A DISSERTATION ON

A Comparative Study to Elucidate Multifunctional Role of Dehydrin Protein in *Spirulina Platensis* Exposed to Abiotic Stress

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
PRATIBHA
M. Tech Biotechnology (IV Semester)
Roll No: 2001361014

UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Pratibha**, a student of **M.Tech Biotechnology** (**2nd year/4th semester**), Integral University have completed my six months dissertation work entitled "**A comparative study to elucidate multifunctional role of dehydrin protein in *Spirulina platensis* exposed to abiotic stress**" successfully from **Integral University** under the guidance of **Dr. Alvina Farooqui, Associate Professor and Head, Department of Bioengineering, Integral University**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Pratibha

Date:

Dr. Salman Akhtar
(Course coordinator)
Associate Professor
Dept. of Bioengineering

CERTIFICATE BY SUPERVISOR

This is to Certify that Ms. **Pratibha (2000101667)** has carried out the research work presented in this thesis entitled **"A comparative study to elucidate multifunctional role of dehydrin protein in *Spirulina platensis* exposed to abiotic stress"** for the award **M. Tech Biotechnology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M. Tech Biotechnology** degree.

I wish her good luck and bright future.

Dr. Alvina Farooqui
Associate Professor and Head
Department of Bioengineering
Integral University, Lucknow

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Pratibha,** a student of **M.Tech Biotechnology** (2nd Year/4th Semester), Integral University has completed his six months dissertation work entitled **"A comparative study to elucidate multifunctional role of dehydrin protein in *Spirulina platensis* exposed to abiotic stress"** successfully. She has completed this work from Integral University under the guidance of **Dr. Alvina Farooqui**, Associate Professor and Head, Department of Bioengineering, Integral University, Lucknow. The dissertation was a compulsory part of her **M.Tech Biotechnology** degree**.**

I wish her good luck and bright future.

Dr. Alvina Farooqui

Head of department (HOD)

Department of Bioengineering

Faculty of Engineering

TO WHOM IT MAY CONCERN

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Dr. Alvina Farooqui
Associate Professor and Head
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Integral University, Lucknow

Acknowledgement

DATE: **Pratibha**

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LIST OF ABREVIATIONS

ABREVIATIONS	NAME
μg	Microgram
μL	Microlitre
μM	Micromolar
mm	Millimeter
mM	Millimolar
rpm	Rotation Per Minute
CCP	C-terminal Carotenoid Protein
EPS	Extra-Cellular Polysaccharides
HCP	Helical Carotenoid Protein
OD	Optical Density
PFD	Photon Flux Density
PMSF	Phenylmethylsulfonyl floride
ROS	Reactive Oxygen Species
SOD	Superoxide Dimutase

INTRODUCTION

The demands of agricultural products are increases with increase in the population globally. To meet the agricultural productivity for population by enhancing the genetically yielding crops and also using synthetically formed manure or green revolutions. A balanced agriculture is practiced including environmental factors and modern agricultural practices are profoundly depends on the manure or fertilizers (biofertilizer) and nitrogen is most important element in agricultural fertilizer and in atmosphere 78% (atmospheric volume) nitrogen present. Utilized in agriculture till came in contact with soil or water. But the major hindrances faced during agricultural practices are the drought, flood and stresses due to rapidly changes in environment, globalization and industrialization. Stress mainly abiotic (salts, osmotic, metals and heavy metals) and biotic (wound depletion) leads to the declination of agricultural products and that cause the major issues to fulfillment of agricultural products to world population. To combat with these natural and man-made calamities, cyanobacteria play promising role in the fulfillment of requirement either food, environment purifier, natural and man-made calamities etc. According to evolutionary data, cyanobacteria present since Paleoarchean era (non-oxygenic but photosynthetic bacteria) and Mesoarchean (oxygenic photosynthesis) era on earth. Cyanobacterial cells not only have properties to release oxygen or CO_2 fixation also capable for nitrogen fixation, in which it converts atmospheric nitrogen or ammonia into the nitrates by the helps of nitrogenase enzyme usually found in the heterocyst(a modified thick cell wall where nitrogen fixation occur(Singh et al., 2011)) containing cyanobacteria like *Spirulina platensis* or *Anthrospora* mostly found in alkaline brakish or saline water and terrestrial region, free floating containing filamentous cylindrical and multicellular organism having special trichomes feature and further nitrates utilize by other higher plants in their cellular metabolic processes(Joshi et al., 2020).

Adaptation rate of cyanobacteria towards harsh environments (Abiotic and biotic stress like cell damage, temperature, metals, osmotic and salts stress), this adaptation occur either by xenobiotically or naturally and their ranges may upto halophilic to

cryophyllic(low temperature) (Yadav et al., 2021). Xenobiotics, abnormal characteristic either physiological or biological caused by any chemical compound above normal range, it may occur by natural (evolution) or rapid (mutation or stress) (Embrandiri et al., 2016). Due to their great adaptation towards harsh environment, many changes seen in their morphology or cell start producing extracellular polysaccharide which provide safety to cells (Malyan et al., 2020). Due to rapid division of cells, it is used for treatment of industrial effluents like food, sugar, paper, textile, pharmaceuticals and distilleries industry. *Cyanothece 16Som 2* and *Nostoc PCC 7936, Nostoc sp.* and *Cyanospiracapsule* are used for the removal of heavy metals like Chromium (Cr), Copper (Cu), *Gloeothece magna, Nostoc linckia* and *Nostoc rivularis* for Cadmium (Cd), Magneese (Mn) an nickel (Ni) (Anabaena sp.) etc during waste water treatment discharge from industries. For removal of phosphorous (P) *Methyl parathion (Fischerella sp.), Chlorpyrifos (Synechocystis sp.), Fenamiphos (Nostoc sp.)* is used (Malyan et al., 2020). Cyanobacteria can have ability to combat with harsh environments or stress (Rezayian et al., 2019). Cyanobacteria combat with these stresses by changes in their physiology, morphology, molecular or biochemical activites. Due to these properties of cyanobacteria , it is used as model for study of defense mechanism/ strategies against stress (Rezayian et al., 2019). Cyanobacteria can have ability to combat with harsh environments or stress (Fujita et al., 2006; Rezayian et al., 2019). To combat with these stresses, cyanobacteria l cells shows modification in their physiology, morphology, molecular or biochemical activities. Due to these properties of cyanobacteria , it is used as model for study of defense mechanism/ strategies against stress (Rezayian et al., 2019; Righini et al., 2022). When stress concentration above the critical tolerance levels for cyanobacteria causes death of cells (Allakhverdiev & Murata, 2008; Malyan et al., 2020; Suzuki et al., 2014; Yadav et al., 2021).Increase of either abiotic stress or biotic stress directly effects on cell metabolism of cyanobacteria or other higher organism's cell (Malyan et al., 2020; Suzuki et al., 2014; Yadav et al., 2021).All these stress leads to release of oxidative stress which causes generation of ROS in the form of superoxide (O_2^-) and peroxide (O_2^{-2}) and H_2O_2 , these all inhibit the water splitting during cellular respiration in organism these all process occur in mitochondria which causes dehydration and ultimately trigger to the apoptosis of cell or cell death (Yadav et al., 2021).Where as in higher organisms to

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combat with the effect \ toxicity or any stresses, an autophagy process exist which work as housekeeper and have capability to maintain homeostasis of cell(Liu et al., 2021; Rocha et al., 2019; Yang et al., 2018). Metals stress (Aluminum stress), leads to the decrease of uptake of phosphorous from medium to the soil in higher plants (Pettersson et al., 1988).These stress directly affects on the PS-I and PS-II in autotrophic cells either plants or algae (Allakhverdiev & Murata, 2008; Liu et al., 2021).Heat sock protein (Hsp); Hsp70, Hsp40, a chaperone protein plays role in the ATP hydrolysis and Hsp40 stimulates the efficiency of Hsp70 approx 1000times, these work in higher photosynthetic organism or plants. Homologous toHsp70, Hsp40, same function perform by DnaK and DnaJ (chaperone) in prokaryotic, which prevents the cells from heat shock (H. F. Xu et al., 2020). Multiple copies of DnaK and a chaperone DnaJ in cyanobacteria helps in the stress out form the stress condition (Huang et al., 2002).HCP (helical carotenoid protein) which mostly found in cyanobacteria that helps in the terrestrial adaptation, homologous to HCP,CCP C-terminal like carotenoid protein found in *Nostoc flagelliforme* (Melnicki et al.2016; Muzzopappa et al. 2017).

Stress leads to the release of ROS; in low concentration leads to signaling and when concentration is high, helps in growth or in adaptation in harsh environments. ROS affects on rate of photosynthetesis, nitrogen metabolism, growth, and in pigments contents (Yadav et al., 2021). To combat with stress, biological and genetical changes occur in cells over expression of specific protein (dehydrin or dehydrin like protein) seen during stress (Rezayian et al., 2019; Sukenik & Kaplan, 2021). To subsist with dehydration or any damage in cells, cyanobacteria l cells start production of polysaccharides and layer to the exterior of cells (extracellular polysaccharides; EPS) by upregulation in cellular activities which like DNA repair, endure protein integrity, and synthesis of solute which help in enduring oxidative stress and prevent cells dehydration (H. Xu et al., 2021). EPS work as masking of cells other cell organelle and greatly work to minimize the abiotic stress.

Due to production of EPS it uplift water holding capacity of cells and internal water loss inhibit, which aid in water recycling, which helps in the recovery of photosynthesis (H. Xu et al., 2021).It work slow in reabsorption of water as compare to dehydration rate, so

EPS act as 1st line defense mechanism in stress condition. EPS work like cementing material between cyanobacteria l cells and soil, it contain many trichome which play major role in stress tolerance (Isanta-Navarro et al., 2021; H. Xu et al., 2021). Trehalose (non- reducing α-glucose, linked by α1, 1-glycosidic bond) is a major solute which can eases the integrity of structure of lipid membrane of cyanobacteria l cells when water is low in soil Aid out with stress tolerance, cyanobacteria expresses many proteins like osmoprotective protein, anhydrin, LEA (late embryogenesis abundant) protein, some unidentified protein. WaspA acidic water stress protein associated with EPS and came up with biofilm formation observed in many Nostoc species (H. Xu et al., 2021). WaspA concentration reported high in stress condition or in condition dehydration. Some chaperon, ELIPs (early light-inducible protein) and homoiochlorophyllus rapidly response in up-regulation in case dehydration (H. Xu et al., 2021).

Due to death and decay of cyanobacteria and mixed in soil, and their fluid part act as binding agents which binds with soil and their texture change. After that soil become more fertile and more porous and rich in nutrient (Joshi et al., 2020). When porosity in soil increases leads to hold more water and easily available for plants and crops. Secondary metabolites a phytochemical compound or plant hormones produce by cyanobacteria , which increase the overall growth of crops and plants (Joshi et al., 2020; Liu et al., 2021)

Cyanobacteria l cell produces primary and secondary metabolites in which some components of secondary metabolites act as antifungal and antibacterial which acts against fungi and bacteria and reduces the diseases caused by them in plants. It has potential to control the pathogenic fungi and plants which cause major damage of crops. *Sclerotinia sclerotiorum* and *Rhizoctonia solani* cause damping-off (disease) in plants and inhibited by *Nostoc muscorum* extract (Joshi et al., 2020). Cyanobacteria notably used as biofertilizer in various crop fields like rice, okra, wheat and pea. It has capability to fix atmospheric nitrogen and easily available for crops like *Nostoc, Anabaena, Tolypothrix* (Joshi et al., 2020; Malyan et al., 2020).

Cellular metabolism leads to primary and secondary metabolites production, primary metabolic product used by cells for itself and for other purpose like biofertilizer,

bioplastic and dyes (Malyan et al., 2020) where as secondary metabolic product like alkaloids, terpenes and cyclic peptides. Secondary metabolites act as antifungal, antibacterial, anti-algal (Lau et al., 2015). Soil salinity is a major problem specially in India, and practically required more effort for filtration, (Linn, 2009; Rai, 2015) Soil salinity is reducing by absorption of sodium ion from soil, cyanobacteria have high capability to combat with high salinity present in soil (Allakhverdiev & Murata, 2008).

2. Review and Literature

Cyanobacteria are a monophyletic, oxygenic photosynthetic, gram negative; prokaryotic bacteria and having nitrogen fixation capability. It is a ubiquitous organism, morphologically diverse found in fresh water, saline water, and terrestrial (Walker & Dundee, 2009). According to evolutionary data, cyanobacteria present since Paleoarchean era (non-oxygenic but photosynthetic bacteria) and Mesoarchean (oxygenic photosynthesis) era on earth (Sánchez-Baracaldo et al., 2022).

Adaptation rate of cyanobacteria towards harsh environments (Abiotic and biotic stress like cell damage, temperature, metals, osmotic and salts stress), this adaptation occur either by xenobiotically or naturally and their ranges may upto halophilic to cryophyllic(low temperature) (Yadav et al., 2021). Xenobiotics, abnormal characteristic either physiological or biological caused by any chemical compound above normal range, it may occur by natural (evolution) or rapid (mutation or stress) (Embrandiri et al., 2016). Major reason of their adaptation due to the composition of outer layers of cell, lased with extra-polymeric, a polysaccharide. This polysaccharide contain many binding site to metals which helps in the accumulation or metabolize in cell and change into less toxic ion compare to previous (Yadav et al., 2021). Mechanism occur in cyanobacteria l cell gave rise a hope for the future in many aspects (Yadav et al., 2021).

2.1. Classification

Classification of cyanobacteria on the basis of morphological appearance and their life cycles are divided into distinctive five subsection lies under Cyanophyceae of algal classification.

2.1.1. Chroococcales: It is unicellular organism and non-filamentous. Present in single cell form or aggregated, reproduce by binary fission or budding. For example-*Synechocystis, Microcystis, Cynobium, Gloeocapsa, Chrococcus,* etc (Walker & Dundee, 2009).

2.1.2. Pleurocapsales: A unicellular, non-filamentous. In both form single and aggregated. Asexually reproduce by multiple fission or binary fission. For example-*Myxosarcina, Stanieria, Hyella, Pleurocapsa,* etc (Walker & Dundee, 2009).

