Study on use of fly ash mixed with Cyanobacteria as Biofertilizer in Wheat

A

Thesis

Submitted to

School of Science & Technology

VardhmanMahaveer Open University, Kota

for thedegree of

Doctor of Philosophy

in

BOTANY

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VMOU/Research/Ph.D/BO/2015/79

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CERTIFICATE

This is to certify that the thesis entitled **"Study on use of fly ash mixed with Cyanobacteria as Biofertilizer in Wheat",**embodies results of original investigation which was carried out by Miss **HemlataVerma** (Reg.No.VMOU/Research/Ph.D./BO/2015/79) under my supervision in the Department of Botany, School of Science and Technology, VardhmanMahaveer Open University, Kota, Rajasthan, India for partial fulfillment of Ph.D. degree to be awarded by VardhmanMahaveer Open University.

She has done her research work duly following UGC Regulation on Minimum Standard and Procedure for the award of M.Phil./Ph.D. Degree Regulation 2009.

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Place: Kota

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The research work embodied in this thesis entitled **"Study on use of fly ash mixed with Cyanobacteria as Biofertilizer in Wheat"** has been carried out by me duly following UGC Regulations on Minimum Standards and Procedure for the award of M.Phil./Ph.D. Degree Regulations 2009 at Department of Botany,School of Science and Technology, VardhmanMahaveer Open University, Kota, Rajasthan, India. The work submitted for consideration for the award of Ph.D. degree is original, based upon the data collected by me. The content of this neither full nor in parts, has not been submitted to other institute or University for the award of any degree or fellowship previously.

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ACKNOWLEDGEMENT

"The essence of all beautiful art, all great art is gratitude"
- Fredrick W Nietzsche

Let me first bow down to **God** almighty with whose grace and blessings, I have been able to accomplish this research work.

I acknowledge with immense gratitude the invaluable assistance and guidance rendered by my Mentor, **Dr. Anuradha Dubey**. Her depth of knowledge, quick feedback and constructive suggestions and constant motivation became a significant source of inspiration and guidance for me. I am incredibly thankful to her for her big-heartedness and eagerness to spare her precious time to help out in my research work.

I would like to wish my gratitude to Hon'ble V.C **Prof. R.L. Godaraa, Prof. Ashok Sharma**(FormerV.C**.), Prof. Vinay KumarPathak,**(FormerV.C**.).** I am thankful of **Prof. P.K. Sharma, Prof. H.B. Nandwana**and **Prof. B. Arun Kumar**.

I would like to express my sincere thanks to **Dr. KshamataChoudhary (**Director Research), **Dr. Subodh Kumar** (Former-Director Research), **Prof. Dinesh Kumar Gupta (**Former-Director Research)and **Dr. Patanjali Mishra** (Deputy Director Research) VMOU, for their insightful comments, encouragement and support.

I am also thankful to**Dr. Anil Kumar Jain, Dr. Keerti Singh, Dr. AnurodhGodha, Dr. Akbar Ali, Dr. Akhilesh Kumar, Dr. AlokChauhan, Dr. Surendra Kulshreshta, Dr. KapilGautam, Sh. Ravi Gupta, Sh.Sushil Rajpurohit, Sh. Sandeep Hooda, Sh. NeerajArora**and **Sh. Rakesh Sharma,** for their guidance on research methodology, support and enlightenment during the Ph.D. course work program.

I am thankful to **Sh. Suresh Saini, Smt.Vijaylaxmi Sharma** and **Sh. Balkishan**for their help and support. I am thankful of Incharge Library, library staff, EMPC staff and all the staff members of VMOU for rendering their help throughout this journey of research.

With a deep sense of gratitude, I am thankful of Prof**. Dr. Sudhir Sexena**, (Head, Department of Blue-Green Algae,ICAR, New Delhi), **Dr. Chandra** (Head, Department of Biotechnology, MD College, Sriganganagar), **Dr. Charan Singh, Dr. Sheenam,** (Department of Biotechnology MD College, Sriganganagar,). I am thankful to**Amrit Pal** (Senior Technician, Suratgarh TPS), **Parminder Singh** (Technician, Suratgarh TPS) for their help.

I am sincerely thankful to **Mrs. SunitaMayal** (Former Sarpanch), **Mrs. Anita Mayal (**Sarpanch), **Mr. PriyavartGaat, Mr. Harivinod Sharma, Mr.Vijay Singh Takhar** (Village Development Officer) and **Mr.VinodTakhar**for allowing me to pursue this research work. I am thankful to all the staff members of my office for their support.

I do solicit my generous gratitude to my friends for giving continuous support, suggestions, guidance and moral strength whenever I needed. Only due to their support and help this difficult task was successfully completed. I am extremely lucky to have my friend **Dr. Monika Talreja** & thankful of her for always

supporting me in each and every aspect of my work.I am thankful to my friend **Dr. Leena Choubisa** for always being there for me, encouraging me and sparing time for me from her business to accomplish the work.

Not a single alphabet can express my deep feeling towards **Mr. SandeepChouhan,** his always being gives me all time encouragement, making me aware of my aims and capacities.

I take this opportunity to remember my Paternal Grandfather **Late Sh. Kalu Ram Ji, Grand Mother Late Smt. Mishri Devi** and **Maternal Grandfather Late Sh. Duda Ram Ji, Grand Mother Late Smt. Gyan Devi** to express my sincere gratitude and homage to them for the affection which they offered to me in my past, the time can never fade them cherishing memories which will remain forever with me.

My father **Late Sh. Rameshwar Dayal Verma**has always been an inspiration to me.This study would not have come to fruition without the cooperation, help, and encouragement extended at all times by my mother **Smt. Sulochana Devi** and in this juncture of time my all family members are stand rock behind me, my elder sisters and brother in laws, **Mrs. Roma & Mr. Subhash Kayath, Mrs. Manju & Mr. Vijay Batu, Dr. Rajni & Dr. JitendraPayal** are the persons to give me all time encouragement. Without their motivation it was impossible for me to reach at this stage of life.I am especially thankful to my most respectable and loving sister **Dr. Rajni** for her unconditional, limitless love. She always has great confidence in me & remained there in my ups and downs of life.

I would like to express my affection towards children of my family **Vinay, Saket, Rachit** and **Harika**for their jolly behavior that cherished and relaxed me.

At last my sincere thanks to each and every one, who have helped me in difficult phase, in several ways in completing the work. I would like to admit that unintentionally if I have failed to give the due credit to anyone then please do exonerate me.

