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Changes in Cell Wall Polyssacharides of *Scenedesmus rotundus* and *Pseudochlorella pringsheimii* to Elevated Levels of Cadmium and Zinc

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***Abstract*: Abiotic stress is considered to be one of the major problems in living organisms beyond the range of normal variation of adverse effects. Different organisms respond to abiotic stresses in different ways. These effects tend to be visible through morphological changes in the organism. Micro algae being the first primitive organism in the web cycle are prompt for the exposed contamination. Many micro algal species growing in metal polluted environments shows ability to tolerate large concentration of metals. The cell wall being at the interface, between the system and surrounding, provides a protective barrier to reduce the entry of the metal into the system. Most studies related to selective adsorption of metal ions to the cell wall have investigated adsorption of metals to dead algal biomass. Studies pertaining to uptake of metals by living cells and subsequent internalization are meagre. This ability of the micro algae is confabulated for survival and is done by activation of various types of altered defense mechanisms. The present study is aimed at understanding the growth pattern and the expression of the polysaccharides of *Scenedesmus rotundus* and *Pseudochlorella pringsheimii* to the elevated level of Cadmium and Zinc.**

***Keywords*: Algae, Cadmium, Cell wall, Metals, Polyssacharides, Toxicity, Zinc.**

1. Introduction

Abiotic stress is the negative impact of environmental factors on living beings in the environment. The stresses include famine, high salt concentration, low or high temperatures, and other environmental factors. Abiotic stresses impact the synthesis, amount of product, transportation and storing of primary and secondary metabolites in algae. The main cause of such abiotic stresses is unfavorable climate which changes agro-ecological conditions and indirectly affects growth.

Plants and algae are subjected to a wide range of environmental stresses which lead to multiple physicochemical in the organism. Micro algae constantly undergo various cellular mechanism changes. They also experience changes production of stress metabolites and they are influenced by the above changes. Micro algae are autotrophs that utilize light and inorganic nutrients to produce biomass rich in value added products such as fats, carbohydrates, proteins etc.

Polysaccharides are high molecular weight carbohydrates with weights of 10kDa to 1000kDa and are the most abundant carbohydrates. They have complex structures and other physico-chemical properties. They are made up of Penrose and/or hexose which are linked by glycosidic bonds which form linear or ramified homopolymer or heteropolymer. They have various biological functions which includes energetic reserves in the form of starch or glycogen, structural components which are used for structural and protective purposes in the form of beta-glucans or peptidoglycan or are excreted outside the cell and may or may not mucilaginous layers and they do not have well-defined functions.

Exocellular microbial polysaccharides, also known as exopolysaccharides (EPS) play an important role as protective barriers. As mentioned above the concentration of the polysaccharide produced can be variable depending on the environmental factors, thus we are inducing an environment with a high metal concentration (Cd, Zn) to observe which environment leads to higher production of the polysaccharide and to study the growth pattern of the algae.

Metal toxicity, also called metal poisoning is defined as the lethal effects of some metals in various types and amounts to living organisms. Heavy metal toxicity can cause serious alterations in plants and animals and can also lead to harmful and permanent mutations. Cadmium of one the most dangerous metals and accumulations of this metals even in low concentrations can cause major damage to the cellular functions of the algae and plants. It can alter the minerals and nutrients of the plants and effects the cellular polysaccharides and proteins in algae. Zinc is another such metal whose toxicity alters the cellular permeability leading to reduction in sodium and potassium content of the cell which is followed by inhibition of

photosynthesis, the nitrogen fixation and lastly cell multiplication in algae.

2. Review of Literature

The algal species used for the experiments *Scenedesmus rotundus* and *Pseudochlorella pringsheimii* are green algae. The main components of green algae are pectin, cellulose, hemicellulose, arabinogalactan proteins (AGPs), extensin and lignin (only in embryophyte cell wall). Charophytes were the first land species and land plants have evolved from charophytes. The cell wall structure of charophytes is as mentioned above. It is easy to carry out studies using charophytes as they are simple algae and easy to study with.

The isolation procedure of these species involves centrifugation and heating at high temperatures with chemicals. This is done in order to break the cell wall and isolate the polysaccharide components. Centrifugation breaks the cell wall and heating separates the polysaccharide making it easier to study. The OD is taken at 490 nm because that is the most suitable wavelength. The OD readings indicate the amount and the range of effect of the metal on the particular on the algal species. Then the graph is plotted and the slope of the graph is found out which is then used to calculate the final concentration.

Marine algae are of extreme benefits in areas such as health. They have biological activities like immunomodulatory, antitumor, antiviral, antibiotic and hypolipidemic. They have excellent use in pharmaceuticals, fuel, feed and fodder etc. due to its cell wall properties and nutritional benefits. The marine algae can be classified into three groups, namely: 1) brown algae (Phaeophycean), 2) red algae (Rhodophyceae) and 3) green algae (Chlorophyceae). These sea weeds and used in diets as well in Asia. The study of metal toxicity will help in study of effects of metal toxicity in higher organisms as well because metals form a major part of our life and its toxicity is harmful to all organisms.