2.1.3. Oscillatoriales: Free-floating, filamentous, multicellular trichomes (Helically coiled or sheath). Heterocyst is not formed or akinetic. For example- *Spirulina, Starria, Limnothrix, Oscillatoria, Athrospira, Cirnalium, Lyngbya, Trichodesmium, Leptolynbya, Borzia, symploca,* etc (Walker & Dundee, 2009).
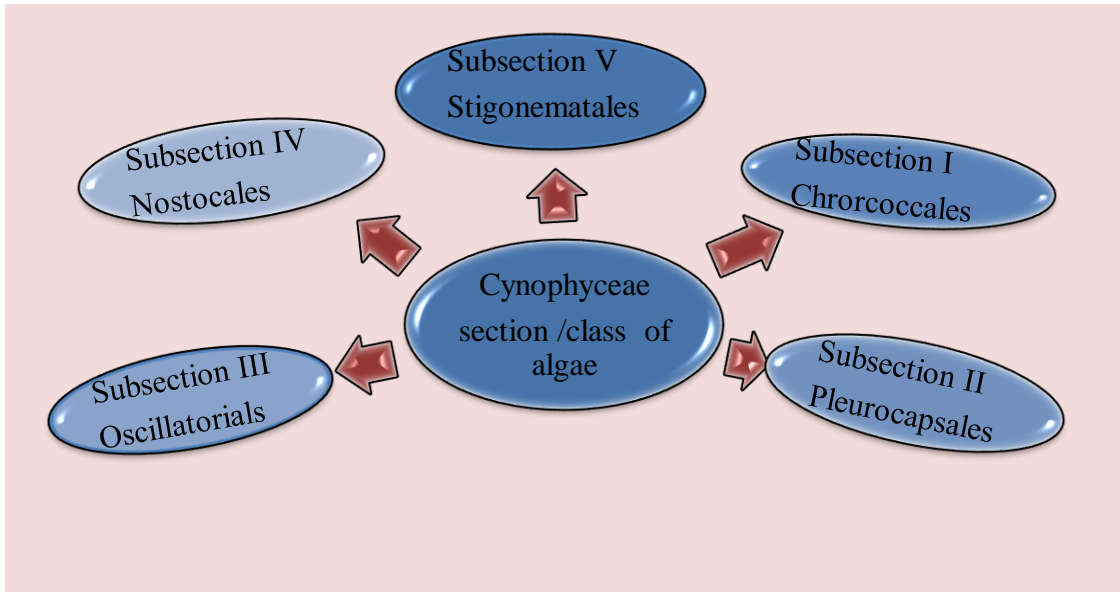
Fig 1: Subclasses of Cyanophyceae.

2.1.4. Nostocales: Cells are filament in Moniliform, both found in fresh and marine. Heterocyst formed when low concentration of nitrogen present in soil or water. Uniseriate trichome with true branching present in many form as taper, spiral, straight, or in gelatinous form. For example- *Nostoc, Scytonema, Calothrix, Tolypothrix, Anabaena, Gloeotrichia, Nodularia, Cylindrospermum*, etc (Walker & Dundee, 2009).

2.1.5. Stigonematales: Cell is always filamentous, present in both truly branched trichome and multiseriate. Heterocyst formed in cell, when amount of nitrogen is low in soil and water. For example- *Geitleria, Nostochopsis, Stigonema, Fischerella, Chloroglopsis*, etc(Walker & Dundee, 2009).

2.2. Merits of cyanobacteria:

2.2.1. Role of cyanobacteria in Earth sphere:
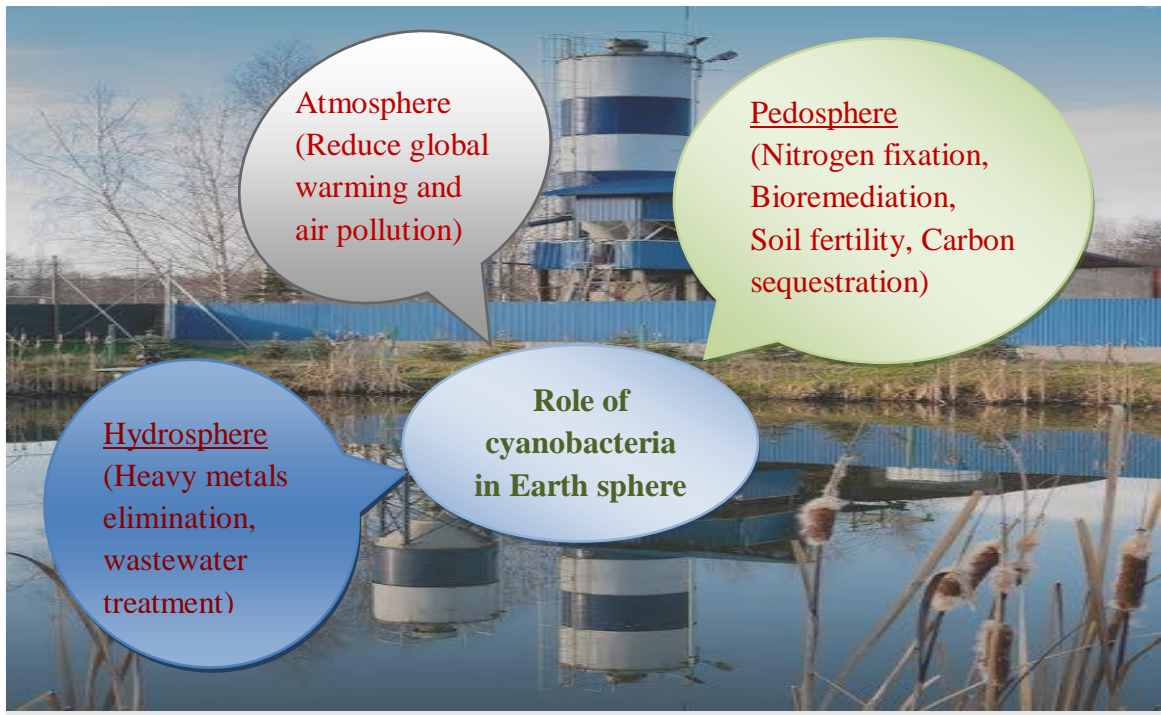
Fig.2: Illustration of role of cyanobacteria in earth sphere.

2.2.2. Role of cyanobacteria in agriculture:

- **Boost soil porosity**: Due to death and decay of cyanobacteria and mixed in soil, and their fluid part act as binding agents which binds with soil and their texture change. After that soil become more fertile and more porous and rich in nutrient (Joshi et al., 2020).

- **Boost water holding capacity of soil**: when soil porosity increases leads to hold more water and easily available for plants and crops.

- **Libration of growth promoting compounds**: Secondary metabolites a phytochemical compound or plant hormones produce by cyanobacteria , which increase the overall growth of crops and plants (Joshi et al., 2020)

- **Biocontrol agents**: Cyanobacteria 1 cell produces primary and secondary metabolites in which some components of secondary metabolites act as antifungal and antibacterial which acts against fungi and bacteria and reduces the diseases caused by them in plants. It has potential to control the pathogenic fungi and plants which cause major damage of crops. *Sclerotinia sclerotiorum* and *Rhizoctonia*

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*solani*cause damping-off (disease) in plants and inhibited by *Nostoc muscorum* extract (Joshi et al., 2020).

- **Biofertilizer**: Cyanobacteria notably used as biofertilizer in various crop fields like rice, okra, wheat and pea. It has capability to fix atmospheric nitrogen and easily available for crops like *Nostoc, Anabaena, Tolypothrix* (Joshi et al., 2020; Malyan et al., 2020).

- **Production of bioactive compounds**: Cellular metabolism leads to primary and secondary metabolites production, primary metabolic product used by cells for itself and for other purpose like biofertilizer, bioplastic and dyes (Malyan et al., 2020) whereas secondary metabolic product like alkaloids, terpenes and cyclic peptides. Secondary metabolites act as antifungal, antibacterial, antialgal (Lau et al., 2015)

- **Reduce soil salinity:** Soil salinity is a major problem specially in India, and practically required more effort for filtration,(Linn, 2009; Rai, 2015)Soil salinity is reducing by absorption of sodium ion from soil, cyanobacteria have high capability to combat with high salinity present in soil (Allakhverdiev & Murata, 2008).

Table 1: List of cyanobacteria species and their potential application.

S. No.	Cyanobacteria	Cyanobacteria use as	References
1.	*Nostoc sp., Anabaena sp., Cylindrospermum sp., Phormidium sp., Oscillatoria sp.*	N2 fixation	(Gonçalves, 2021b; Múnera-Porras et al., 2020)
2.	*Anabaena sp., Calothrix sp., Nostoc sp., Oscillatoria sp., Phormidium sp.*	Phosphate solubilization	(Gonçalves, 2021b, 2021a)
3.	*Anabaena sp., Calothrix sp., Nostoc sp., Phormidium sp., Hapalosiphon sp., Aulosira sp., Tolypothrix sp., Oscillatoria sp., Plectonema sp.*	Phytohormone production	(Gonçalves, 2021b; Múnera-Porras et al.,

			2020)
4.	*Anabaena sp., Calothrix sp., Nostoc sp., Phormidium sp., Hapalosiphon sp., Aulosira sp., Tolypothrix sp., Oscillatoria sp., and Plectonema sp.*	Biotic compound excretion	(Múnera-Porras et al., 2020; Rocha et al., 2020)
5.	*Nostoc sp., Anabaena sp., Cylindrospermum sp., Phormidium sp.,Synechococcussp.,Chlorogloeopsis sp., Spirulina sp.*	Symbiotic associations	(Múnera-Porras et al., 2020; Rajeev et al., 2013)
6.	*Nostoc sp., Anabaena sp., Calothrix sp. Scytonema sp., Microcystis sp., Oscillatoria sp., Synedchoccus sp.*	Biocontrol agents	(Righini et al., 2022)

2.2.3. Role of cyanobacteria in bioremediation (Heavy metals absorption):

Cyanobacteria have great adaptation towards harsh environment, reported that in harsh environment cell produce extracellular polysaccharide which provide safety to cells (Malyan et al., 2020). Due to rapid division of cells,it is used for treatment of industrial effluents like food, sugar, paper, textile, pharmaceuticals, and distilleries industry. Cyanothece 16Som 2 and Nostoc PCC 7936, Nostoc sp. and Cyanospira capsule are used for the removal of heavy metals like Chromium (Cr), Copper (Cu),*Gloeothece magna,Nostoc linckia*and *Nostoc rivularis* for Cadmium (Cd), Magneese (Mn) an nickel (Ni) (*Anabaena sp.*) etc. during waste water treatment discharge from industries. For removal of phosphorous (P) Methyl parathion (Fischerella sp.), Chlorpyrifos (*Synechocystis sp.*),Fenamiphos (*Nostoc sp*.) is used (Malyan et al., 2020).

Table 2: List of Cyanobacteria under different metal stresses its effect and response towards it.

S. No.	Cyanobacteria	Stress	Effects on cyanobacteria	Response of cyanobacteria towards stress	References
1.	*Oscillatoria sp.*	Cd	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outerlayer by EPS (extracellular polymeric substances)	(Katircioğlu et al., 2008; Yadav et al., 2021)
2.	*Nostocminutum, Anabaena spiroides*	Pb, Cd, Ni	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metaloprotein, composed of outerlayer by EPS	(Al-Sherif et al., 2015; Yadav et al., 2021)
3.	*Pseudanabaenas p. Catenatasp.*	Sr	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Ashworth et al., 2018; Yadav et al., 2021)
4.	*Arthrospira platensis*	Pb	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Duda-Chodak et al., 2013; Yadav et al., 2021)
5.	*Graesiellaemersonii*	U, Ra	ROS generation, protein degradation,	Generation of stress protein,	(Heidari et al., 2018; Yadav et

			protein synthesis inhibition and genotoxicity	metallothionein, composed of outerlayer by EPS	al., 2021)
6.	*Cyanobacterium metallothionein*	Cd	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Yadav et al., 2021; Yang et al., 2015)
7.	*Nostoc sp.*	Cr	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Warjri & Syiem, 2018; Yadav et al., 2021)
8.	*Pithophoraoedog onia, Spirogyra neglecta*	Pb, Cu	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Singh et al., 2007; Yadav et al., 2021)
9.	*Scytonema sp.*	As	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Prasad et al., 2006; Yadav et al., 2021)
10.	*Chroococcus Multicoloratus, Oscillitoriatricho ides*	Pb	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Miranda et al., 2013; Yadav et al., 2021)
11.	*Lyngbyaputealis HH-15*	Cr	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Kiran & Kaushik, 2008; Yadav et al., 2021)

12.	*Nostoc punctiforme A. S/S4, Chroococcidiopsi sthermalis S.M/S9*	U, Cd, Ra	ROS generation, protein degradation, protein synthesis inhibition and genotoxicity	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Heidari et al., 2017; Yadav et al., 2021)
13.	*Spirulina platensis*	Zn	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Mane et al., 2018; Yadav et al., 2021)
14.	*Pseudanabaena Catenate*	Sr	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outerlayer by EPS	(Foster et al., 2020; Yadav et al., 2021)
15.	*Lyngbyawollei*	Cu	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Bishop & Rodgers, 2012; Yadav et al., 2021)
16.	*S. platensis and Aphanothece flocculosa*	Hg	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Cain et al., 2008; Yadav et al., 2021)
17.	*S. muticum*	Hg	ROS generation, protein degradation, protein synthesis	Generation of stress protein, metallothionein	(Carro et al., 2011; Yadav et al., 2021)

			inhibition, genotoxicity and apoptosis	composed of outer layer by EPS	
18.	*Tetraselmischuii and Spirulina maxima*	Cd	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Da Costa & De França, 2003; Yadav et al., 2021)
19.	*Arthrospira platensis*	Pb	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Gupta & Rastogi, 2008; Yadav et al., 2021)
20.	*N. muscorum Oscillatoria sp.*	Cr	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed ofouter layer by EPS	(Singh et al., 2007; Yadav et al., 2021)
21.	*Phormidium sp., Lyngbya sp., Aulosira sp., Scytonemasp*	Cu, Cd, Pb	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Foster et al., 2020; Yadav et al., 2021)
22.	*Synechococcus sp.*	Cd	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Carro et al., 2011; Yadav et al., 2021)