HemlataVerma

LIST OF ABBREVIATIONS

&	-	And
^{o}C	-	Degree Centrigrade
µg	-	Micron gram
µg/g	-	Micron gram per gram
BGA	-	Blue Green Algae
ds/m	-	Decisiemens/ meter
Cd	-	Cadmium
Cu	-	Copper
EC	-	Electrical Conductivity
ESP	-	Electro Static Precipitator
FA	-	Fly ash
FAM	-	Fly Ash Mission
FAUP	-	Fly ash Utilization Program
FBC	-	Fluidized Bed Combustion
FLAA	-	Flame Atomic Absorption Spectrometry
FYM	-	Farmyard Manure
g/kg	-	Gram per kilogram
GFAA	-	Graphite Furnace Atomic Absorption
ICP-MS	-	Inductively Coupled Plasma Mass Spectrometry
INM	-	Integrated Nutrient Management
IR-Sensor	-	Infrared Sensor
$K_2Cr_2O_7$	-	Potassium chromate
Mg	-	Milligram
mg/g	-	Milligram/gram
mg/m^3	-	Milligram/$metre^3$
ML	-	Milliliter
Mn	-	Manganese
MT	-	Metric million per tons
N	-	Normality
Ni	-	Nickel
Pb	-	Lead
Ppm	-	Parts per million
pH	-	PotentiaHydrogenii
RSSC	-	Rajasthan State Seed & Corporation

SOC	-	Soil Organic Carbon
STPS	-	Suratgarh Thermal Power Station
t/ha	-	Ton per hectare
WHC	-	Water Holding Capacity
Yr	-	Year
Zn	-	Zinc

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CHAPTER 1

INTRODUCTION

Fly ash is a fine coal dust which is generated as byproduct from the boilers of the coal fired thermal power plants. The particle size of fly ash varies from one sub-micron to several micrometers. The physico-chemical properties of fly ash depends on the nature of the coal burnt (Rout, 2004).

According to Page *et al*. (1979) coal fly ash is a powdery material made up of tiny glass spheres and consists primarily of Silicon, Aluminium, Iron and Calcium Oxides. They stated that coal fly ash consists of practically all the elements present in the soil except nitrogen. It can act as soil ameliorating material (Sheoran*et al*., 2014) because of its physical condition and presence of macro and micro nutrients.

The American Society for Testing and Materials C618 (www.theconstructor.org) specified two categories of fly ash, Class C and Class F depending on the type of coal and the resultant chemical analysis.The burning of anthracite and bituminous coal typically produces F fly ash which is pozzolanic in nature and contains less than 7% lime (Calcium oxide).Class C fly ash is made-up from the burning of lignite or sub-bituminous coal. In addition to having pozzolanic properties this class also have some self-cementing properties. The Thermal Power Plants generate both type of fly ash.

The pH of fly ash varies from 4.5-12.0 units depending largely on the sulphur content of the parent coal (Plank and Martens, 1974). Many researchers have done the

addition of fly ash in the soil to evaluate the long term consequence of fly ash on soil ecology and crop productivity (Singh & Singh, 1986; Kesh*et al*., 2003).

The physical, chemical and mineralogical properties of most of the common soil are more or less similar in many respects. Fly ash has been found more effective in raising soil pH to levels conductive to maximum plant growth than that of weathered ash in a given soil (Phung*et al.*, 1979). The effect of fly ash on chemical properties of soil is influenced by original pH of both fly ash and soil.

Fly ash is a useful soil-amending agent with agronomic and environmental benefits (Zhang *et al.*, 2004). The utilization of fly ash in agriculture in an effective manner has become essential to prevent soil deterioration and to replenish other soil nutrients and is an alternative approach for fly ash management (Cheung *et al.,* 2000; Jala and Goyal, 2006; Pandey, 2010).

Fly ash may be applied in agriculture as a fertilizer. However, the favourable effect of fly ash on soil properties and plant growth has been pronounced by few workers like Deepti& Mishra, 2014; Lal*et al.,* 1996; Rajkumar, 2000 and Thakare, 1996.

Fly ash can be used as a potential nutrient supplement. Addition of fly ash to the soil has brought about a boon of nutrient ions Copper, Nickel, Zinc, Iron, Phosphorus, Potassium and Sodium. The concentration of Calcium, Magnesium, Sodium and Potassium are observed greater in plant life grown on fly ash amended soil whilst compared to crop grown only on soil (Padmakaran*et al*., 1994).

Fly ash contains several nutrients including Sulphur, Boron, Calcium, Iron, Copper, Zinc, Manganese and Phosphorus which are beneficial for plant growth

(Elseewi*et al*., 1981) such as Chromium, Lead, Mercury, Nickel, Arsenic and Barium. Although fly ash contains traces of harmful elements and heavy metals, as soil amendments and soil conditioner and enhances plant growth. The toxic effect of coal ash is found to be insignificant and the concentration of harmful elements is found to be within the permissible limit on utilization in plants (Kumar and Chauhan, 2008).

In addition it increases the availability of Sodium, Potassium, Calcium, Magnesium, Boron, Sulphate and other nutrients (Dalmau, 1990; Elseewi, 1981) except nitrogen.Fly ash contains higher concentration of essential plant nutrients like Calcium, Potassium, Molybdenum, Zinc and Boron but a low content of available N; therefore, an application of fly ash to agriculture or forestry fields should be accompanied with supply of nitrogen (Doran and Martens, 1972). In fact, fly ash consists of practically all the elements presenting soil except nitrogen (Kumar *et al*., 2000; Rai *et al*., 2000).

As a fertilizer fly ash is deficient in nitrogen (Kumar *et al*., 2000; Rai *et al*., 2000), this nitrogen deficiency in fly ash can be fulfilled by using cyanobacteria mixed with soil and used as Biofertilizer.

Cyanobacteria are a diverse group of prokaryotes, having oxygenic photosynthesis and are amongst the most successful and the oldest life forms present on the planet earth.They comprise about 150 genera and 2,000 species ranging from unicellular, colonial, filamentous to branched filamentous forms and are divided into five subsections, i.e. Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales (Boone and Castenholz, 2001).

Cyanobacteria are photosynthetic bacteria. They are relatively minor and usually unicellular, though they often grow in colonies.Cyanobacteria are often called as "blue-green algae". According to Bergey's classification (Bergey, 2001) cyanobacteria are relatives of the bacteria.They are prokaryotic and it is only the chloroplast in eukaryotic algae to which the cyanobacteria are related.

Cyanobacteria can play a crucial role in plant and soil fertility, as nitrogen-fixing microorganisms and producers of several natural substances positively affecting growth. Recently around the world, considerable progress took place in the development of cyanobacteria based biofertilizer technology (Saadatnia and Riahi, 2009).

The economic importance of cyanobacteria lies in their agronomic significance as biofertilizers due to the nitrogen deficiency solving ability that enables them to develop in habitats where little or no combined nitrogen is available (Singh and Saxena, 2013). Cyanobacteria play an essential role in the enlargement of agriculture (Singh, 2011; Singh *et al.,* 2011). Cyanobacteria are the group of photosynthetic organisms that can easily survive on the bare minimum needs of light, carbon dioxide and water (Woese, 1987; Castenholz, 2001).

