 mM DCPIP, 5 mM ascorbate, 1 mM azide, 10 µM DCMU, and 0.5 mM MV. In all assay cells equivalent to 15 µg of Chl were used. All the photochemical activities were measured at saturated light intensity of white light (410 Wm -2) under continuous stirring. Low light intensity when required was provided by passing the light through calibrated neutral density filters. Chl content was estimated by following the methods of [11] by using methanol extraction.

3. Results and Discussion

In this investigation to characterize the alterations in photosynthetic activity of the cyanobaterium, *Spirulina platensis* electron transport measurements were taken initially. The whole chain photosynthetic electron transport activity was

measured for the fly ash treated and control samples. MV is known to accept the electron from A_0 in the photosynthetic electron transport chain [12]. Therefore the electron transport has been measured by using MV as terminal acceptor. Control cells exhibits a high rate of oxygen consumption 252 µmoles of $O_2 \downarrow$ mg^{-1} Chl h^{-1}, (Table 1). The treated cell to different concentration of thermal fly ash caused the inhibition in the electron transport activity. At 2.50% concentrations, 56% inhibition at whole chain electron transport was noticed. The reason for the loss of whole chain electron transport could be alteration either at the level of P700 as has been observed by Wong and Govindjee [13] or at PS I or both. These results are in agreement with the observations of Murthy and Prasanna Mothanthy [14].

Table 1: Effect of Ash content on whole chain electron transport ($H_2O \rightarrow MV$) of the cyanobacterium, *Spirulina platensis.*

% of fly ash	Whole chain electron transport activity ($H_2O \rightarrow MV$) µ moles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}	Percentage inhibition
Control	252 ± 21	0
0.25	214 ± 19	15
0.50	170 ± 16	29
2.50	111± 10	56
5.00	98± 8	38

Therefore to identify the target photosystem, an attempt has been made to study the alteration of fly ash in PS II catalysed electron transport. The PS II activity was measured by using *p*-BQ as a Hill electron acceptor. Control cells exhibited a high amount of activity which is equal to 361 µ moles of O_2 evolved $\uparrow$ mg^{-1} Chl h^{-1}. The increase in the concentration of fly ash caused gradual inhibition in PS II catalysed electron transport (Table 2). In the presence of low concentration of fly ash no appreciable changes in the Ps II activity but almost 30% loss was noticed after treating the cells at 5.00% concentrations. The loss in the PS II catalysed electron transport activity could be either due to alterations in the water oxidation complex or due to changes in the reaction centre or in the phycobili proteins.

Table 2: Effect of ash content on PS II catalysed electron transport activity ($H_2O \rightarrow$ *p*-BQ) of the intact cells of the cyanobacterium *Spirulina platensis.*

% of fly ash	PS II electron transport activity ($H_2O \rightarrow$ *p*-BQ) µ moles of $O_2\uparrow$mg Chl^{-1} h^{-1}	Percentage inhibition
Control	361 ± 33	0
0.25	282 ± 26	22
0.50	209 ± 19	42
2.50	126 ± 11	65
5.00	110± 9	30

Unlike the situation with the whole chain electron transport and PS II catalysed electron transport, PS I catalysed reaction could not be assayed in intact cells of *Spirulina* as reduced DCIP/ TMPD/ DAD did not readily enter into intact cells. Therefore, thylakoid membrane fragments were prepared to study the effect of fly ash concentrations on PS I catalysed electron transport. The rates were matching with the rates of chloroplast thylakoid membrane, with reduced DCPIP as donor. Therefore we have selected $DCPIPH_2$ as electron donor system and studied the effect of ash content on PS I catalyzed electron transport activity. Murthy and Mohanthy [15] showed the similar inhibition in PS II photochemistry due to detachment of phycobilinosomes from the thylakoid membrane under heavy metal stress. To verify the susceptibility of PS I, we have studied the effect of fly ash on PS I activity was measured using reduced $DCPIPH_2$ as donor and MV as acceptor. Control thylakoid exhibited O_2 consumption which is equal to 429 µmoles s (Table 3). The increase in the concentrations caused marginal inhibition in PS I catalysed electron transport by 10% at 2.50% concentrations. At 5.00% concentration only 17% loss in PS I catalysed electron transport was noticed. The reason for the loss of PS I catalysed electron transport could be due to the changes in the reaction centre, P_{700}. Golbeck *et al*., [16] indicated that the heavy metals inhibitory affect on the P_{700} which could be one of the reason for the loss of PS I activity. Thus thermal fly ash which is a by-product of coal combustion shows multiple effects on photosynthetic electron transport depending on the extent of concentrations for which the cells are exposed.

Table 3: Effect of ash content on PS I catalysed electron transport activities of the ($DCPIPH_2 \rightarrow MV$) intact cells of the cyanobacterium, *Spirulina platensis.*

% of fly ash	PS I electron transport activity ($DCPIPH_2 \rightarrow MV$) µ moles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}	Percentage inhibition
Control	429 ± 41	0
0.25	410± 40	4
0.50	403 ± 38	6
2.50	390 ± 37	10
5.00	356 ± 33	17

4. Conclusion

Heavy metals are able to cause alterations in whole chain, PS II and PS I catalysed electron transport in the thylakoid membranes of intact cells of *Spirulina*. Between the two photosystems (PS), PS II seems to be more susceptible to fly ash (crude) than PS I at low concentrations. As *Spirulina platensis* is a very important and high protein yielding alga for the production of Single Cell Protein (SCP), we suggest that the water contaminated with ash and heavy metals are not advisable for the growth of *Spirulina platensis* or any other algae or cyanobacteria. Further work should be carried to analyze (qualitative and quantitative) the concentrations of these heavy metals concentration in the fly ash (crude), which metals are responsible and at which targets sites there are showing the inhibitory activity in this cyanobacterium; *Spirulina platensis* or in other algal species.

5. References

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