23.	*Cyanothece sp.*	Cu, Cr, Ni	ROS generation, protein degradation, protein synthesis inhibition, genotoxicity and apoptosis	Generation of stress protein, metallothionein, composed of outer layer by EPS	(Micheletti et al., 2008; Yadav et al., 2021)
24.	*Nostoc sp., Anabaena sp. ,Oscillitoriasp., spirullina sp., Lyngbya sp.*	Salinity or salt stress	ROS generation, dehydration, protein synthesis inhibition, protein degradation,	Synthesis of extracellular polymeric substances or polysaccharides, stress protein, many unknown protein	(Poveda, 2020)
25.	*Synechocystis sp., Nostoc sp., Anabaena sp., Oscillitoria sp., spirullina sp., Lyngbya sp.*	Osmotic stress glucose , sucrose threalose, maltose, sorbitol, etc.	Dehydration of cells, ROS generation, protein degradation, protein synthesis inhibition	Synthesis of extracellular polymeric substances or polysaccharides, stress protein, many unknown protein	(Allakhverdiev & Murata, 2008)
26.	*Synechocystis sp., Nostoc sp., Anabaena sp., Oscillitoria sp., Spirulina sp., Lyngbya sp.*	Aluminum	Dehydration, ROS generation, inhibit PS-I, PS-II	Synthesis of extracellular polymeric substances or polysaccharides, stress protein, many unknown protein	(Kannaujiya et al., 2020; Shamim et al., 2020; Sukenik &Kaplan, 2021)

2.3. Cyanobacteria l response towards stress:

Cyanobacteria can have ability to combat with harsh environments or stress (Rezayian et al., 2019). Cyanobacteria combat with these stresses by changes in their physiology, morphology, molecular or biochemical activities. Due to these properties of cyanobacteria , it is used as model for study of defense mechanism/ strategies against stress (Rezayian et al., 2019).
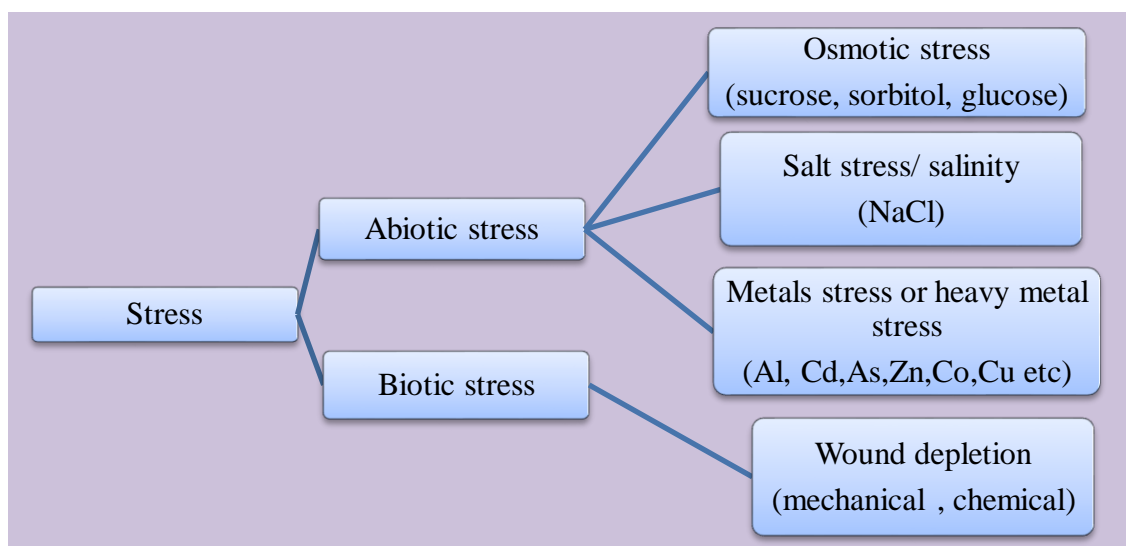
Fig. 3: Illustration of role of cyanobacterial response towards stress

2.3.1. Metal stress or heavy metal stress (Oxidative stress): Metals which have high atomic number (>20) or atomic mass (>~63.5 g mol^{-1}) or atomic density (5g cm^{-3}) undergoes heavy metals categories, it may be metals or metalloids. For example Aluminum (Al), Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Nickel (Ni), Copper (Cu), Iron (Fe), Selenium (Se), Lead (Pb), Palladium (Pd), Mercury(Hg), Zinc (Zn), etc (Yadav et al., 2021).

Human got exposed to heavy metals either by food or drinkable water or polluted air while breathing, person who work in factories or industries where heavy metal discharge as by product or waste came direct contact with it and suffered serious health issues like cancer, asthma, eye sight loss, etc. some heavy metal which elevate disease like Anosmia, renal failure, neurological problems, and bone disease are reported in male caused by

increase of Cadmium (Cd) concentration in human body (Yadav et al., 2021). Acute poisoning (affected area may be brain, blood cells, gastrointestinal tract and heart) and chronic poisoning (cause keratosis and irregular pigmentation in skin) occur when Arsenic concentration increase. Short term exposure to As imbalance leukocytes and erythrocytes regulation, nausea, vomiting, long-term exposure to As lead to hypertension, cancer, heart disease, neural disorder (central and peripheral neural disorder) and lastly cause death. Exposure to copper, zinc, cadmium and magnesium causes improper regulation in of body metabolism (Yadav et al., 2021).

Plants have high ability to fight with heavy metals stress as compared to human but less as compared to cyanobacteria, as we already know that Cyanophyceae have great ability to combat with harsh environment i.e., heavy metals stress or with oxidative stress. These stress leads to the activation of ROS (Reactive Oxygen Species) (Liu et al., 2021) from mitochondria (procreation of ROS), released by target cells and act like signal for healthier cells for upcoming damage and that's way act as housekeeper (Liu et al., 2021).

2.3.2. Salts stress or soil salinity: Salt stress is one of the most common stresses occur due to the presence of sodium chloride in ions form (Rai, 2015). Maximum range of salts tolerance depends on the strain of cyanobacteria that came under which categories like, for halophilic, moderate and low salt tolerance level is 2.5-3.5 molar, 1.25 molar and 0.7 molar respectively (Rezayian et al., 2019).

Salt stress mainly cause water and ion imbalance in cell and leads to activate primary stress i.e., osmotic stress and ionic stress and for long duration cause oxidative stress known as secondary stress. In cell there is ion balance between extracellular membrane and cytosolic space, when stress given or naturally occur it cause dehydration of cell and due to this cell turgidity loss and cells get shrink (Allakhverdiev & Murata, 2008; Rezayian et al., 2019; Yadav et al., 2021). Salt stress usually affects in cells process as photosynthesis (photo system I and photo system II) and metabolic respiration. Changes in cell metabolism observed modification occur in protein or in genetic level. At primary level modification in protein occur to combat with stress and later goes to genetic level (Rezayian et al., 2019). Modifications in protein mainly occur due to ROS generation, due to which either bond formation or bond breakage between amino acids. For example

in stress condition cysteine change into cystine and methionine into degraded photodamaged D1 protein (Rezayian et al., 2019).

2.3.3. Osmotic stress: Earlier, salt stress and osmotic stress term used as synonym, because for osmotic stress NaCl frequently used to study about their effects due to osmolyte. But in recent both are different not only action also their effect on cyanobacteria l cells (Allakhverdiev & Murata, 2008). When cyanobacteria l strain treated to0.5 molar of sorbitol, about 30% to 50% decrease in cytoplasmic volume observed within less than 5minute and again treated to 1.0 molar of sorbitol within 10-minute cytoplasmic volume decrease upto 50%. In both case causes cell shrinkage. Loss in cytoplasmic volume observed due to presence of p-chloromercuriphenyl-sulfonic acid (CSA), whereas in normal condition it returns to its normal volume when osmotic stress is removed. Osmotic stress block activity of photo system-I (PS-I) and photo system-II (PS-II)about 70% and 40% respectively (Allakhverdiev & Murata, 2008).

2.4. Demerits of stress on cyanobacteria:

When stress concentration above the critical tolerance levels for cyanobacteria causes death of cells (Allakhverdiev & Murata, 2008; Malyan et al., 2020; Suzuki et al., 2014; Yadav et al., 2021).Increase of either abiotic stress or biotic stress directly effects on cell metabolism of cyanobacteria or other higher organism's cell (Malyan et al., 2020; Suzuki et al., 2014; Yadav et al., 2021).All these stress leads to release of oxidative stress which causes generation of ROS and H_2O_2 , these all inhibit the water splitting during cellular respiration in organism (Yadav et al., 2021). Metals stress (Aluminum stress), leads to the decrease of uptake of phosphorous from medium to the soil in higher plants (Pettersson et al., 1988).These stress directly effects on the PS-I and PS-II in autotrophic cells either plants or algae (Allakhverdiev & Murata, 2008; Liu et al., 2021).

2.5. Merits of stress on cyanobacteria:

Stress leads to the release of ROS; in low concentration leads to signaling and when concentration is high, helps in growth or in adaptation in harsh environments. ROS effects on rate of photosynthesis, nitrogen metabolism, growth, and in pigments contents (Yadav et al., 2021).To combat with stress, biological and genetical changes occur in

cells over expression of specific protein (dehydrin or dehydrin like protein) seen during stress (Rezayian et al., 2019; Sukenik & Kaplan, 2021).

Abiotic stress
- Osmotic stress
- Salt stress
- Metal stress

Action on mitochondria
- ROS generation
- $O_2 + SOD \longrightarrow H_2O_2 + POD \longrightarrow H_2O_2 + O_2$

Upregulation of Dehydrin protein
- Protect organelle, protein and DNA from oxidative damage.
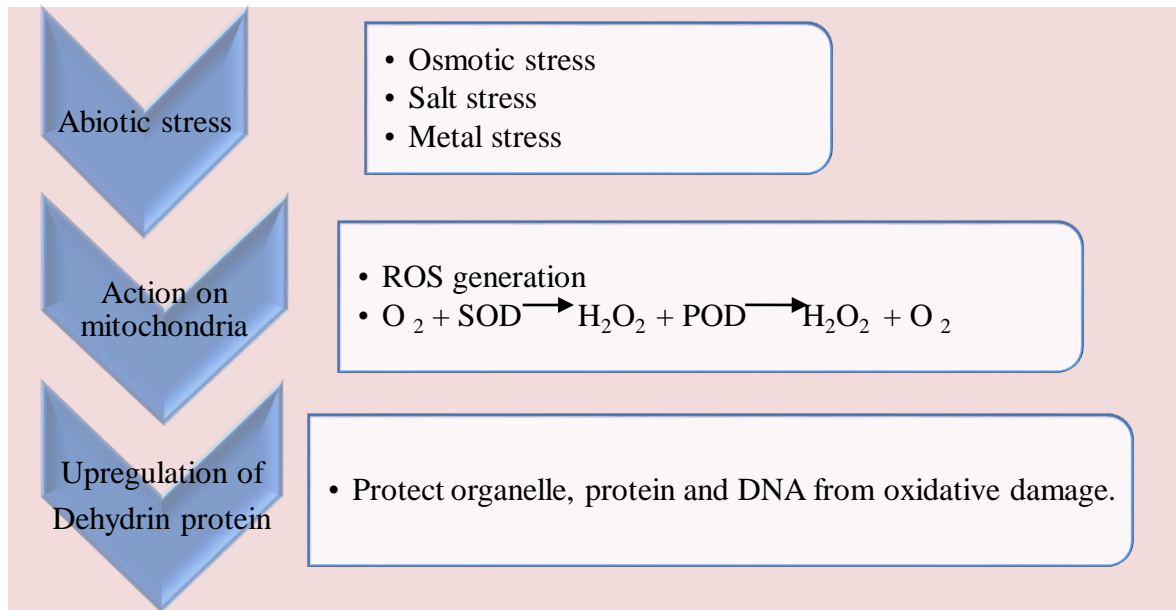
Fig. 4: Effects of stress on cyanobacterial cells.

2.6. Effects of stress on cyanobacterial cell:

Abiotic and biotic stress has led to the increase the toxicity in environment, due to which their effects shown the growth of the cyanobacteria, also seen when treated with different stress in-vitro culture. These stresses cause many alterations in the synthesis of protein, enzyme, and other biomolecules, which create hindrance in the cell signaling which directly effects on the metabolic process of cell (Yadav et al., 2021).When the toxicity of stress increases above the critical concentration, lead to changes in many physiological processes as in pigment contents, growth and photosynthetic rates. These changes cause the generation of ROS (Reactive Oxygen Species) in the form of superoxide and peroxide and release of ROS have both negative and positive effect on cell. ROS in low concentration act as the signaling for stress that cells have to be prepared for that. And high concentration of ROS trigger the apoptosis processes cell and cause the death of the cyanobacteria l cells (Liu et al., 2021).To combat with these stress like metals (AlCl$_3$.6H$_2$O.), salts (NaCl), osmotic (sucrose, sorbitol) cyanobacteria l cells start to enhance the production of some special protein or dehydrin protein, enzymes and also

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cells has to produce the large amount of EPS which protect the cell wall from outer harsh environment (Wen et al., 2019; Xu et al., 2021).

2.7. Role of dehydrin protein: To subsist with dehydration or any damage in cells, cyanobacteria l cells start production of polysaccharides and layer to the exterior or cells (extracellular polysaccharides; EPS) by upregulation in cellular activities which like DNA repair, endure protein integrity, and synthesis of solute which help in enduring oxidative stress and prevent cells dehydration (Xu et al., 2021).In research it was reported that; water holding capacity of EPS is high, which aid in water recycling, in *Nostoc commune* EPS not only uplift water holding or absorption it also enhanced in recovery of photosynthesis (Xu et al., 2021).

It works slowly in reabsorption of water as compared to dehydration rate, so EPS act as 1st line defense mechanism in stress condition. EPS work like cementing material between cyanobacteria l cells and soil, it contain many trichome which play major role in stress tolerance (Isanta-Navarro et al., 2021; Xu et al., 2021).Trehalose (non- reducing α-glucose, linked by α1, 1-glycosidic bond) is a major solute which can eases the integrity of structure of lipid membrane of cyanobacteria l cells when water is low in soil Aid out with stress tolerance, cyanobacteria expresses many proteins like osmoprotective protein, anhydrin, LEA (late embryogenesis abundant) protein, some unidentified protein. WaspA acidic water stress protein associated with EPS and came up with biofilm formation observed in many Nostoc species (Xu et al., 2021). WaspA concentration reported high in stress condition or in condition dehydration. Some chaperon, ELIPs (early light-inducible protein) and homoiochlorophyllus rapidly response in up-regulation in case dehydration (Xu et al., 2021).

Objectives

1. Maintenance and growth standardization of Cyanobacterial strain *Spirulina platensis*.

2. Growth, photosynthetic ability, defense mechanism of *S. platensis* under different abiotic stress (Aluminum, NaCl, Sucrose).

3. Elucidation of molecular underlying mechanism with special focus on induction of stress protein, dehydrin and its protective role to prevent DNA damage under different abiotic stress.