Cyanobacteria offer an economically attractive and environmentally pleasant alternative to chemical fertilizers which increase the soil productivity immediately and indirectly (Singh and Saxena, 2013; Thatoi*et al.,* 2013). The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter.

The application of cyanobacteria as biofertilizers in the cultivation of wet-land rice has a beneficial effect on the growth and yield (Renaldo *et al.,* 1971;

Venkataraman, 1979; Swaminathan, 1982; Watanabe & Roger, 1984; Grant *et al.,* 1986). Reports on the effect of cyanobacteria on the growth of other crops than rice are however scarce (Henricksson, 1971; Witty, 1974; Pachpande, 1990; Nanda *et al.*, 1991).

According to Mohammadi*et al.* (2010) the application of cyanobacteria had a significant effect on nutrient uptake in wheat. Swarnalakshmi*et al.* (2013) also found correlation between nitrogen fixation and increased Phosphorus uptake with the use of cyanobacteria by the wheat plant.

Nostoc and *Anabaena* are two the most common cyanobacteria that survive well in soil. They are broadly characterized by their unbranched filaments and the presence of heterocysts which are the sites of nitrogen fixation. They are naturally found in most paddy soils and improve the fertility and texture of the soil in addition to the rice yield at no cost (Prasanna *et al.,* 2013).

They are widely used in a rice field as they fix atmospheric nitrogen to the soil. In rice farming, nitrogen is the second limiting component after water. In rice cultivation, it is thoroughly impossible to observe the role played by chemical fertilizer. Still, yet least it is becoming essential for the alternative like cyanobacteria which would mix fertility to the soil, facilitate soil health and eco-friendly to the environment (Watanabe and Roger, 1984). These both genera are available in natural conditions and are used as biofertilizers. These photosynthetic microorganisms can be cultured, harvested and used as a natural source of biofertilizer (Chittora*et al.,* 2020).

In the present work, the both genus *Anabaena* and *Nostoc* are considered as

component for a nitrogen resource in biofertilizerto study their effect on wheat.

Biofertilizers, being crucial components of organic farming, play an important role in keeping long-term soil fertility and sustainability by fixing atmospheric nitrogen, mobilizing fixed macro and micronutrients, or converting insoluble phosphorus in soil into forms receivables to plants, thereby increasing their efficiency and availability (Sahu*et al*., 2012).

Biofertilizers does not pollute the soil, even not disrupt the ecological balance and as a result they are ecofriendly (http://www.niir.org). Use of biofertilizer allows farmers to reduce the use of chemical fertilizers and pesticides, which are dangerous to the environment and enhance the risk for human health (Sahu*et al*., 2012).

A big question before present-day agriculture is to increase the agricultural production to meet the present and future food needs of the population within the limited available resources, without deteriorating the environmental quality (Singh and Strong, 2016). Sustainable agriculture practices can fulfill the growing need for food as well as ecological quality (Mason, 2003).

The present study of sustainable agriculture will be helpful in eco-friendly, low-cost farming with the help of microorganisms. Apart from the increase in growth and no use of nitrogen chemical fertilizer this will also result in improved soil physicochemical properties, lesser residual soil nitrogen, carbon will built up gradually, improved soil pH and electrical conductivity.

The current work was undertaken to find out the possibility of using biofertilizers for wheat. Present research was performed to assess the ability of fly ash mixed with *Anabaena* (cyanobacteria) and *Nostoc* (cyanobacteria) used as

biofertilizers, to improve the biochemical composition of wheat *(Triticumaestivum*) plants, under conditions of no use of chemical fertilizers.

The research was conducted on wheat as it is an important cereal crop of India. Most of the research works with the cyanobacterialbiofertilizer has been performed on Rice. But wheat is the main cereal used in most of the part of India. It fulfills the need of food and used in different preparations like chapaties, bread, pizza, biscuits etc. Wheat is rich in reactant components, mineral salt, Calcium, Magnesium, Sulfur, Potassium, Chlorine, Arsenic, Manganese, Zinc, Silicon, Iodine and Copper. This abundance of supplements is the reason it is regularly utilized in our daily diet.

They have saturated and trans-fats which enhance cardiovascular diseases, while omega-3 fats decreases cardiovascular disease risk. Wheat is immeasurable and less effective in patients with metabolic disorders. Common types of metabolic syndromes comprise visceral obesity, also known as the "pear-shaped" body, high triglycerides, low levels of protective low density lipoprotein cholesterol and high blood pressure. Wheat defends against all of these conditions.

It is the most famous and readily available bulk laxative. Three cups of wheat consumption per day are enough for an individual to live a long, healthy and disease-free life. For maintaining a fibre-rich diet comprising wheat bread and cereals high in bran, problems such as constipation and digestion will be alleviated

Rajasthan has the fifth rank in the production of wheat in India. Rajasthan has (www.healthline.com).In Rajasthan, wheat is the most important food crop.7.49% of the total wheat production and 7.24% of India's wheat area (www.agriculture.rajasthan.gov.in). More than 20 districts are producing wheat and

11 are significant producers. Sriganganagar, Hanumangarh, Bharatpur, Kota, Alwar, Jaipur, Tonk, Chittorgarh, Sawaimadhopur, Udaipur and Pali are important wheat producing districts of Rajasthan(http://www.agriculture.rajasthan.gov.in).

1.1 Site of Study

The present research study was conducted in Rajasthan state at Sriganganagar district. The district has the highest area of wheat production in Rajasthan (www.agriculture.rajasthan.gov.in) and also having fly ash generation as the byproduct of Suratgarh Super Thermal Power plant. The economy of the Sriganganagar is based on agriculture; its main crops are wheat, mustardand cotton. Industries in Sriganganagar are primarily based on agriculture like cotton ginning and urgent factories, mustard oil generators, Rajasthan State Ganganagar Sugar Mills, wheat flour turbines etc.

The Suratgarh Super Thermal Power Plant is located close to the city. It isthe oldest Thermal Power Plant in Rajasthan which is generating 1500 MW of electricity, which is highest in the state. The fly ash is generated in huge quantity (60MT) in this power plant (www.energy.rajasthan.gov.in). Cement manufacturing factories like Shree Cement Ltd &Bangur cement are well known which are using fly ash to produce PPC, OPC & top rate cement. But the agriculture & ecofriendly use of fly ash is very limited and it is the need of present scenario.