3. Materials and Methods

A. *Materials*

1) *Bold's Basal Medium (BB)*

STOCK NO.	Components	PER 400 ML
1.	$NaNO_3$	10 g
2.	$MgSO_4.7H_2O$	8 g
3.	NaCl	1 g
4.	K_2HPO_4	3 g
5.	KH_2PO_4	7 g
6.	$CaCl_2.2H_2O$	1 g
7.	Trace elements	PER LITRE
7.1	$ZnSO_4.7H_2O$	8.82 g
7.2	$MnCl_2.4H_2O$	1.44 g
7.3	MoO_3	0.71 g
7.4	$CuSO_4.5H_2O$	1.57 g
7.5	$Co(NO_3)_2.6H_2O$	0.49 g
8.	H_3BO_3	11.42 g
9.1	EDTA	50.0 g
9.2	KOH	31.0 g
10.1	$FeSO_4.7H_2O$	4.98 g
10.2	H_2SO_4 (Conc.)	1.0 ml

MEDIUM	PER LITRE
Stock solution 1 – 6	10ml each
Stock solution 7 - 10	1ml each

Chemicals for tests and Standardization of Glucose
Ethanol
Hydrochloric Acid
Phenol

B. *Methods*

1) *Estimation of Sugar*

The Estimation of Sugar was done by using Phenol Sulphur acid method Gabriela et. al., 2002.

1. Glucose solution was taken in varied concentration of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.00ml. Then the volume was made up to 1ml using water.
2. The test tubes were marked to make the identification easier. Added 0.3ml of phenol to all the test tubes. And vortex stirred.
3. Then 1.5ml of concentrated sulphuric acid was added.
4. It was incubated either for 30 minutes at 80°C or for 15 minutes at 110°C.
5. After incubation, it was cooled down. Distilled water was taken as blank. The absorbance was calculated at 490 nm.

2) *Estimation of Polysaccharide*

Estimation of EPS:

1. A known volume of the algal samples was centrifuged at 5000g for 15 minutes. It was then filtered using Whatmann filter paper.
2. The filtered liquid was mixed with 95% ethanol and stirred vigorously and left overnight at 4°celsius.
3. The sample was centrifuged at 10,000 rpm for the time of 15 minutes.
4. The supernatant was discarded and the pellet was hydrolyzed in 2M HCl and incubated for 2.5 hours at 105°celsius.
5. Then it was cooled down. Distilled water was taken as blank. The absorbance was recorded at 490 nm.

Estimation of IPS:

1. The pellet was homogenized by adding cold methanol and with a glass rod.
2. 100mg of the diluted cells in 50 ml of distilled water. Then the solution was filtered.
3. The filtered liquid was mixed with 95% ethanol overnight. The solution was centrifuged at 10,000 rpm for the time of 15 minutes.
4. The pellet was hydrolyzed with 2M HCl and incubated for 2.5 hours at 105°celsius.
5. Then it was cooled down. Distilled water was taken as blank. The absorbance was recorded at 490 nm.

4. Results and Discussion

A. *Growth and Maintenance of unialgal forms*

The algal species *Scenedesmus rotundus* and *Pseudochlorella pringsheimii* were cultured and maintained in unialgal forms in the laboratory. These cultures were

continuously monitored for the survival and harvested on Day 12 which showed the maximum exponential count.

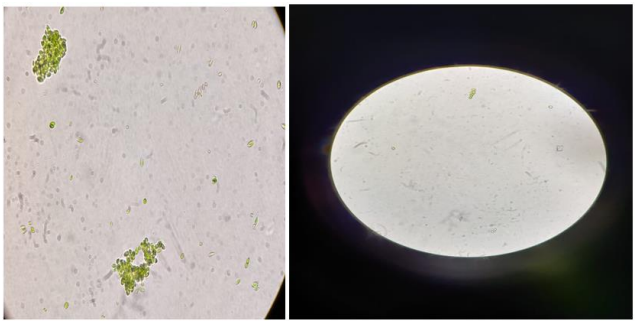


Fig. 1. Microscope image of the algal species *Scenedesmus rotundus* and *Pseudochlorella pringsheimii*

The readings for different number of days for the species were taken along with standard readings.

Table 1
Estimation of sugar and Calculations

Concentration (ml)	OD at 490 nm
0.2	0.020
0.4	0.109
0.6	0.238
0.8	0.300
1.0	0.411