3. MATERIALS AND METHODS

3.1. Experimental organisms

The experimental organism was previously isolated from rice paddy field 2 of Integral institute of Agricultural Science and Technology (IIAS&T). After isolation the strain was purified and Identification was confirmed based upon the keys given by Desikachary for microscopic parameters and maintained at $25\pm 5°C$ temperature, 2000 Lux light (14 hours) in the cyanobacterial culture room in Department of Biotechnology, Integral University, Lucknow. Zarruok medium was used for the growth of *Spirulina platensis*

3.2. Chemicals

All the chemicals were of lab grade and were purchased from standard company like Sigma, Himedia, Merck, etc.

Table 3: List of chemicals used

S. No	Chemical Name	Make
1.	K_2HPO_4	HIMEDIA
2.	$MgSO_4.7H_2O$	HIMEDIA
3.	$CaCl_2.2H_2O$	HIMEDIA
4.	Na_2CO_3	SIGMA
5.	Ferric ammonium	SIGMA
6.	Citrate	SIGMA
7.	Citric acid	HIMEDIA
8.	EDTA	REMI
9.	Na_2NO_3	HIMEDIA
10.	$NHCl_2.4H_2O$	REMI

11.	$Na_2MoO_4.2H_2O$	REMI
12.	$ZnSO_4.4H_2O$	REMI
13.	$CuSO_4.5H_2O$	HIMEDIA
14.	H_3BO_3	MERCK
15.	$Co(NO_3)_2.6H_2O$	MERCK

3.3. Glassware's and Equipment Used

For stock culture: 100-150 mL Erlenmeyer flasks (Borosil, India), For batch culture: 1.0-2.0 L round bottom flasks (Borosil, India), Non absorbent cotton was used to plug the flasks, For Large scale cultivation, 5 liter-glass vessel Self-designed was used, pH meter, Centrifuge, Autoclave, PC based Double beam UV-Vis Spectrophotometer (2202, Systronics).

3.4. Sterilization of medium

All the glasswares and culture media were sterilized in an autoclave at 15 lb inch^2 pressure and at 121 ^{0}Ctemperature for 15 min. Before autoclaving, the desired quantity of media has been poured into suitable sized glass wares properly plugged with cotton.

3.5. Isolation and purification

Standard microbiological techniques were used to isolate and raise the cultures of cyanobacteria into clonal and axenic cultures. For that cyanobacterial strains were collected by gentle scraping using sterile blades and needles. The specimens were stored in screw cap bottles. A pinch of the sample was homogenised and added to 150 ml Erlen mayer flasks containing 50 ml of sterile medium with or without combined nitrogen and incubated at room temperature under fluorescent light. Clonal and axenic populations were obtained by serial dilution.

3.6. Incubation and maintenance of cultures

3.6.1. The stock culture

For maintenance of laboratory culture, 2- 3 mL of a 3 weeks old cyanobacterial stock culture was used as inoculum in 50 mL of autoclaved Zarrouk medium in 150 mL Erlenmeyer flasks. These samples were maintained at 27 ± 2 °C under 75 µmol m^{-2}s^{-1} photon flux density (PFD) by cool fluorescence lamps with a photoperiod of 14:10 h. The stock cultures were maintained for 20-30 days.

3.6.2. Agar Slants

Cyanobacterial strains were inoculated into sterilized tubes containing agar prepared in the above medium. After 15 days of incubation in light and in darkness, cultures were examined microscopically and absence of bacteria was confirmed. Such bacteria free clones were selected and maintained on different agar slants. During the course of experimental work, cultures were tested for bacterial contamination from time to time and in case of contamination the culture was discarded and new slant from original stock was used for further study.

3.6.3. The batch culture

During this work 5 cyanobacterial strains (see 2.2.1) were cultured in batch cultures. Aliquots of 50 mL from the stationary phase stock cultures were used to inoculate 500 mL of autoclaved Zarrouk medium in 1.0 liter round bottom flasks. These samples were cultivated at 27 ± 2 °C under 75 µmol m^{-2}s^{-1} photon flux density (PFD) by cool fluorescence lamps with a photoperiod of 14:10 h. The batch cultures were regularly shaken twice a day. Log phase cultures were used as inocula as well as throughout experimental studies.

3.7. Metal treatment

Stock solution of AlCl$_3$.6H$_2$O was prepared in sterilized double distilled water and the solution was further sterilized by passing through Millipore membrane filter (0.22 µm). From the stock solution various required concentrations 2, 4, 8 and 16 µM of metal were prepared in the Zarrouk medium.

3.8. Salt treatment

Stock solution of NaCl was prepared in sterilized double distilled water and the solution was further sterilized by passing through Millipore membrane filter (0.22 μm). From the stock solution various required concentrations 5, 10, 15and 20 mM of metal were prepared in the Zarrouk medium

3.9. Sucrose treatment

Stock solution of$C_{12}H_{22}O_{11}$was prepared in sterilized double distilled water and the solution was further sterilized by passing through Millipore membrane filter (0.22 μm). From the stock solution various required concentrations 25, 50,75 and 100 mM of metal were prepared in the Zarrouk medium

Aluminum chloride

Molecular formula	-	$AlCl_3.6H_2O$
Molecular weight	-	133.34 g/mol
Physical state	-	Solid

Sodium chloride

Molecular formula	-	NaCl
Molecular weight	-	58.44 g/mol
Physical state	-	Crystalline solid

Sucrose

Molecular formula	-	$C_{12}H_{22}O_{11}$
Molecular weight	-	342.3 g/mol
Physical state	-	Crystalline Powder

3.10. Growth measurement

For growth measurement, cells from each treatment were harvested and centrifuged at 5000 rpm for 10 min and the pellets were then placed in dry, pre weighed glass vials. They were dried to constant weight (3-4 days) at 65 ^{0}C in an oven. Glass vials containing pellets were transferred to a desiccator and then weighed on a chemical balance. Specific growth rates (μ) were then calculated as

$\mu=[\ln(B_f/B_i)]/10$

Where B_i is the initial biomass B_f is the biomass at the end of the 10th day of incubation.

3.11. Photosynthetic pigment determination

3.11.1. Chlorophyll a and Carotenoids (organic soluble pigments)

For extraction of Chl-a and carotenoids, equal volume of each organism was centrifuged and pellet was suspended in a desired volume of 80% (acetone: water, v/v). After overnight incubation at 4^0C, suspension was centrifuged and supernatant was used for measuring Chl-a and carotenoids. The absorbance of pigment extracts was read spectrophotometrically for Chl-a at 665 nm and carotenoid at 480 nm. The specific coefficients as given by Myers and Kratz (1955) were used for the calculation of Chl-a and carotenoids concentrations in cultures. Quantitative estimation of these pigments in terms of g/l was done using formulae given below.

$$C = D/d \ \alpha \text{----------------------------------- (1)}$$

Where α = absorption coefficient (value of α for chl-a is 82.04 and for carotenoids is 200).

D = Optical density.

d = inside path length of spectrophotometer in (cm)

C = Concentration of pigment in gL^{-1}

3.11.2. Phycocyanin (water soluble pigments)

A known volume of cyanobacterial cultures were centrifuged and the pellet was re-suspended in 80% Acetone. After 1 h incubation in dark, cultures were again centrifuged and their residues were used for measuring phycocyanin. The residue so obtained was washed with 2.5 mM phosphate buffer (pH 7-0) and re-suspended in the same. After repeated freezing and thawing, phycocyanin content was extracted and supernatant was obtained after centrifugation and absorbance was recorded spectrophotomerically at 620 nm as per the method of Blumwald and Tel-Or (1982).

3.12. Enzymatic antioxidants and free radicals

3.12.1. Estimation of superoxide

Estimation of superoxide (free radical) in $AlCl_3.6H_2O$, NaCl and sucrose treated and untreated cells of *Spirulina platensis* as per the method of Elstner and Heupe l(1976).10 ml treated and untreated cells of *Spirulina platensis* was thoroughly homogenized in phosphate buffer (2ml) and centrifuged at 8000X g. To 1 ml of supernatant, 0.9ml phosphate buffer and 0.1ml hydroxylamine was added and incubated at 25^0C. After incubation 1ml sulphanilmide and 1ml NEDD was added and optical density was measured at 530 nm incubated after 20 mins of incubation.

3.12.2. Enzyme SOD (superoxide dimutase) activity

Activity of superoxide dimutase was measured spectrophotometrically by the method of Giannopolitis and Ries (1977). Four days of treated cynobacterial cells were collected by centrifugation and washed twice with 100 mM EDTA-phosphate buffer, pH 7.8. The cellular pellet was ground in an ice cold mortar with 100mM EDTA-phosphate buffer, pH 7.8. The homogenate was centrifuged for 20 min at 8000 rpm. The supernatant fraction was used as the enzyme source. The reaction mixture 3 ml contained 1.3 μM riboflavin, 13 mM L-methionine, 0.05 M Na_2CO_3, (pH10.2) 63 μM-p-nitroblue tetrazolium chloride and 0.1 ml of crude extract. Reaction was carried out in similar test tubes under illumination from fluorescent lamp at 25 ^0C. The initial rate of reaction as measured by the difference in increase in absorbance at 560 nm in the presence and absence of extract was proportional to the amount of enzyme. The unit of superoxide dismutase activity was defined as the amount of enzyme which caused a 50% inhibition of the reaction observed in the absence of enzyme. For the blank the reaction was run in darkness.

3.13. Non enzymatic Antioxidants

3.13.1. Total phenol: 10 mL of cyanobacterial culture was centrifuged and cells were homogenized at 4˚C in 2 mL of 80% of methanol. The extracts were then centrifuged at 8000 rpm and to 0.5 mL supernatant fraction, 2 mL of sodium bicarbonate, 0.3 mL DDW, 0.2 mL Folin reagent was added. The resulting reaction mixture was incubated in water bath till blue colour appeared. The O.D. was recorded at 750 nm to measure the amount of Phenol

3.13.2. Proline

Proline was determined following Bates et.al. (1975). 20 mL of exponential phase culture was centrifuged at 8000 rpm and the pellets were homogenised in 10 mL of 3% sulfosalicylic acid. The extracts were then centrifuged for 10 min at 8000 rpm. Further, 2 mL each of acid ninhydrin and glacial acetic acid was added to 2 mL of supernatant and incubated for 1 h at boiling temperature. The resulting mixture was extracted with toluene, and from the organic phase proline was spectrophotometrically quantified at 520 nm.

3.14. Quantification of protein

Protein was measured by the (modified) method developed by Marian M. Bradford (1976).

Take out 150ml treated culture and centrifuge

↓

Pellet is gathered in one tube and add 2ml PBS centrifuge and desgarde supernatant

↓

Transfer pellet into glass tube with the help of 400µl PBS

↓

Total volume in glass tube will be approx 1ml

↓

Kept tube in -80^0C for freezing , than thawed by dipping tubes in water at 37^0C and repeat 4 to 5 times

↓

Green extract turn into purple after thawing , add PMSF 100µl of 10mM or 1ml of 1mM

↓

Glass powder added so that extract is 1-2mm above glass powder which settles at bottom

↓

Tubes were vertexed for total 10min. (in cycles of each 10sec. vertex and 10sec. keeping in ice)

↓

Extract was transferred in eppendorf tubes

↓

Centrifuge at 4^0C, 3500rpm for 5min.

↓

Purplish supernatant appear on the top of settled glass powder

↓

Supernatant transfered in another eppendrof and again centrifuge at 4^0C, 3500rpm for 1min.

↓

Protein extract was kept at -20^0C

↓

Quantification of protein, take 200µl bradford dye with 20µl of extracted protein sample in 96 wells ELISA plate and kept it for 45min. ,then take ELISA reading.

3.15. Analysis of proteins by SDS-PAGE

SDS-PAGE of cyanobacterial protein was performed on 12% polyacrylamide resolving gel after ten days of metal treatment and carried out in a vertical system (Bangalore Geni) according to the method of laemmli (1970).

SDS-PAGE is used for analyzing mixtures of proteins. In this technique, proteins are made to react with an anionic reagent, Sodium Dodecyl-sulfate (SDS), to form negatively

charged complexes. The amount of SDS bound by protein, and so the charge on the complex, is proportional to its size. The proteins are denatured and solubilized due to their binding with SDS. Thus, proteins of either acidic or basic pI form negatively charged complexes that can be separated on the basis of differences in charge and size by electrophoresis through a sieve like matrix of polyacrylamide gel.

3.16. Reagents for SDS Gel Electrophoresis:

(Sambrook and Russell):
1. 30 % Acrylamide and Bisacrylamide: (29:1) (100 ml)
Acrylamide   29 gm

Bisacrylamide   1gm

Prepared in deionized warm H_2O (pH = 7.0 or > 7.0)

Storage: Store the solution in dark bottles at RT

2.   SDS (10%): (10 ml)
1 gm SDS in 10 ml
Storage: Stored the solution at RT
3. Ammonium Per Sulfate (APS) (10%) (1 ml)

0.1 gm APS in 1 ml dH_2O
Storage: Store the solution at 4°C
Note: Prepare it fresh before use
4. Solution for preparing resolving gels for 12 % Tris- Glycine SDS
PAGE:

Reagents	Resolving Gel 12 % (5 ml)	Stacking Gel 12 % (2ml)
H_2O	1.6 ml	1.4 ml
30 % acrylamide mix	0.33 ml	500 μl
1.5 M Tris (pH8.8)	1.3 ml	--
1 M Tris (pH 6.8)	-	250 μl
10 % APS	50 μl	20 μl
10 % SDS	50 μl	20 μl
TEMED	2μl	2μl

2 X SDS gel loading Buffer (10 ml)

Tris (0.5M, pH=6.8)	2.5ml
SDS (10% ml)	4.0ml
Glycerol 100%	2.0ml
β -mercaptoethanol	0.8ml
Bromophenol blue (1%)	0.7ml

Tris Glycine SDS sample buffer (2x). Store at 4°C

10% SDS,

100% glycerol,

0.5M Tris-Cl, pH 6.8,

2 mM ethylene diamine tetraacetic acid (EDTA), and

0.1 mg/ml bromphenol blue dye.

5 X Tris glycine electrophoresis buffer: (stock solution) (500 ml)

Tris Base7.55 gm

Glycine (pH 8.3)47 gm

Dissolved it in 400 ml of dH_2O.Then added 25 ml of a 10 % (w/v) stock solution of SDS and adjusted the volume to 500 ml.