1.2 Objectives of Research

This study is proposed to be undertaken with the following objectives:

1. To prepare bio-fertilizer by mixing of fly ash and cyanobacteria

2. To use of bio-fertilizer in plants: wheat

3. To produce wheat without chemical fertilizers

1.3 Significance of Research Work

The present research is going to cover the effect of fly ash mixed with cyanobacteria as biofertilizer on the wheat plant. Hence the present work is an attempt to investigate if there is any beneficial change by using fly ash mixed with cyanobacteria on wheat**.** This work will be useful to provide improved growth and properties of wheat besides reducing the high demand of nitrogen fertilizer.

This study will be helpful to state that fly ash can be used as a potential source of integrated plant nutrient supply system. This study will be useful to improve economic condition of the farmers by encouraging them for the use of biofertilizer. The study will find out the useful effect of biofertilizer fly ash mixed cyanobacteria on wheat. The study will build a foundation for further investigations on the use of fly ash mixed with cyanobacteria biofertilizer on wheat.

It may provide some useful suggestion for the disposal of fly ash with use of microorganism as biofertilizer which in turn can help in replacement of chemical fertilizers in future by providing the useful plants nutrients for the growth and development of plant. It may be also helpful in cleaning the environment by utilizing the fly ash which is considered as the waste product of coal fired thermal power plants.

CHAPTER 2

REVIEW OF LITERATURE

A Thermal Power Station is the source of electric power. The Thermal Power Plant is also designated to like Coal Thermal Power Plant and steam turbine Power Plant. In India, bituminous coal, brown coal and peat are used as fuel for boiler. The bituminous coal used as boiler fuel has an uncertain matter from8 to 33% and ash current of 5 to 16%.About 125 thermal power plants in India form the source of country's primary fly ash (Kumar, 2006). The Indian coal constitutes about 30-40% fly ash after complete burning (Kumar *et al.*, 2000).

According to the report of American Coal Ash Association in agriculture, wasteland reclamation and civil engineering purposes use 32% of the fly ash, 30% of the bottom ash, 94% of the boiler slag and 9% of flue gas desulfurization sludge (http://www.acaa-usa.org.).Fly ash particles are empty spheres (cenospheres) filled with smaller amorphous particles and crystals (plerospheres). The cenospherefraction constitutes as much as 1% of the total mass and gets easily airborne (Hodgson and Holliday, 1966). Its generation in the country has enhanced from 40 Million ton (MT)/yr. (1994) to about 235 MT/yr. (2013). It is presumed to be 1000 MT/yr. (2031-32).

As it is generating in huge amount and creating a problem for the environment so there is a need to explore it. Soil properties as influenced by fly ash application have been studied by many researchers (Aitken *et al*., 1984; Sikka and Kansal, 1994; Grewal*et al*., 2001; Deshmukh *et al*., 2000; Nidhi, 2003; Inam, 2007) for utilizing

this industrial waste as an agronomic amendment. The literature on the fly ash application and cyanobacteria from abroad and India were gained and reviewed. The review was also aimed to identify a research gap that can be used to find out the use of fly ash as biofertilizer.

2.1 International Studies on Fly ash and Cyanobacteria

Doran and Martins (1972) and Page *et al*.(1979) revealed that fly ash application to agriculture soil is beneficial as it contains micronutrients essential for plant life and crops. Fly ash could correct the deficiency of several micronutrients and prevent some metal ions toxicity through the neutralization of soil acidity.

Dominati*et al*.(2014) found that manageable properties, such as organic carbon content or pH, are easily modified by management on shorter time scales. Land utilization and management primarily affect these manageable characteristics of soils and through capacity of soil to contribute to ecosystem services provision.

Wong and Wong (1989) highlighted that the electrical conductivity and pH of sandy soils and sandy loam soils were increased, but more so for the sandy soil due to fly ash application. The increase in electrical conductivity may limit soil water availability because of the high osmotic pressure and the increased pH would alter the availability of micro-elements to plants.

Non-significant higher uptake of metals in fly ash treated plots was due to their presence in oxide form and so insoluble in water for becoming readily available for their uptake (Page *et al*., 1979).Zhi*et al*. (2011) also recorded an increase in the soil pH and soil electrical conductivity by applying fly ash.

Several fields and greenhouse experiments indicate that many chemical constituents of fly ash may benefit plant growth and improve soil agronomic properties (Elseewi*et al*., 1980; Wong and Wong., 1989).

Adriano *et al*. (1980) stated that forestry attracts fly ash utilization for growing few economically essential trees such as pulp and paper trees, biodiesel crops firewood and plywood trees. Indian fly ash has been found useful for plants growth due to several plant nutrients.

Research and experts (Wong and Wong, 1989; Page *et al.*, 1979; Zhi *et al.,* 2011) view proved that fly ash having both the soil amending and nutrient enriching properties that improve crop growth and yield in low fertility soils.

As fly ash contains trace amounts of heavy metals (Wong and Wong, 1989), soils application has been investigated for its safe use for crop production in human consumption (Page *et al.*, 1979; Doran and Martins, 1972; Wong and Wong, 1989).

In tomato, application of coal fly ash showed increase in the shoot dry weight and the foliar Nitrogen content but decreases in the foliar and stems phosphorus content at the flowering stage. However, the foliar Nitrogen, Phosphorus and Potassium content increased at the harvest stage (Zhi *et al.,* 2011).

Xian *et al*. (2011) stated that fly ash was used as a coating agent to prepare control release fertilizer from typical compound fertilizer ($N:P_2O_5: K_2O$ -15:15:15). When applied in Chinese cabbage, this slow-release fertilizer increased Chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and decreased stomatal limitation during its late growth stage and showed improvement in the plant parameter characteristics and biomass. The coated controlled release fertilizers

decreased NO_3. N-significantly and organic acid contents to a certain extent in the plant's functional leaves and improved sugar acid and soluble sugar levels.

Shou-Chen *et al*. (2011) analyzed Chlorophyll content in the Soybean plant. The photosynthesis rate at the flowering and seed-filling stages was more significant when fly ash was applied in combination with dairy manure than fly ash alone.A similar increase in yield, plant Chlorophyll content and other physiological parameters was observed by Shou-Chen *et al*. (2011) in soybean plants when fly ash was applied in combination with farmyard manure.

Rautaray*et al*. (2003) studied direct effect of fly ash, organic wastes and chemical fertilizers on rice (*Oryzasativa*) and their residual effects on mustard (*Brassica napusvarglauca*) grown in sequence. The integrated use of all the three amendments was found to show an increase in rice-mustard yield by 14%, compared to use with fertilizers 10% and fly ash alone at 3%, respectively.

Zhi *et al.* (2011) studied that coal fly ash applications increased the shoot dry weight and significantly increased foliar and stem N, P and K content in spring wheat at harvest compared with the control.

Rautaray *et al*. (2009) found out that integrated fertilization with fly ash, organic materials and mineral fertilizers to soil improved pH, bulk density, organic carbon and available nutrients. It may further helpful to improve crop quality.It is stated that fly ash has nitrogen deficiency (Kumar *et al*., 2000) and in the present work it is mixed with cyanobacteria to compensate it.