Slope- 0.35
Blank- 3.101
Control- 3.191
Cadmium- 2.924
Zinc- 3.141

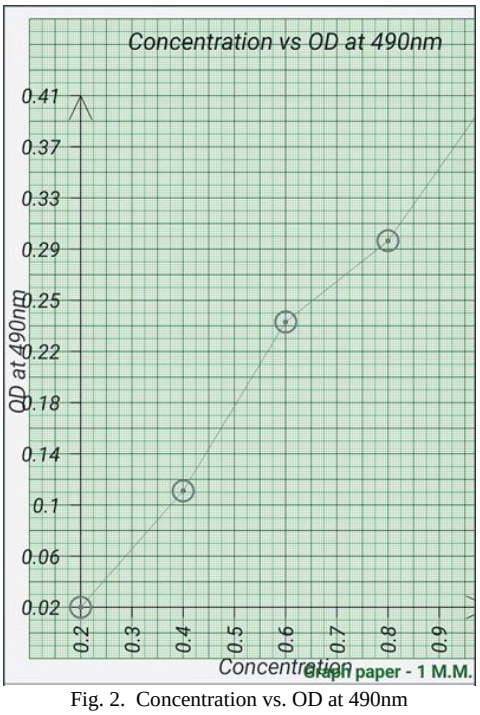


Fig. 2. Concentration vs. OD at 490nm

Calculations:
Final concentration= OD/Slope of the graph

Table 2

Concentration in ml	OD at 490 nm	Final Concentration (mg/ml)
0.2	0.020	0.057
0.4	0.109	0.311
0.6	0.238	0.680
0.8	0.300	0.857
1.0	0.411	1.174

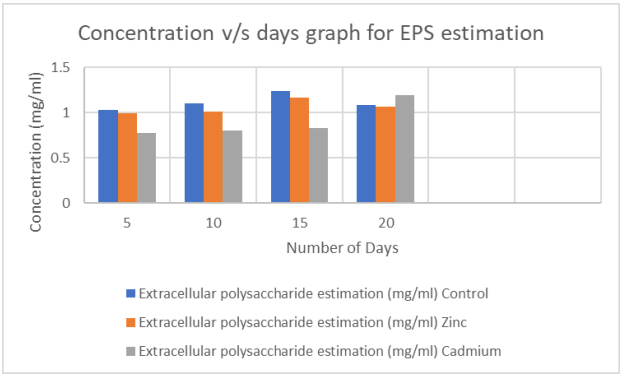
For EPS and IPS estimation:

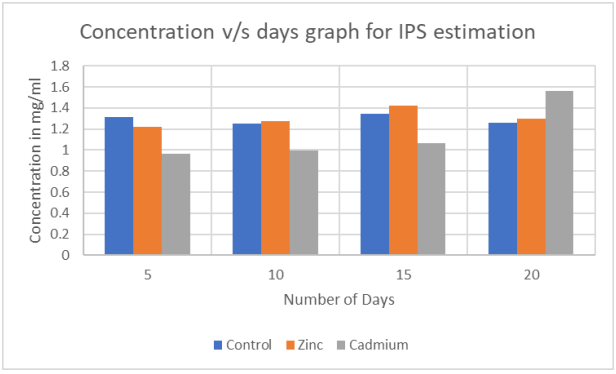
Table 3
Estimation of EPS

Days	Concentration of Extracellular polysaccharide (mg/ml)		
	Control	Zinc	Cadmium
5	1.026	0.993	0.776
10	1.102	1.011	0.798
15	1.243	1.164	0.825
20	1.086	1.065	1.189

Table 4
Estimation of IPS

Days	Concentration of Intracellular polysaccharide (mg/ml)		
	Control	Zinc	Cadmium
5	1.31	1.22	0.962
10	1.248	1.278	0.998
15	1.346	1.425	1.066
20	1.256	1.298	1.563





Calculations:
Final Concentration =OD/Slope of the graph
Find the slope from the graph by plotting a line graph

Discussion:

Metal toxicity, also defined as metal poisoning is defined as the lethal effects of metals on living organisms in various types and amounts. Some metals can be lethal only when they form toxic soluble compounds. The effect of toxicity may vary from metal to metal. Different metals affect different kinds of life forms in different ways.

Cadmium is a heavy metal used in various industries and can be harmful to life forms. It inhibits the growth of algae and is said to alter the cellular polysaccharides. Zinc is a metal found in very minute quantities inside the body. Zinc toxicity in algae can lead to alteration in cell permeability, caused to alterations in cellular polysaccharides and proteins, which is followed by a reduction in amounts of sodium and potassium in the cell which leads in inhibition in photosynthesis, followed by inhibition of nitrogen fixation, and finally results in inhibition of cell division.

As per the graph of estimation of the extracellular and intracellular polysaccharides, the effect of cadmium increase as the days progress and the effect is most pronounced on the 20th day for both the species. This may be because of cadmium is a heavy metal and is more toxic than zinc and hence has more pronounced effects than zinc whose effects remain more or less constant. The cell population and contamination must be monitored throughout the culturing of both the species and must be sub-cultured if needed.

5. Conclusion

The algal species were able to survive in the elevated concentration of Cadmium and Zinc. When the metals interact with the cells either it adsorbs on to the surface or gets absorbed into the cell where it is vacuolized. Cell wall being the barrier of absorption changes occur on it to prevent the entry of the heavy metals and for the survival. This leads to changes in the rigidity in the cell wall. Polysaccharides being one of the components on the cell wall may alter due to the metal stress. In our study changes in the concentration of polysaccharides is observed in the Cadmium and Zinc treated cells when compared to the Control. Further studies have to done to analyze the type of polysaccharides produced in the defense action.

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