1.5 M Tris base (pH 8.8) (30 ml)

Tris9.1 gm

H_2O30 ml

(Adjusted the pH 8.8 with HCl)

1 M Tris base (pH 6.8) (20 ml)

Tris	3.63 gm
H_2O	20 ml

(Adjust the pH 6.8 with HCl)

Staining solution (250 ml)

Commassive blue	300 mg
Methanol	80 ml
Glacial acetic acid	20 ml

dH$_2$O   100 ml

Dissolved the dye in methanol first and then add acid and water

Distaining solution: (200 ml)

Methanol   l60 ml

Glacial acetic acid   20 ml

ddH$_2$O   120 ml

3.17. SDS-Gel Electrophoresis

Thoroughly cleaned, dried glass plates and three spacers were taken and assembled properly. The spacers were set with 1 mm distance from the edges of the glass plates. The construction was held together with bull-dog clips. The chamber was clamped in an upright, level position. A sufficient volume of separating gel mixture (5ml) was prepared. The freshly mixed solution was carefully pipetted or poured into the chamber without generating air bubbles. It was poured to a level about 1 cm below, where the bottom of the well-forming comb would rest when it would be in position. A thin layer of water or methanol was poured to prevent oxidation of resolving gel. The layer was discarded when resolving gel was polymerized. The mixture was left to stand until it was set.

 Stacking gel (2 ml) was prepared properly. The solution was pipetted into the chamber. The comb was inserted and the gel was allowed to stand until set. When the stacking gel was set, the comb was removed without distorting the shape of the wells. The clips, holding the plates together, were removed and gel was installed in the apparatus. The apparatus was filled with reservoir buffer. Samples were prepared for electrophoresis, while the gel was setting/ polymerizing. The sample solution was heated in boiling water for 2 m and cooled before loading. The gel was finally loaded. Required volume of sample solution was taken up in a pipette and carefully injected into a sample well through the reservoir buffer.

After loading all the samples, electrophoresis was started by turning on power (dc) on a gel of about 1mm thickness and about 14 cm length, with an applied voltage of about 80 V when samples are in stacking gel then on 120 V through running gel. At the end of electrophoresis (when the dye front reached the bottom of the gel), protein bands in the gel are visualized by staining. The gel was removed from the glass plates and immersed

in the protein stain immediately. The gel was left there with gentle agitation until the dye had penetrated the gel. Dye that was not bound to the protein was removed by transferring the gel to a distaining solution. After about 24 hr, with gentle agitation and several changes of distaining solution, the gel background became colorless and protein bands of blue, purple color is visible.

Finally, the whole cell protein profiles of the samples were visualized under Trans white light and captured using Gel Doc. All experiments were performed in three independent replicates and only those bands present in at least two gels of the independent set were taken for analysis.

3.18. DNA fragmentation assay

3.18.1. Reagents required

(a) Lysis buffer

> 1M Tris HCl (pH 7.2)
> 0.5M EDTA (pH 8.0)
> 5M NaCl
> SDS 20%

(b) Proteinase K   20 mg/ml

> Chloroform: Isoamyl alcohol 24:1
> RNAseA
> Isopropanol

Cells were treated with cadmium at a conc. of 20 µM for 24 hours. DNA was extracted by proteinase K digestion method (Sambrook and Russell,2001 modified). Cells were resuspended in 567 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) by repeated pipetting. Lysis of the cells was performed using 30 µl of 10 % SDS and 3 µl of 20 mg/ml proteinase K. The mix was subsequently incubated for 1 h at 37 °C. 100 µl of 5 M NaCl was added. The samples were gently mixed by inversion and incubated for 10 min at 65 °C. Nucleic acids were thereafter isolated by chloroform: isoamyl alcohol (24:1) separation. DNA was finally recovered by precipitation using 0.6 volumes of isopropanol and centrifugation (5 min, 4 °C, and 15000 rpm). The DNA pellets were washed with 1

ml of cold 70 % ethanol. Finally, the tubes were centrifuged one last time 5 min (4 °C, 15000 rpm), the supernatant was discarded, and each pellet dried before being re-suspended in 100 µl TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

4. RESULTS AND DISSCUSIONS

4.1. Growth measurement

Firstly for the measurement of growth pattern of treated *Spirulina platensis.* The culture were firstly treated with different concentration of $AlCl_3.6H_2O$ (2, 4, 8) µM; (5, 10, 15) mM NaCl and (25, 50, 75) mM $C_{12}H_{22}O_{11}$. *Spirulina platensis* was found less sensitive to Al and more to the sucrose at all the concentrations. The Growth pattern of *S. platensis* was monitored at regular intervals continuously for 10 days in zarrouk medium by estimating the chlorophyll content.

S. platensis was observed that highly sensitive towards Sucrose and less sensitive towards NaCl and Aluminum respectively (Fig. 4(a), (b), (c)). Show the growth pattern of *S. platensis* under various treatment doses of aluminum (0, 2, 4, and 8 µM), Sodium chloride (0, 5, 10 and 15) and Sucrose (0, 25, 50 and 75). An Aluminum concentration of 2 and 4 µM, NaCl at 5 and 10 mM and sucrose at 25 and 50 mM did not caused significant effect even after 10 days of exposure (Fig.4.1). Further, the effect became prominent after 8 µM, 15mM and 50mM exposure of aluminum, sodium chloride and sucrose respectively.
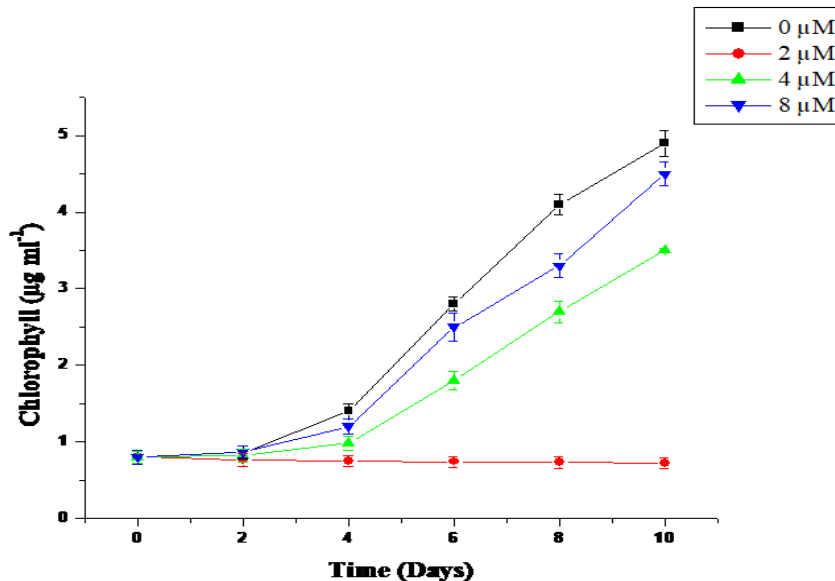
Fig.4.1 (a): Effect of different concentration of Aluminum chloride on the growth of *S. platensis.* Values are mean ± SE with n=3.

35
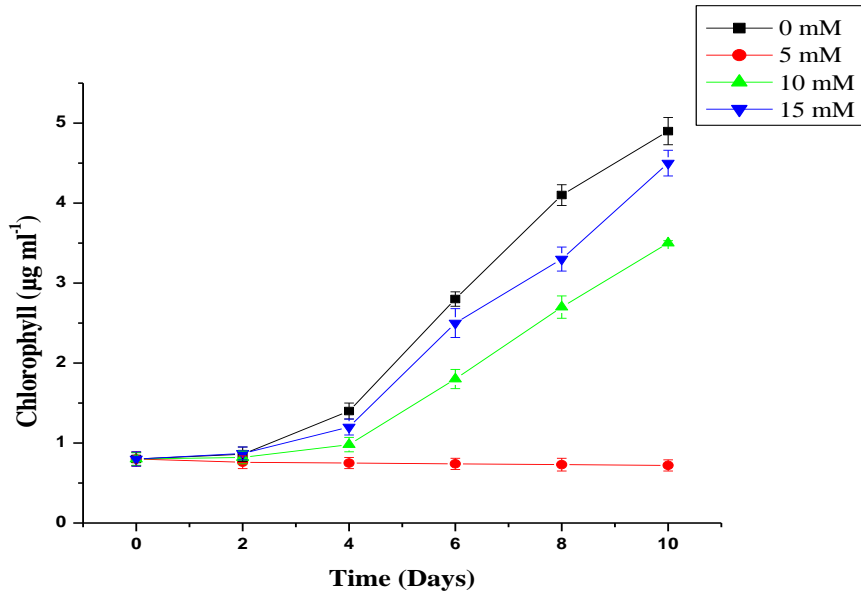
Fig.4.1 (b): Effect of different concentration of Sodium chloride on the growth of *S. platensis.* Values are mean ± SE with n=3.
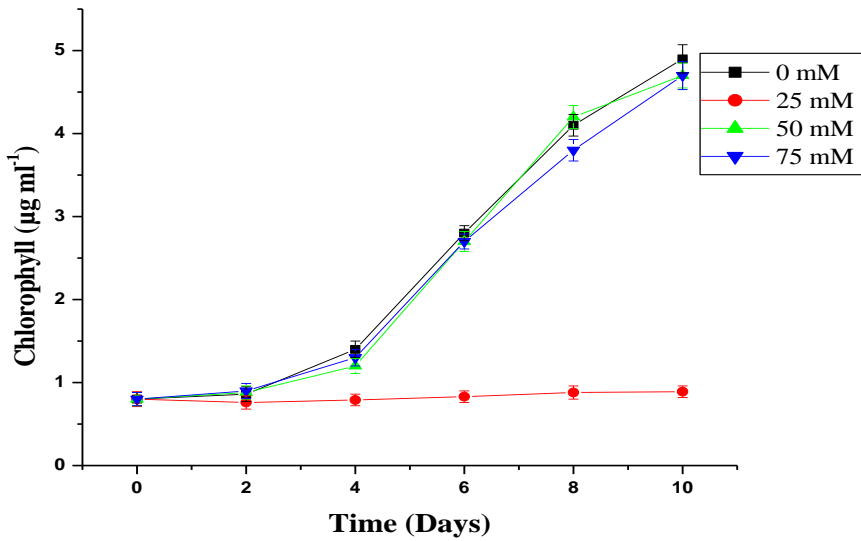
Fig.4.1(c): Effect of different concentration of sucrose on the growth of *S. platensis.* Values are mean ± SE with n=3.

Similar result were observed by Kannaujiya and their fellow (Kannaujiya et al., 2020) when they treated *Nostoc sp.* with aluminum at different concentration.

Maximum growth rate was observed, when cynobacterial culture "*Spirulina platensis*" was treated with 8 μM AlCl$_3$. Present knowledge in this area suggests that on increasing the concentration of Al beyond toxicity level resulted in a gradual decrease in the growth. A similar finding was reported by Pradhan and their fellow worker (Pradhan et al., 2020) , In response to different concentrations (0–100 μM) of AlCl$_3$, the growth rate of *G. amphibium* was decreased significantly. With increase in the dose of the AlCl$_3$; decline in growth of *G. amphibium* was observed by them. Decrease in their growth due to toxicity created by Al which inhibit the photosystem II, this was studied by Yang and his fellow worker (Yang et al., 2015), their study demonstrated that the chl-a content maybe by implication hindered by Al application. Similar study reported by Shamim and their coworkers (Shamim et al., 2020), that Al directly cause effects on photosystem II, which decrease the rate of photosynthesis. Earlier it has been reported by Farooqui(Farooqui et al., 2015) that *N. muscorum* was moderately sensitive among the various cyanobacterial strains under metal stress and hence can be a very suitable test system to study both the underlying mechanism of metal toxicity as well as tolerance.

Further *Spirulina platensis* treated with NaCl, maximum specific growth rate was observed at the 15 mM concentration of NaCl. And when the concentration or molarity of salt (NaCl) was increases, rapid declination was observed. This observation occur due the dehydration happen in cell when the concentration of salts increases, when NaCl concentration in surrounding is more than inside cells due to efflux and influx or sodium-potassium pump present in cell wall was open. This triggers the influx of sodium and chloride ions inside cell, which cause dehydration of cell and hindered photosynthesis that decrease the growth of *S. platensis*. Similar effects seen by Allakhverdiev & Murata in the (Allakhverdiev & Murata, 2008), when they treated *Synechococcus sp.* with NaCl. They observed the decrease in cytoplasmic volume at 0.5M concentration of NaCl. When different concentration of NaCl treated soil used for the growth of different species of cynobacteria *Calothrix* spp., *Nostoc calcicola*, *Anabeana sp.* by Venus singh (*Cyanobacteria Modulated Changes and Its Impact On*, 2015).He observed the same effects on the growth of different cynobacterial species.

S. platensis were treated with sucrose, maximum specific growth rate was observed at the 50 mM concentration of sucrose. And when the concentration or molarity of sucrose ($C_{12}H_{22}O_{11}$) was increases, rapid declination of growth in *S. platensis* was observed. This observation occur due the dehydration happen in cell when the concentration of sucrose increases, when sucrose concentration in surrounding is more than movement of water occur from inside cell to the outside. That causes shrinkage of cell due to water loss in *S. platensis*. In experimental study by Allakhverdiev & Murata suggested that the water loss from cell major cause by the sorbitol, when they treated *Synechococcus sp.* with sucrose. They observed the decrease in cytoplasmic volume at 1 M concentration of sucrose (Allakhverdiev & Murata, 2008).

4.2. Photosynthetic pigment determination

Table-4: Effect of $AlCl_3.6H_2O$, NaCl and $C_{12}H_{22}O_{11}$ on photosynthetic pigments of *S. platensis* measured after four days of treatment. Means ± SE. Values in parenthesis are [%] decrease. All the treatments are significantly different ($P < 0.01$) from control (Student's *t*-test).