Roger and Reynaud (1979) defined cyanobacteria as the organisms that lie taxonomically between prokaryotes and eukaryotes and are capable of photosynthesis and nitrogen fixation in aerobic conditions.

Durrell (1956) studied that upland soils in arid climates are inhospitable to many microorganisms because of high temperature and little water. Yet, cyanobacteria are incredibly resistant to such adverse conditions and form the dominant component of microflora in many cases (Fogg *et al*., 1973).

Sixty-two algal species were recorded from 120 soil samples collected from the Gulf of Mexico and areas of Ecuador and Colombia; of these 46 species were cyanobacteria with 23 nitrogen fixers that included the population of *Nostoc muscurum* (21%), *Nostoc paludosum* (13%) and other nitrogen fixing cyanophytes (4%).

The most relevant factors for the occurrence of cyanobacteria in addition to light are soil moisture, pH, mineral nutrients and combined nitrogen (Granhall, 1975).It has been reported that N availability to plants is increased due to the application of cyanobacteria in agriculture ecosystems, particularly the rice fields (Stewart *et al*., 1968; Peters *et al*., 1977).

Cyanobacteria are more abundant in the tropical soils due to their higher temperature optima (Castenholtz, 1989).Obana*et al*. (2007) studied the effects of *Nostoc sp.* on soil characteristics, plant growth and nutrient uptake.The results showed an enhancement of organic carbon, the nitrogen content of the surface soil and an increase in the system's productivity when the soil temperature and moisture were maintained.

A survey of 102 soil samples from four countries has shown an abundance of heterocystous forms, positively correlated with pH and available phosphorus content of the soils (Roger *et al*., 1993).

Chunleuchenon *et al*. (2003) investigated Nitrogen-fixing cyanobacteria from Thai soil.They found higher nitrogen fixing-cyanobacteria in the agricultural soil, which generally increased in the rainy season and decreased during a dry season. Environmental factors such as pH, moisture content and temperature influenced the variability of cyanobacterial population density.

Kapustkaand Rosowski(1976) studied the responses of *Cylindrospermum* species to different sources of nitrogen.They found that the occurrence and position of heterocyst, which are the main criteria to distinguish the genus, were dependent upon the N-source. Intercalary heterocysts were observed in the young cultures of the isolate.

The three primary heterocystous*viz*., *Anabaena, Nostoc* and *Calothrix* species responded differently to different irradiation levels. Most cyanobacteria appeared to be different in the rain moistened and flooded rice fields of Bangladesh, though mats of *Scytonema mirabilis* were ordinary under both conditions (Rother and Whitton, 1989; Whitton *et al*., 1988).

Cyanobacteria are known to excrete several extra-cellular compounds like polysaccharides, peptides and lipids during their growth in soil. These compounds diffuse around soil particles, glue and hold them together in the form of micro-aggregates. Besides polysaccharides are made of fibres, which can also entangle clay particles and form clusters. These clusters or micro-aggregates, in turn, grow and take the shape of macro-aggregates and subsequently, of larger soil aggregates. The

interwoven nature of growing algal filaments may also help bind the soil particles along with the organic Carbon added through algal biomass (Rao and Burns, 1990; Rogers and Burns, 1994).Many workers have indicated the importance of these compounds in soil-aggregate formation or soil stabilization.

Garcia-Meza *et al.* (2006) reported that the colonization by cyanobacteria and green algae was helpful during the first stage of remediation of mine tailings and other degraded terrestrial environments.

Cyanobacteria can improve bioavailability of phosphorus to the plants by solubilizing and mobilizing the insoluble organic phosphates present in the soil with the help of phosphatase enzymes. Cyanobacteria can solubilize the insoluble form of $Ca_3 (PO_4)_2$, $FePO_4$, $AlPO_4$and hydroxyapatite [$Ca_5 (PO_4)_3OH$] in soils and sediments (Doriche*t al*., 1985; Wolf *et al*., 1985; Cameron and Julian, 1988).

The abundance of cyanobacteria in rice fields was first observed by Fritsch (1907). More than half of cyanobacterialheterocystous forms are found growing at or floating above the surface, particularly in wetland rice fields, which supply 86% of the world's rice requirements (Ladha and Reddy, 1995).

Terraces for rice cultivation are widely distributed in North America, France, Central America, China, Japan and India. Roger *et al*. (1986) carried out a quantitative estimation on nitrogen-fixing autotrophs in rice terraces in the Philippines, wherein they found a decrease in BGA at increasing elevation. Terraces have been disappearing due to difficulties in restoration and cultivation.

Yamaguchi (1979) mentioned soils in floodplain used for rice production maintain a moderate degree of nitrogen fertility without nitrogen. Researches show

that tropical rice soil fertility was due to the cyanobacterial abundance. Studies on paddy fields in several countries viz., Japan, Thailand, China, the Philippines, Bangladesh and India by Roger and Kulasooriya (1980) revealed cyanobacteria's dominance presence. Cyanobacteria constitute 86% of the total algal flora in southern Iraq (AL-Kaisi, 1976), 75% in Indian rice fields and 70% in Italian soils (Singh and Kour, 2009).

Ariosa*et al*. (2005) worked on developing cyanobacterial blooms in Valencian rice fields, Spain. Blooms of *Gloeotrichia*, *Gloeocapsa*, *Microchaete* and *Nostoc* were found to be small and dispersed and appeared for a few weeks. Siahbalaei*et al*. (2011) reported Nostocalean cyanobacteria from the rice fields of Golestan Province in North-East of Iran. Pereira *et al*. (2005) surveyed heterocystous cyanobacteria in Chilean rice fields. Physico-chemical parameters in a submerged rice field. They found microbial biomass not correlated with physicochemical parameters.

Roger and Reynoud (1982) described free-living cyanobacteria in tropical soils. They narrated that the paddy field environment provides a favorable environment for cyanobacteria growth due to high temperature, light, water and nutrient availability. Cyanobacteria are known to be essential nitrogen fixers that occur freely and in the association, e.g. *Azolla* described by Giller and Wilson (1991).

Saadatnia and Riahi (2009) isolated, identified and multiplied cyanobacteria from paddy fields of Iran and investigated their application as biofertilizer.

Innok *et al*. (2009) studied cyanobacterialakinete induction and its application as a biofertilizer for rice cultivation and proposed that akinete induction might be an appropriate as cyanobacterial inoculum. Pereira *et al*. (2009) worked on filamentous nitrogen-fixing cyanobacteria for the development of biofertilizer.

The abundance of heterocystous species was significantly correlated with available Phosphorus in paddy fields of Bangladesh (Mandal *et al*., 1992).