Abiotic stress	Source	Concentration	Chlorophyll ($\mu g\ mL^{-1}$)	Phycocyanin ($\mu g\ mL^{-1}$)	Carotenoids ($\mu g\ mL^{-1}$)
Metal Stress	Aluminum chloride ($AlCl_3.6H_2O$)	(4μM)	1.01 ± 0.01 (5)	0.45 ± 0.001 (+4)	15 ± 0.30 (10)
Salt	Sodium chloride (NaCl)	(10mM)	0.99±0.02 (5)	0.35±0.01 (+4)	12±0.041 (10)
Osmotic stress	Sucrose ($C_{12}H_{22}O_{11}$)	(50mM)	0.96±0.015 (5)	0.31±0.002 (+4)	10±0.51 (10)

S. platensis when treated with aluminum, sodium chloride and sucrose at concentration 4 μM, 15mM and 50mM respectively, maximum photosynthetic pigments (Chlorophyll-a, Carotenoids, and Phycocyanin) was observed in aluminum treated *S. platensis* where as

the lowest photosynthetic pigments was observed in sucrose treated *S. platensis*. In response to different concentrations (0–100 μM) of AlCl₃, the growth rate of *G. amphibium* was decreased significantly. With increase in the dose of the AlCl₃; decline in growth of *G. amphibium* was observed by them. Decrease in their growth due to toxicity created by Al which inhibit the photosystem II, this was studied by Yang and his fellow worker (Yang et al., 2015), their study demonstrated that the chl-a content maybe by implication hindered by Al application. Similar study reported by Shamim and their coworkers (Shamim et al., 2020), that Al directly cause effects on photosystem II. Similar effects seen by Allakhverdiev & Murata in the (Allakhverdiev & Murata, 2008), when they treated *Synechococcus sp.* with NaCl. They observed the decrease in cytoplasmic volume at 0.5M concentration of NaCl. That causes shrinkage of cell due to water loss in *S. platensis*. In experimental report of Allakhverdiev & Murata(Allakhverdiev & Murata, 2008), reported that the water loss from cell major cause by the sorbitol, when they treated *Synechococcus sp.* with sucrose.

4.3. Enzymatic antioxidant and free radicals

4.3.1. Total Superoxide and Superoxide dismutase (defense enzyme) in aluminum treated *S. platensis*:

Total superoxide was measured in *S. platensis* after 4 days of exposure to Al (2-8μM), the results obtained are confer in Fig.4.2 (b), the increment was found based on dose in both the free radicals i.e. superoxide (1 % - 33 %). Low Al was unable to promote total superoxide content even after 4 days of exposure. Whereas, the high doses (8μM) of Al ROS significantly, indicating that the primary response of organisms is the generation of ROS upon exposure to high levels of heavy metal.

SOD activity in *S. platensis* under, treatment is conferred in Figure 4.2 (a). SOD activity in untreated *S. platensis* was 6.0 ± 0.22 Unit (mg protein)-1 change in OD at 430 (mg protein)$^{-1}$ min^{-1} respectively. Low concentrations of Al (2 and 4) μM did not show significant increase in the SOD activity whereas high concentration Al (8μM) stimulated the enzyme activity by 25% in *S. platensis*.
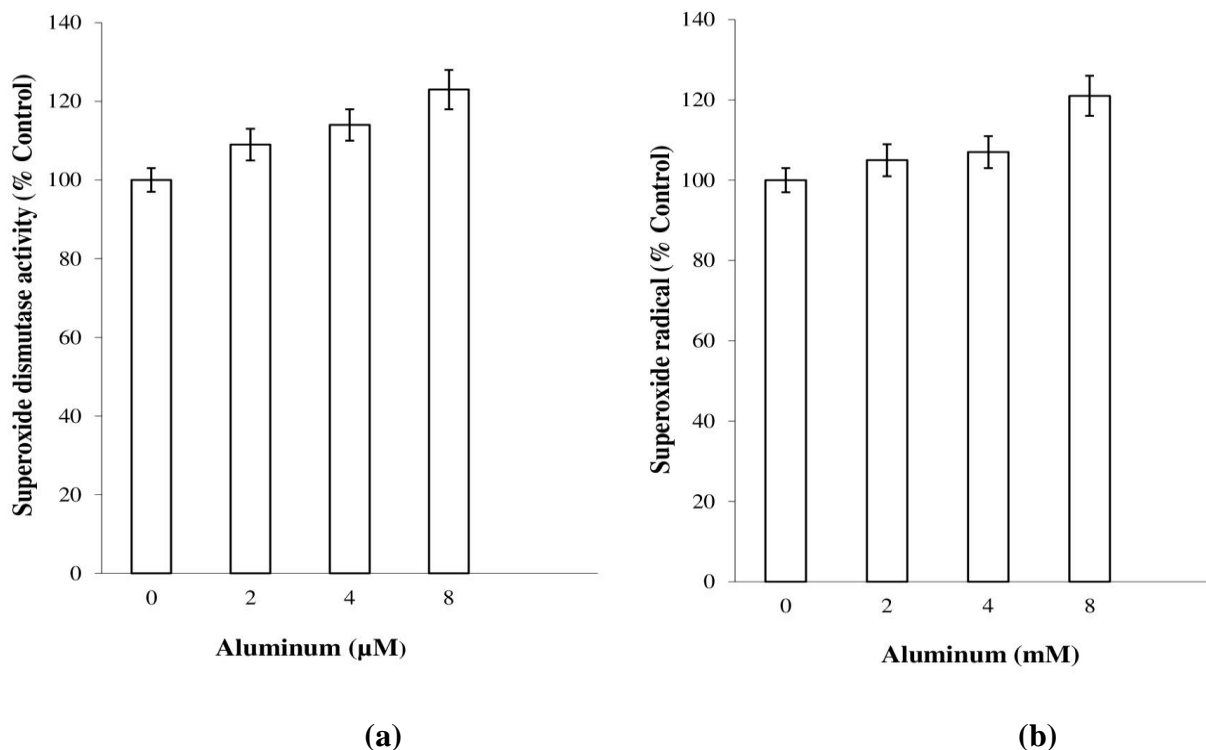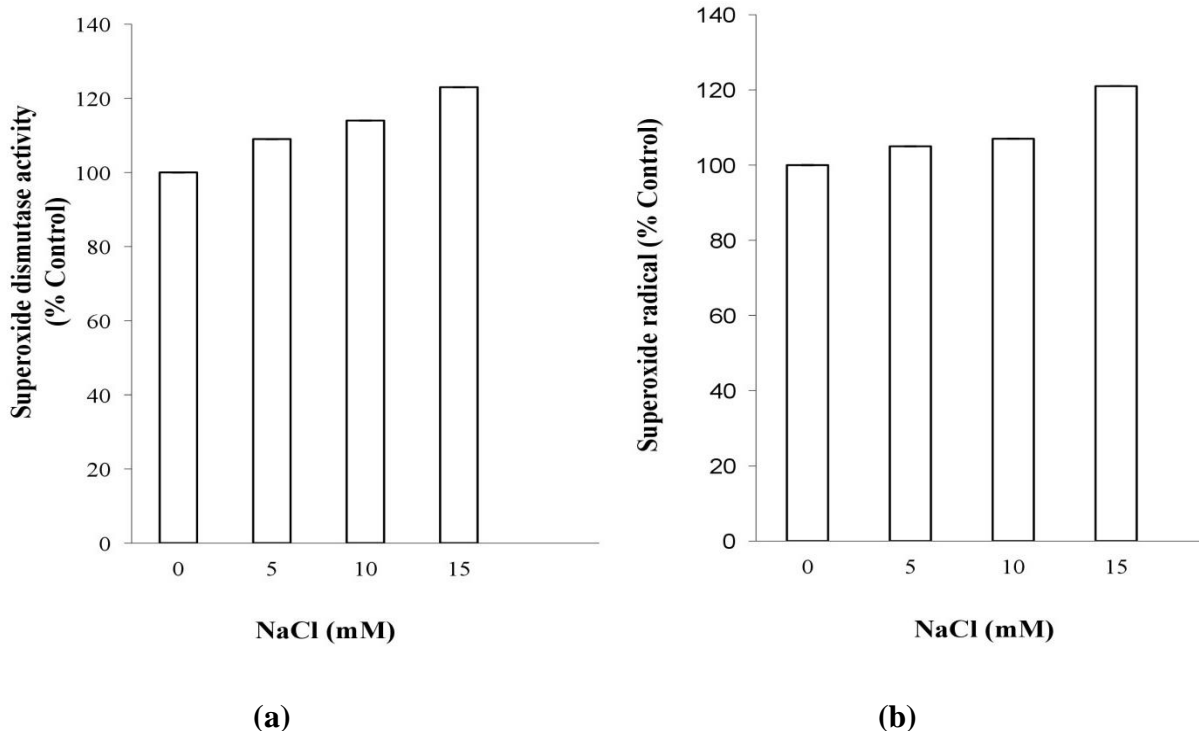
Fig.4.2: Effect of different concentrations of Al on **(a)** Superoxide dismutase activity and **(b)** Superoxide radical of *S. platensis*. Values are mean ± SE with n=3.

4.3.2. Total Superoxide and Superoxide dismutase (defense enzyme) in Sodium chloride treated *S. platensis*:

Total superoxide were measured in *S. platensis* after 4 days of exposure to NaCl (5-15 mM), the results obtained are confer in Fig.4.3 (b) the increment was found based on dose in both the free radicals i.e. superoxide (2 % - 36 %). Low NaCl was unable to promote total superoxide content even after 4 days of exposure. Whereas, at the high doses (15mM) of NaCl stimulate ROS significantly.

SOD activity in *S. platensis* under, treatment is conferred in Fig.4.3 (a). SOD activity in untreated *S. platensis* were 6.2 ± 0.21 Unit (mg protein)$^{-1}$ change in OD at 430 (mg protein)$^{-1}$min^{-1} respectively. Low concentrations of NaCl (5 and 10) mM did not caused significant increase in the SOD activity whereas at high concentration, NaCl (15 mM) stimulate the enzyme activity by 27% in *S. platensis*.

Fig.4.3: Effect of different concentration of NaCl on **(a)** Superoxide dismutase activity and **(b)** Superoxide radical of *S. platensis*. Values are mean ± SE with n=3.

4.3.3. Total Superoxide and Superoxide dismutase (defense enzyme) in Sucrose treated *S. platensis*:

Total superoxide was measured in *S. platensis* after 4 days of exposure to Sucrose (25-75 mM), the results obtained are confer in Fig.4.4 (b), the increment was found based on dose in both the free radicals i.e. superoxide (2 % - 37 %). Low Sucrose was unable to promote total superoxide content even after 4 days of exposure. Whereas, the high doses (50 mM) of Sucrose stimulate ROS significantly SOD activity in *S. platensis* under, treatment is conferred in Fig.4.4 (a). SOD activity in untreated *S. platensis* were 6.3 ± 0.24 Unit (mg protein)$^{-1}$ change in OD at 430 (mg protein)$^{-1}$ min-^{1} respectively. Low concentrations of Sucrose (25) mM did not show significant increase in the SOD activity whereas high concentration Sucrose (50 mM) stimulates the enzyme activity by 30% in *S. platensis*.
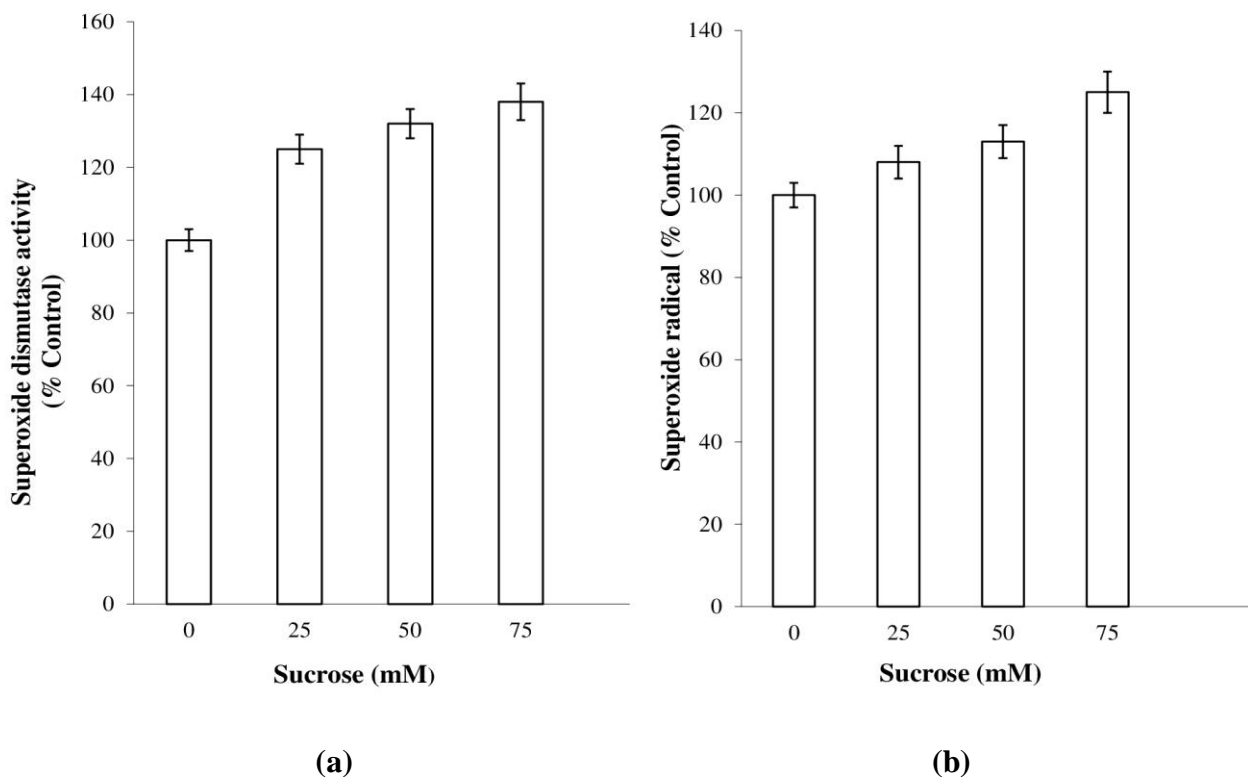
Fig.4.4: Effect of different concentration of Sucrose on **(a)** Superoxide dismutase activity and **(b)** Superoxide radical of *S. platensis*. Values are mean ± SE with n=3.

Low conc. of $AlCl_3.6H_2O$, NaCl and $C_{12}H_{22}O_{11}$ (2 μM, 10mM and 25mM) does not show the significant response in the generation of ROS a free radical (superoxide and peroxide)after 4[th]days treatment with different stress, but when doses increase $AlCl_3.6H_2O$ (8 μM), NaCl (15mM) and$C_{12}H_{22}O_{11}$ (50mM), generation of ROS was stimulated significantly. ROS majorily effects on the photosystem (PS-II). That causes the inhibition of the synthesis of other photosynthetic pigments (carotenoids and Phycocyanin). (Latifi et al., 2009)he agreed that the source of generation of ROS is chloroplasts and mitochondria. Generation of ROS hindered the photosynthesis processes and oxidative phosphorylation in response it releases the free reactive oxygen species (Shrikanta et al., 2015).