It is not easy to assess the impact of Phosphorus fertilization on cyanobacteria in paddy fields since other fertilizers, particularly Potassium, are added simultaneously. The highly significant increase in cyanobacterial biomass of the cyanobacterial genera, i.e. *Aulosira*, *Aphanotheceand Gloeotrichia,* was explicitly shown with addition of the phosphate (Bisoyi and Singh, 1988).

Pszczolkowski*et al*. (2012) andGrzesik and Romanowska-Duda, (2014) confirmed previously described interdependencies between plant growth enhancement and the presence of cyanobacterial strains or methods of their application.This indicates that the studied cyanobacterial strains contain a potential source of bioactive compounds that activate several metabolic processes, regulating growth and development of plants.Their results showed the positive influence of microalgal cell suspensions on cutting rooting, metabolic activity, plant development of grapes or seed germination and seedling growth of sunflower and corn.

The positive impact of the used strains on cutting rooting and plant growth might have been caused not only by the increased concentration of different bioactive compounds present in cyanobacteria (Markou and Nerantzis, 2013) but also by their ability to assimilate atmospheric nitrogen and indolic compounds, as it was found in research performed on rice, wheat, gillyflower, grapevine and *Virginia*fanpetals.

Reduced electrolyte leakage from leaves was observed indicating that the investigated cyanobacteria lower the permeability of cytomembrane. (Spiller and Gunasekaran, 1990; Obreht*et al*., 1993; Haroun and Hussein, 2003; Grzesik*et al*.,

2009; Shanan and Higazy, 2009; Romanowska-duda*et al*., 2010; Pszczolkowski*et al*., 2012).

Cyanobacteria fix atmospheric nitrogen by forms, i.e. free-living and symbiotic associations with partners such as water fern *Azolla*, cycads, *Gunnera*etc. Some cyanobacterial members are endowed with the specialized cells known as heterocyst – thick-walled modified cells, which are considered the site of nitrogen fixation by nitrogenase enzyme (Bergman *et al*., 1997). Teaumroong *et al.* (2002) analyzed the diversity of nitrogen-fixing cyanobacteria in Thailand's various ecosystems.

Several researchers have investigated that inoculation of cyanobacteria (*In-vitro*) in wheat crops could enhance the plant shoot/root length, dry weight and yield (Spiller and Gunasekaran, 1990; Obreht*et al*., 1993).

Gantar*et al*. (1995) observed that extracellular substances released by cyanobacteria that colonize wheat plant roots showed a significant effect on plant growth, though the agronomic efficiency was not evaluated. Due to their natural diversity, cyanobacteria's capacity to grow in various locations, even those unfit for agriculture, could be exploited. The fast cyanobacterial cell growth and simple nutritional requirements, mainly water, sunlight and CO_2 provide a broad scope for commercial application of cyanobacterial species as plant growth promoters (Ruffing, 2011),

Obreht*et al*. (1993) suggested that co-inoculation of cyanobacteria with wheat enhanced root dry weight and Chlorophyll. For a long time, the importance of cyanobacteria was recognized; a considerable amount of research has been carried out

to evolve methods and means to effectively utilize these organisms as biofertilizers (Brouers*et al*., 1987; Shi and Hall, 1988).

The ecology, physiology and molecular genetics of cyanobacteria were elaborately studied by Cohen (2006). They conclude that the proper understanding of ecology, physiology and molecular genetics of cyanobacteria can help to explore their uses in different fields.

2.2 Indian Perspective on Fly ash and Cyanobacteria

Khungar (1998) studied coal as a prime energy source for power production with a high amount of fly ash as a by-product. Its proper disposal is a challenge. Fly ash contains essential plant nutrients inclusive of micronutrients and elements like Potassium, Sodium, Calcium, Zinc, Magnesium and it is able for agriculture due to its efficiency to improve soil health and crop performance (Basu *et al*., 2009).

The use of fly ash is attracting the attention of scientists and farmers. Fly ash is a heterogeneous mixture of amorphous and crystalline phases and generally considered as Ferro aluminosilicate. It comprised about 69% silt and clay size fractions. A low value of its particle density established its potential for dust formation (Sharma *et al*., 2001). The high water holding capacity of ash was due to its dominant silt and clay size fractions. Fly ash contained about 93% of silica and sesquioxides (Al_2O_3 and Fe_2O_3). In the remaining portion, Ca_2^+ was the dominant cation, followed by Mg_2^+, Na^+ and K^+. The bulk density of fly ash was 1.01 mg/m3.The water holding capacity of fly ash was 56.9% and electrical conductivity values were 0.65 dS/m, (Sharma *et al*., 2001).

Water transmission characteristics of soil (saturated hydraulic conductivity) decreased, but water retention improved with increasing fly ash (Agarwal and Sharma, 2009).

Many researchers added fly ash in the soil to evaluate the long-term consequences of fly ash on soil environment (Garg*et al*., 2003; Kalra*et al*., 1997;Kesh*et al*., 2003; Singh & Singh, 1986) and crop productivity (Kesh*et al*., 2003). Fly ash incorporation in the sandy loam soil (up to 40%) modified the soil environment, mainly moisture retention, release and transmission behaviour, pH, EC and organic carbon.

The soil-ash admixture texture remained sandy loam up to 10% ash application; beyond this level, the texture turned to the loamy soil (Kesh*et al*., 2003).

Kumar *et al*.(2005) conducted a study in field demonstration projects taken at more than 50 locations by fly ash mission (FAM), now known as fly ash utilizationprogramme (FAUP) in varying agro-climatic conditions and different soil crop combinations, supported with laboratory investigations.

It showed that significant fly ash application does not have any adverse impact on soil health, the presence of trace heavy metals and radionuclides in fly ash. The presence of these elements is too low to make any harmful impact.

Fly ash amendment of soil could improve nutrient status of the soil as it contains considerable amounts of vital plant nutrients like potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and phosphorous (P) (Kalra *et al*., 1997; Singh and Yunus, 2000).

Sengupta (2002) analyzed that fly ash has been used as a source of essential plant nutrients like calcium, magnesium, potassium, phosphorus, copper, zinc, manganese, iron, boron and molybdenum and also for boosting crop growth and yield. It has been successfully applied in different agro-climatic conditions and soil types in other parts of the country.

Saraswat and Chaudhary (2014) worked on the effect of fly ash in improving soil quality and increasing crop production efficiency. Several authors have investigated the property of fly ash (FA). The degree of soil pH change on the FA application is dependent on the factors like the difference between the pH of FA and soil, the buffering capacity of the soil and the FA capacity as determined by the amount of CaO, MgO and Al_2SiO_5 present. On the addition of 200-400 t/ha of FA to sandy loam soil, a significant development in the permeability, field moisture-holding capacity, total transferable bases and a reduction in bulk density and acidity, to the benefit of crop production.

Maize crop grown at IARI, New Delhi, India, under varying levels of fly ash mixed either through application in the soil at the time of sowing or through dusting the amounts split and applied in various stages of the crop growth showed an increase in yields when compared with control in fly ash treated plots through soil application up to 10 t/ha application and after that decrease in trend was noticed. Dusting crop canopies with ash decreased the yield in proportion to the amount applied and the values were lower than obtained under control. The decrease in products obtained under dusting treatment might be activity, due to fly ash deposition on the crop canopies which caused reduced plant growth. The increased yields obtained under fly

ash application in the soil up to 10 tons/ha rates might be due to improved soil structure and enhanced nutrient availability.

Panda *et al*. (2015) studied that there was ample scope for the safe utilization of fly ash in agriculture without severe deleterious effects but fly ash varied widely in its physical and chemical composition; therefore, the mode of use in agriculture was different and depended on the characteristics of the soil or soil type. The study suggested a careful investigation of the accumulation of heavy metals (present in fly ash) in soil as such in the edible portions of other vegetables.

The magnitude of heavy metal adsorption by plants depends upon heavy metal content in fly ash, the soil type, pH, the plant species, etc. Among all of the species grown on fly ash-amended soils, Boron showed a significant increase in the legume while Se increased principally in grasses and Mo showed a consistent increase in all the species.Selenium, Copper and Lead contents were high in plants. Molybdenum and Sulphur components of fly ash was also easily assimilable and are likely to show accumulation in plants.

Kumar (1987) however did not observe any depressing effect of fly ash application (up to 10%) on the Cadmium contents of lettuce. The potentiality of alkaline fly ash in detoxifying Cadmium in the soil-plant system can be exploited; it is without any effect of other parameters of plant growth. Trace elements (Zinc, Copper, Iron, Manganese and Cadmium) were used as massive metal indicators by the crop when grown under fly ash added soil (Sharma *et al*., 2002 &Kalra*et al*., 1998).

Treatments of coal ash at 100 g/kg of calcium carbonate at 1.0 g/kg promoted the height, bearing spikelets, grains per spike, 1000 grains weight in wheat observed by Patil*et al*. (2010). In the cotton-wheat cropping system, in light-textured soil,

Singh *et al*. (2009) reported increased seed cotton yield with the application of fly ash during the first year and a residual beneficial effect on the subsequent wheat crop.

Gupta and Singh (2009) analyzed the physicochemical characteristics (pH, electrical conductivity and trace element concentration) of fly ash applied in the soil in the Arpa irrigation project area of Bilaspur District, Chhattisgarh, India. The increased value of these soil properties was favourable and recommended for the cultivation of chickpea in acidic soil.

Similarly, the various crops like rice, wheat, gram, lentil and mustard were grown on varying level of fly ash, encouraging the crop growth and subsequently its yield (Sharma *et al*., 2002; Sharma, 1998 and Sharma *et al*., 2001; Khan and Singh, 2001).

Its amendment in the soil brings about an increase in growth and yield of cucumber, maize, okra, potato, tomato, wheat as by observed by Kausar, 2007; Khan and Khan, 1996; Mishra and Shukla, 1986; Raghav and Khan, 2002).

Numerous studies report the impact of fly ash addition on the yields of different crops with either depressions or enhancements in yield (Sharma *et al*., 2002; Sikka&Kansal, 1994; Kalra*et al*., 1997; Sharma, 1998; Deshmukh *et al*., 2000; Grewal*et al*., 2001 and Garg*et al*., 2003). Whereas depression in yield has been largely reported to occur due to Boron toxicity, Phosphorus and Zinc deficiency (Chatterjee and Ratan, 1987), improvements have been attributed principally to Boron supply in Boron deficient soils improvements in sulfur supply and available water capacity. FA amended the acidic soil. FA and acidic soils were found to have high P-fixation capacities and mixing of the two was found to resolve the P-fixation problem to a great extent.

Effect of fly ash and sewage sludge (treated city waste) applied to peanut *(Arachishypogaea* L.) crop on microbial status changes, nitrogen fixation and crop production in lateritic soil. Such wastes in different doses and the combination of wastes to agricultural lands after the nutrient cycling processes, particularly for leguminous crops, where nodulation, N_2-fixation, and N-uptake is mainly governed by a group of microorganisms. Nodule number and N accumulation in nodules were higher in fly ash-treated soil than that of city waste (Sarkar, 2001).

When applied in combination with organic wastes and chemical fertilizers, fly ash increased the pod yield in groundnut in lateritic acid soils to the extent of 24.7% compared to the control (Basu *et al*., 2010).

An investigation was carried out during the dry season (February-May) of 1996 and 1997 at the Indian Institute of Technology, Kharagpur, to study the effect of paper factory sludge and fly ash on groundnut (*Arachishypogaea* L.) and to find out their suitable time of incorporation in lateritic acid soils. Paper factory sludge and fly ash and chemical fertilizers increased the dry matter accumulation, leaf area index and nodule number/plant compared to farmyard manure and their combination along with fly ash and chemical fertilizers. The beneficial effect was also recorded in yield attributes, yield, oil content in the kernel, nutrient uptake and chemical properties of soil. Their incorporation at 15 days before sowing or at sowing was more advantageous than that at 30 days before sowing (Karmakar *et al.,* 2005).

Fly ash applied as bio-compost (Sludge + fly ash + Coir pith) in vegetable cowpea significantly increased various growth parameters viz., crop growth rate (19.5%) and relative growth rate (32%), pod (26%) and haulm (22.3%) yield (Prasanthrajan and Kannan, 2007).

Rizvi and Khan (2009) reported that 20% level of fly ash and 30% level of brick kiln dust amendments in the soil to be ideal for better plant growth and yield of eggplant. Biomass accumulation in the stems and roots of *Populus* plant increased with an increase in fly ash application rate up to 20%. In contrast, the biomass accumulation in leaves and total biomass accumulation in the plant increased with fly ash application up to a level of 10% only.

Bioconcentration of micronutrient Iron, Manganese and Zinc in stem and leaves of *Populusdeltoides* displayed higher values up to 10% of fly ash application. After that, it declined by a magnitude of 78%, 71% and 62%, respectively (Jala and Goyal, 2006).

Kumar and Singh (2010) demonstrated that fly ash could be utilized safely as a carrier in bio-fertilizer formulations (*Azotobacter*and *Azospirillum*) and finding an effective alternative use for fly ash. *Rhizobium* strains isolated from plants grown in fly ash contaminated soil registered tolerance to fly ash (Chaudhary *et al*., 2011) and improved plant growth when it was inoculated in 100% fly ash conditions. This study suggested that an integrated approach employing biotechnological means and inoculation of plants with host-specific fly ash tolerant *Rhizobium* strain may prove a stimulus to a fly ash management program.