AlCl$_3$.6H$_2$O, NaCl and C$_{12}$H$_{22}$O$_{11}$ cause the oxidative stress which inhibit the protein synthesis, damaged synthesized protein, lipids and DNA (Birben et al., 2012). To overcome of this stress cell enhances the synthesis the specific enzyme superoxide dimutase (SOD), which repair or remove the damaged biomolecules from the cell. Maximum activity of SOD was observed at the high concentration of respective stress AlCl$_3$.6H$_2$O (8 µM), NaCl (15 mM) and C$_{12}$H$_{22}$O$_{11}$ (50 mM).

4.4. Non-enzymatic antioxidant and free radicals

4.4.1. Total phenols and Proline (defensive, non-enzymatic) antioxidant in aluminum treated *S. platensis*

Fig.4.5 (a) and (b) data point out the total phenol contents and proline in treated *S. platensis* at different concentration of aluminum chloride (2, 4, and 8) µM. After four days treatment a dose-dependent increment in free phenols and proline was observed but the enhancement was insignificant at low dose of aluminum chloride (2 and 4) µM. A significant increment of 41% in total phenol content with 31% in proline content at (8) µM observed respectively.
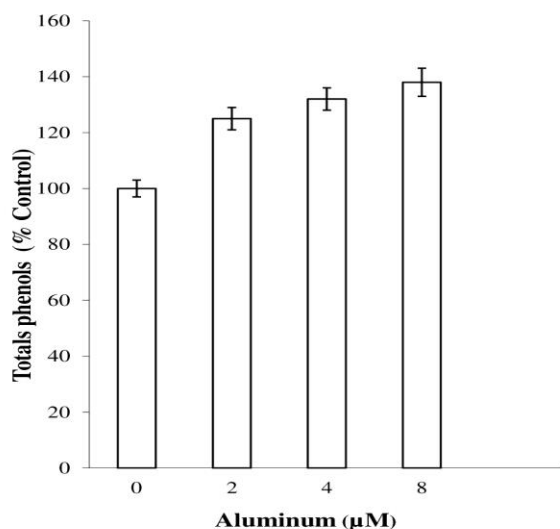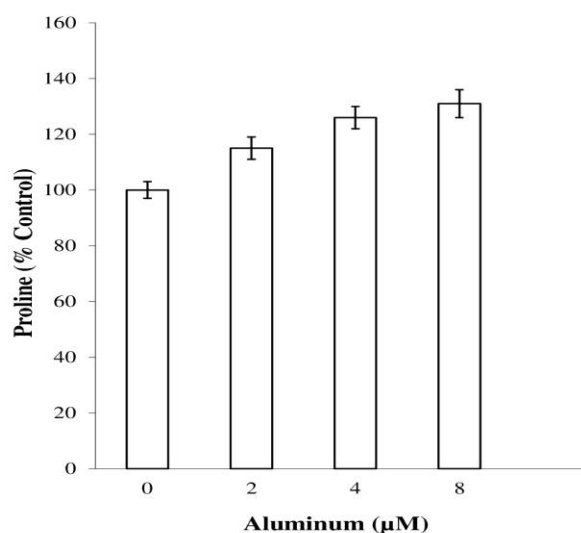
(a)   (b)

Fig.4.5: Effect of different concentration (0-8) µM of Al on *Spirulina platensis* **(a)** Total phenols. Total phenol in untreated control was 0.6±0.03 mg (g dry weight)[-1]. **(b)** Proline content, amount of proline in untreated control was 12.5±0.5 µg (g dry weight)[-1]. Values are means ±SE of three replicates.

4.4.2. Total phenols and Proline (defensive, non-enzymatic) antioxidant in sodium chloride treated *S. platensis*

Fig.4.6 (a) and (b) data point out the total phenol contents and proline in treated *S. platensis* at different concentration of NaCl (5, 10 and 15) mM. After four days treatment a dose-dependent increment in free phenols and proline was observed but the enhancement was insignificant at low dose of aluminum chloride (5 and 10) mM. A significant increment of 45% and 59% in total phenol content with 41% in proline content at (15) mM observed respectively.

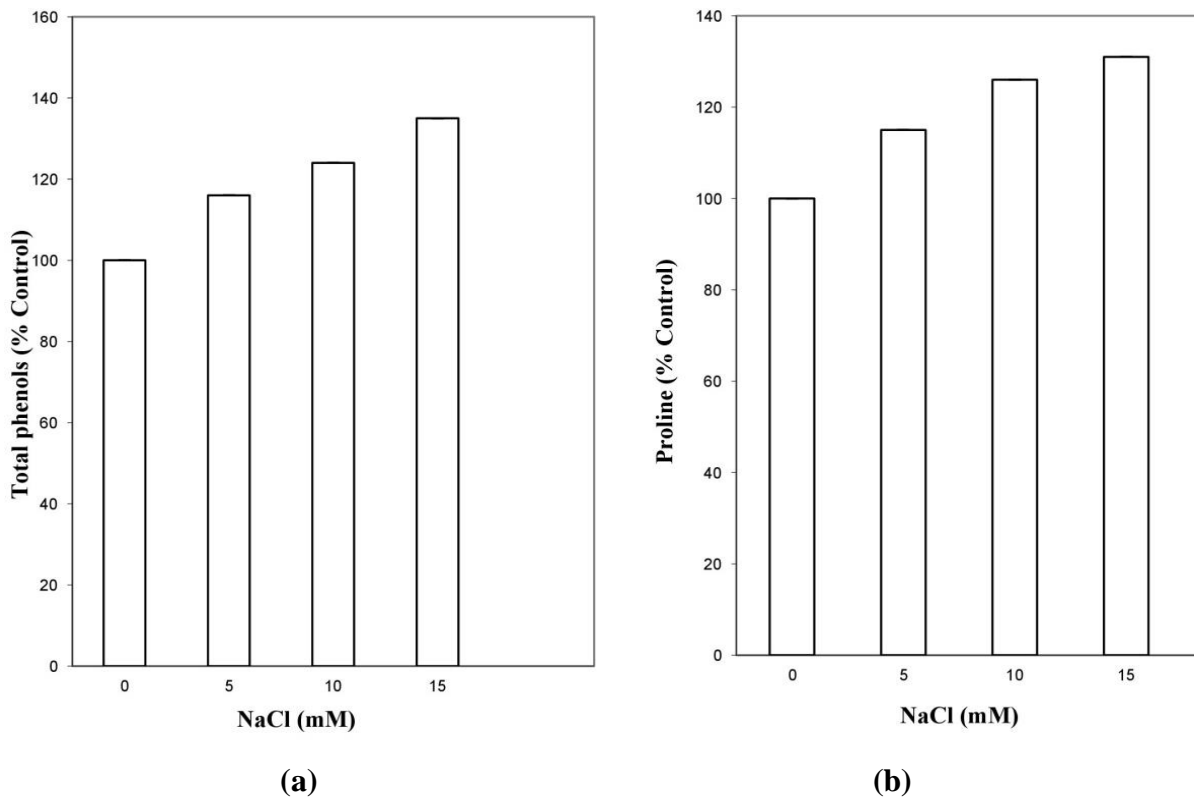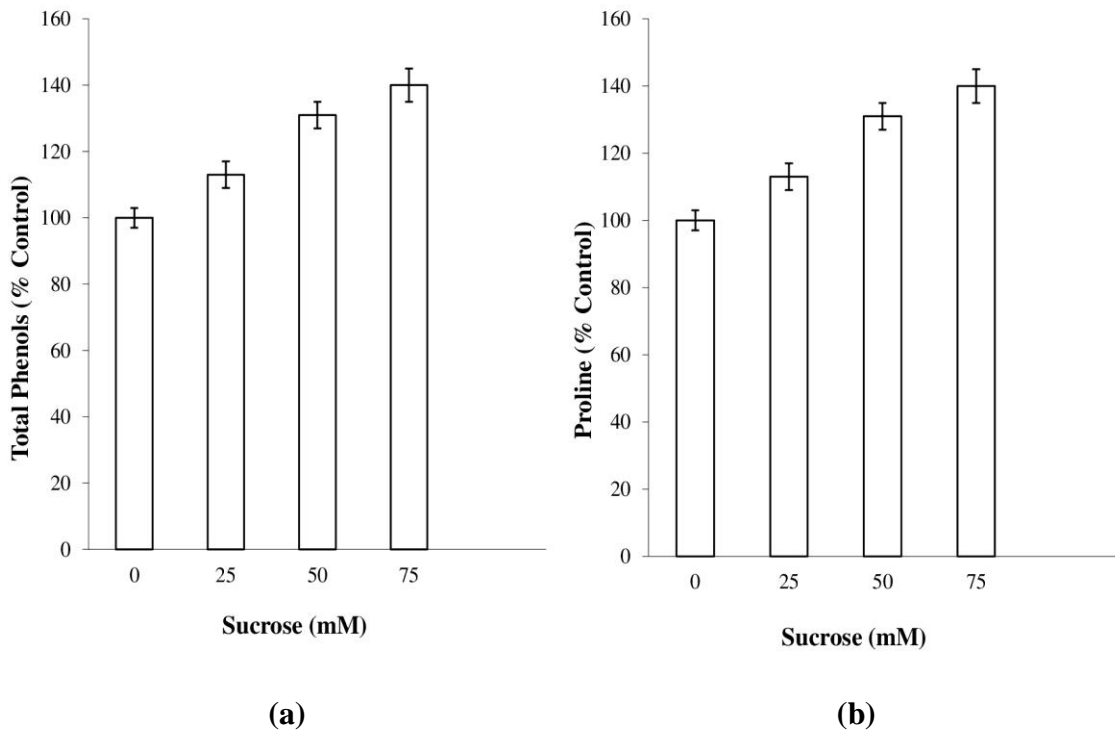(a)   (b)

Fig.4.6: Effect of different concentration (0-15) mM of NaCl on *Spirulina platensis* **(a)** Total phenols. Total phenol in untreated control was 0.7±0.04 mg (g dry weight)[-1]. **(b)**

Proline content, amount of proline in untreated control was 13.5±0.5 μg (g dry weight)⁻¹.
Values are means ±SE of three replicates.

4.4.3. Total phenols and Proline (defensive, non-enzymatic) antioxidant in Sucrose treated *S. platensis*

Fig.4.7 (a) and (b) data point out the total phenol contents and proline in treated *S. platensis* at different concentration of sucrose (25, 50 and 75) mM. After four days treatment a dose-dependent increment in free phenols and proline was observed but the enhancement was insignificant at low dose of aluminum chloride (25 and 50) mM. A significant increment of 47%and 61% in total phenol content with 44% in proline content at (75) mM observed respectively.

(a)   (b)

Fig.4.7: Effect of different concentration (0-75) mM of sucrose on *Spirulina platensis* **(a)** Total phenols. Total phenol in untreated control was 0.7±0.04 mg (g dry weight)⁻¹. **(b)** Proline content, amount of proline in untreated control was 13.5±0.5 μg (g dry weight)⁻¹. Values are means ±SE of three replicates.

In parallel to enzymatic antioxidants, non-enzymatic enzyme which have low molecular weight such as phenol, ascorbic acid, proline and glutathione compare to enzymatic plays vital role in association with enzyme or alone (Liang et al., 2013) found in the treated cynobacterial cells which maintain the osmotic compression of the cell. Proline, an amino acid which majorly associated with metal, salt and osmotic ($AlCl_3.6H_2O$, NaCl and $C_{12}H_{22}O_{11}$) stress tolerance. It was reported that the increase in proline concentration act as indicator of stress tolerance (Hayat et al., 2012). It works as chelator, inhibit peroxidation of lipids, radical scavengers. At concentration (8 Mµ, 15 mM, and 50 mM) of $AlCl_3.6H_2O$, NaCl and $C_{12}H_{22}O_{11}$proline biosynthesis increases due to increase of enzyme (glutamate kinase) activity in *Spirullina platensis*. In Sundaram and Soumya **(**Sundaram and Soumya, 2011) shows that the proline concentration increases in heterocyst containing cynobacteria under stress.

Activity of proline is regulated by the phenolic acid which serves as the antioxidants during stress (Mazid et al, 2011). In scarce reports, a phenol shows Aluminum stress tolerance in cynobacteria *N. muscorum* were been demonstrated and had correlation with the enhancement of activity of antioxidant by alternation in mechanism to overdue with stress Mazid et al, 2011.

4.5. Analysis of protein by SDS-PAGE

4.5.1. Alteration in polypeptide pattern of *Spirulina platensis* under Aluminum stress

Aluminum treated cells showed a totally different protein profile (Fig.5.1). None of the polypeptides showed disappearance or even down regulation after being treated with 8µM of Al for 7 days. A total of four polypeptides showed upregulation out of which the 37, 45, 54.23 and 75 kDa showed significant enhancement in intensity. The densitometric analysis (Fig.5.2) showed that the intensities of the above bands increased and the increase was directly proportional to the concentration of the metal used.

240kDa →

74 kDa
54.23 kDa
45 kDa

37 kDa

52 kDa →

3.5kDa →

L   **C**   **Aluminum**
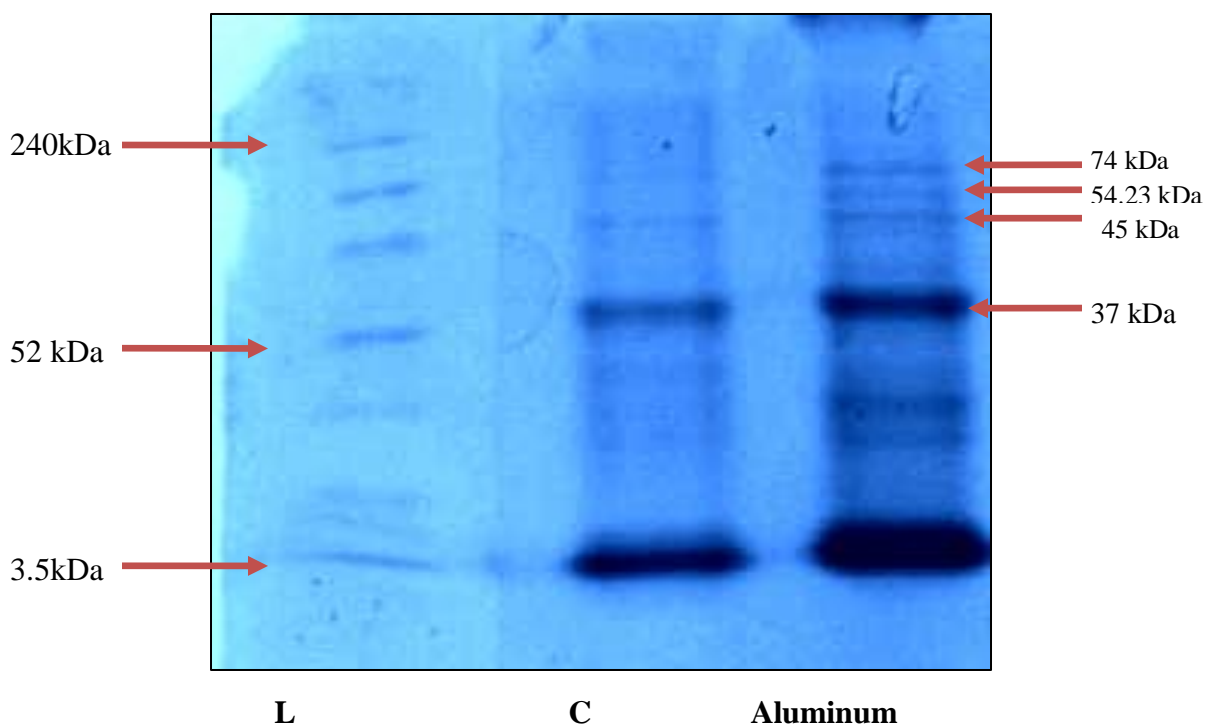
Fig.5.1: Protein profiles of *Spirulina platensis* at different abiotic stresses of a (Lane 1, Marker (L); Lane 2, Control (C) Lane 3 Al treated. Represent profile under Al stress (20 μM)

4.5.2. Alteration in polypeptide pattern of *Spirulina platensis* under Sodium chloride stress

Sodium chloride treated cells showed a totally different protein profile (Fig.5.2). None of the polypeptides showed disappearance or even down regulation after being treated with 15mM of sodium chloride for 7 days. A total of two polypeptides showed upregulation out of which the 37 and 71 kDa showed significant enhancement in intensity. The densitometric analysis (Fig 5.1) showed that the intensities of the above bands increased and the increase was directly proportional to the concentration of the salt used.

Fig.5.2: Protein profiles of *Spirulina platensis* at different abiotic stresses of a (Lane 1, Marker (L); Lane 2, Control (C) Lane 3, NaCl treated. Represent profile under NaCl stress (15 mM).

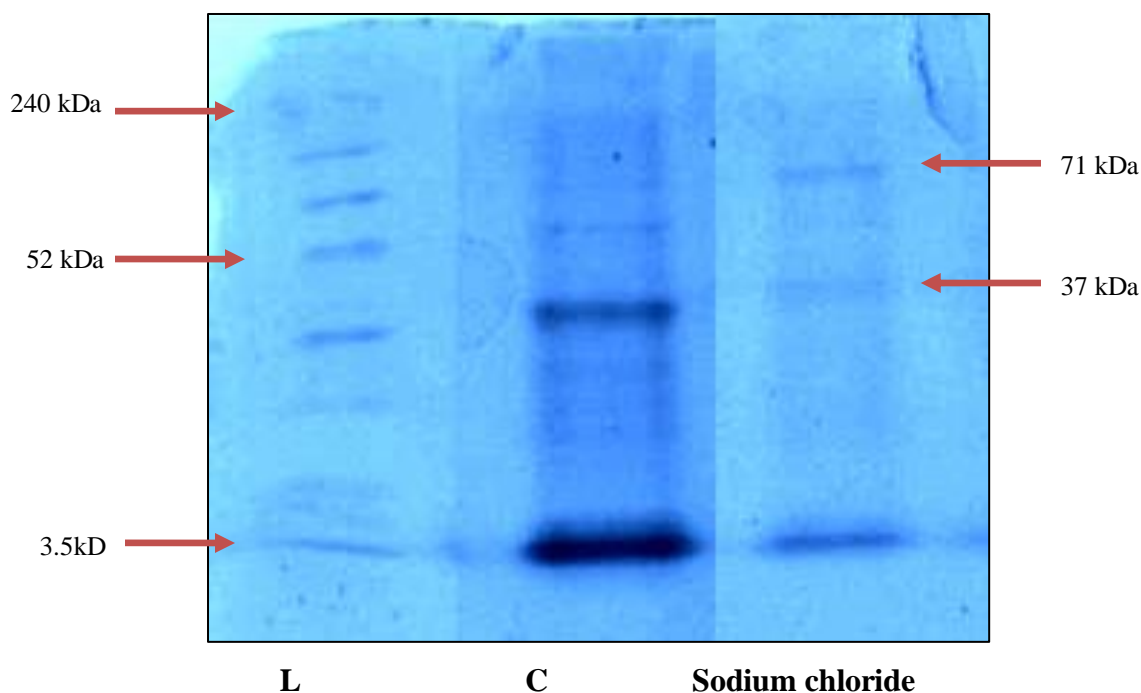### 4.5.3. Alteration in polypeptide pattern of *Spirulina platensis* under Sucrose stress

Sucrose treated cells showed a totally different protein profile (Fig.5.3). None of the polypeptides showed disappearance or even down regulation after being treated with 50mM of sucrose for 7 days. A total of two polypeptides showed upregulation out of which the 37 and 54.23 kDa showed significant enhancement in intensity. The densitometric analysis (Fig 4.4) showed that the intensities of the above bands increased and the increase was directly proportional to the concentration of the metal used.
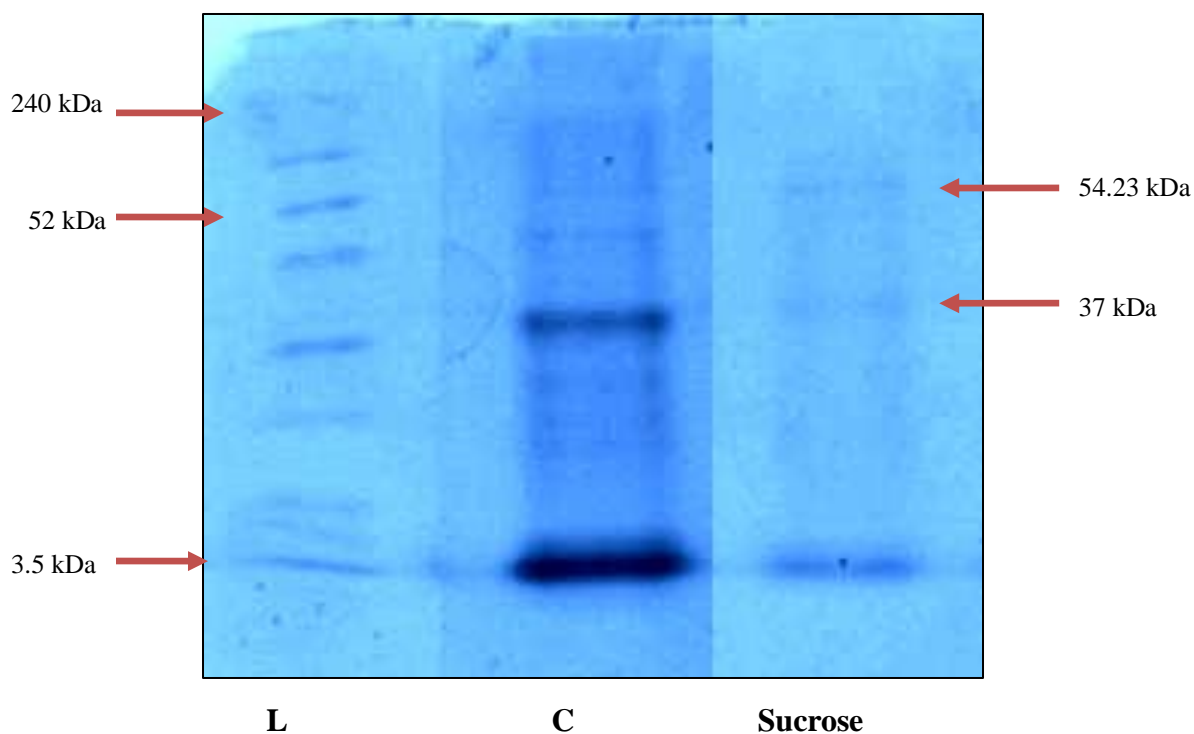
Fig.5.3: Protein profiles of *Spirulina platensis* at different abiotic stresses of a (Lane-1, Marker (L); Lane 2, Control (C) Lane 3, sucrose treated.Represent profile under sucrose stress (0.5M).

Different stress treated aluminum, sodium chloride and sucrose causes the noticeable change in profiling of protein of *Spirulina platensis* at concentration 20 μM, 15 mM and 0.5 M respectively. At many point compare to control and ladder new band was observed in the treated sample. And this new band could be due to the interference of oxidative ions or metals ions at transcriptional level, post-transcriptional level and translational level of changes which cause the synthesis of specific protein that combat with the stress. Exposure to Al caused noticeable changes on protein profile of *N. muscorum* and demonstrated suppression of a protein band in *N. muscorum*. Supression of protein bands could be due to the interference of metal ions at the level of transcription, post-translational degradation and translational level (Rai et al., 1995).

4.6. Analysis of DNA fragmentation under heavy metal (Cadmium) treated _Spirulina platensis_ using agarose gel-electrophoresis

This was done for the investigation that wheather metals can induced DNA fragmentation or not. Cadmium and Aluminum treated _S. platensis_ with 20 μM concentration for 24 hr., than DNA isolation was done as procedure mention in materials and methods. 1.5 ratio of absorbance at 260nm and 280nm, indicator of good DNA quantity and which further used in studies of fragmentation. Isolated DNA was run at 1% agarose gel and and photograph geted under UV light.
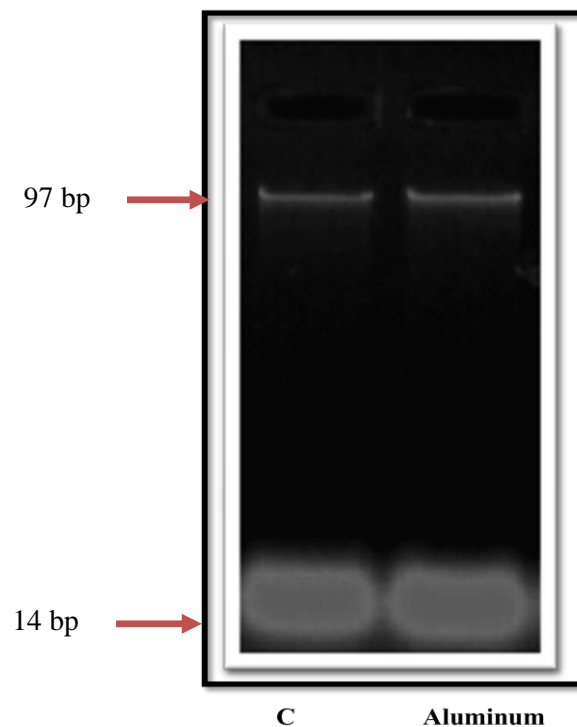
Fig 6.1: DNA fragmentation pattern in _S. platens_. Represents the DNA in the presence of 20 μM Al (Lane 1 DNA from untreated _S. platens_ Lane 2 is after 24hrs).
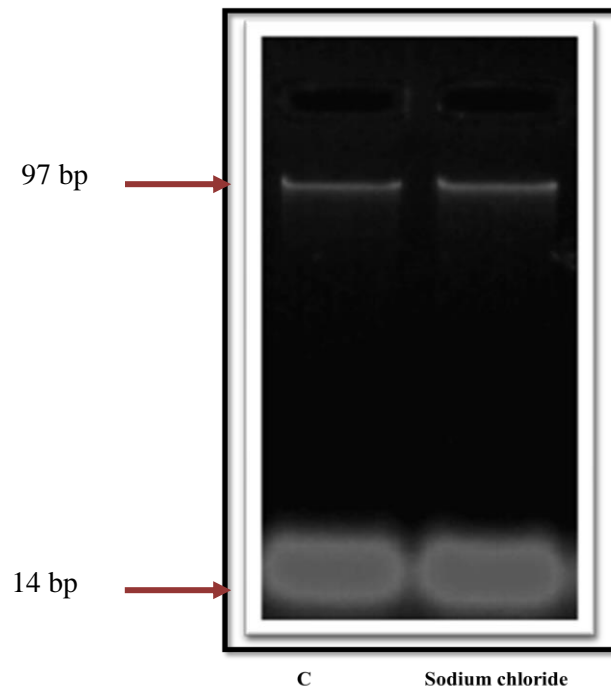
Fig 6.2: DNA fragmentation pattern in *S. platens.*Represents the DNA in the presence of 15 mM NaCl (Lane 1 DNA from untreated *S. platens* Lane 2 is after 24hrs).

Metal (Al), salt (NaCl) and osmotic stress ($C_{12}H_{22}O_{11}$) may cause DNA damage while prolong exposure of these stress where as heavy metals cause rapid DNA damage whether may be for short time exposure. DNA Damage is not always caused by ROS, in some cases it has been observed that the exposure of hydroxyl radical (OH^{-1}) induce by ionizable irradiation leads into DNA damage. Hydroxyl radicals can bind to the C-8 position of guanine which produce 8-hydroxydeoxyguanosine by oxidation, it attacks on other carbon position of guanine or adenine to form hydroxyadenine (Kasprzak, 2002; Ames, 2001; Poulsen, 2000; Halliwell, 1999).

5. CONCLUSION

Cynobacteria are highly tolerant to biotic and abiotic stress. Expression of dehydrin, a dehydration stress protein is a common mechanism to avert stress induced toxicity. It can be concluded that metals cause oxidative stress as evidenced by the increase in ROS Moreover, the data demonstrated a significant increment in the activities of three major enzymes, which are involved in the detoxification of ROS (SOD). However, the magnitude of this activity's increase varies with both the chemical nature of the test metal and concentration. Antioxidant. Variations in the antioxidant levels can serve as a signal for the modulation of ROS scavenging mechanisms and ROS signal transduction and adaptation of cells is very much dependent on the antioxidant defense system. Our results enabled us to show a correlation between responses of antioxidant enzymes as well as non enzymatic antioxidants activities. The genomic and proteomic studies of the cyanobacterial response to stress show that there are few specific proteins whose synthesis is induced or repressed by a specific stress, whereas most of the proteins with enhanced expression belong to the known group of general stress induced proteins, whose alteration is common to different stress conditions.

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