Reports on germination studies showed that fly ash had a positive and negative impact on seed germination. Lower levels of fly ash application enhanced seed germination and seedling growth and at higher levels, either delayed or inhibited these processes drastically in *Viciafaba* (Singh *et al*., 1997).

In another report, increasing concentration of fly ash effluent enhanced germination rapidly in maize (Yeledhalli*et al*., 2008). However, germination and

early growth were affected adversely in wheat but did not cause any harmful effect in *Sorghum* (Agarwal and Sharma, 2009) when fly ash was used. Physiological mechanisms and biochemical constituents in plants had both significant positive and negative changes due to fly ash application.

Yunusa *et al.* (2009) reported that there was uncertainty as to the rates of coal fly ash needed for optimum physiological processes and growth. The addition of 10 t/ha fly ash increased growth rates and concentrations of Chlorophylls a and b but reduced carotenoid concentrations in barley (*Hordeumvulgare*) and ryegrass (*Secalecereale*), canola (*Brassica napus*), radish (*Raphanussativus*), field peas (*Pisumsativum*) and lucerne (*Medicago sativa*).

Transpiration in barley was increased due to fly ash application. There was no consistent pattern of change in pigment concentrations or instantaneous rates of photosynthesis as compared to plant dry weight such as the amount of fly ash applied. Hence plant dry weight was a more reliable parameter for assessing growth in plants supplied with fly ash.

Nagajyoti *et al*.(2009) consider that the treatment of groundnut in pot culture, with 25% of the effluent, had a stimulatory effect on all the biochemical parameters. Carbohydrates, starch, amino acids, protein, nitrate and nitrite reductase enzymatic activities increased in 10, 15, 20 DAS (days after sowing) and decreased thereafter. This study indicated that the power plant effluent had a stimulatory effect on all the biochemical contents at the lower concentration and at higher concentrations, they had deleterious effects.

Fly ash effluent, when applied to maize, increased Chlorophyll a, Chlorophyllb and the carbohydrate content in leaves of maize. In addition to this biochemical

enhancement, the percentage of germination and growth parameters had a positive correlation with Chlorophyll and carbohydrate contents due to nutrients in fly ash effluents in Maize (Yeledhalli*et al*., 2008).

Fly ash improved root length, Chlorophyllcontent, grain yield per plant and average seed weight of chickpea (*Cicerarietinum*) (Gupta and Singh, 2009). Fly ash application in*B.campestris* increased the total Chlorophyll content of the leaf significantly, whereas carotenoid content showed a non-significant increase as compared to control. Translocation of most of the tested metals (Lead, Manganese, Cadmium, Nickel and Iron) in the shoot of the plant was found higher except Chromium, Copper and Cobalt (Gupta *et al.,* 2010).

Vigna radiata and *Vigna angularis* grown in fly ash inoculatedwith *Rhizobium* showed a marked increase in root-shoot length, biomass yield, photosynthetic pigment, protein content and nodulation frequency compared to an uninoculated plant grown in 100% fly ash (Chaudhary *et al*., 2011).

Singh *et al*. (1981) compared *Azolla*'s efficiency, Blue-Green Algae and other organic manures with nitrogen and phosphorus availability in a flooded rice soil. They found composted *Azolla* and blue-green algae had the highest release of Ammonium nitrogen.

Hazarika *et al*. (2014) worked on the ecological assessment of algal growth with particular reference to cyanobacteria from the upper Brahmaputra valley of Assam, North-East India and describes that both classes of water holding capacity and availability of water influence algal growth.

Ahmed *et al*. (2010) worked on cyanobacteria associated with crop plants and concluded that the non-heterocystous cyanobacteria also could increase crop productivity and growth.

In an all India survey out of 2213 soil samples from rice fields, 33% were found to harbour nitrogen-fixing cyanobacteria (Venkataraman, 1975).It has been reported that N availability to plants is increased due to the application of cyanobacteria in agriculture ecosystems, particularly the rice fields (Singh and Singh 1987).Most of the studies on the plant growth-promoting effects of cyanobacteria-related to paddy crops revealed that cyanobacterial inoculation could enhance rice seed germination root and shoot growth (Misra and Kaushik, 1989a, &1989b).

Ghadai *et al*. (2010) explored cyanobacteria in paddy fields of Gunupur, Orissa, India and encountered rich cyanobacterial diversity in the particular rice growing ecosystem.

Dhar *etal*.(2007) studied three carriers based on blue-green algal biofertilizer's comparative performance for sustainable rice cultivation. The study had a clear view that with the addition of biofertilizers, there is also a need to add chemical fertilizer as a supplement to increase crop productivity, reducing the addition of chemical fertilizers and sustaining soil fertility.

Jha *et al*. (2001) investigated the effects of fertilization and crop rotation of cyanobacteria in the paddy fields. They found high fertilizer inhibits the growth of nitrogen-fixing cyanobacteria, thereby indicating that indiscriminate use of chemical fertilizer in the long term can reduce the soil fertility and disturb the ecological balance.

Singh *et al*. (2011) reported the contribution of cyanobacteria in agriculture productivity. They mentioned that cyanobacteria are the most adaptable to drought and desiccation due to the ability to form spores or akinetes.

Prasanna *et al*. (2008) reported on the potential option of using cyanobacteria for environmental sustainability, especially on remediation andamelioration of soil andwater.

Researchers also analyzed through a quantitative study of the algal flora of dried soil samples from upland fields (pH 7.8-8.3) at the Indian Agricultural Research Institute (IARI), New Delhi cyanobacteria were found to dominate in all soil samples (Dutta& Venkataraman, 1968;Mishra&Pabbi (2004)

Tiwari *et al*.(2015) assessed cyanobacterial diversity from India's North-Eastern region and investigated their biochemical properties concerning their application as biofertilizer, particularly for terrace rice field conditions. A total of 450 unialgal cyanobacteria were isolated from five states of the North-Eastern region, which included 35 strains from the state of Assam.

Prasanna and Nayak (2007) worked on rice soil ecology and its effect on cyanobacterial diversity and abundance. They suggested practical need to utilize the native cyanobacteria in the paddy fields to establish better in their niche and better productivity.

The periodicity of cyanobacteria in rice fields in Uttar Pradesh and Bihar was investigated by Singh (1961) and he found three primary filamentous and heterocystous forms, *i.e., Aulosirafertilissima, Anabaena ambigua* and *Cylindrospermum ghorakpurease.*

More than 100 strains of heterocystous cyanobacteria that belong to the genera of *Anabaena, Nostoc, Nodularia, Cylidrospermum, Scytonema, Calothrix, Anabaenopsis, Mastigocladus, Fischerella, Tolypothrix, Aulosira, Stigonema, Haplosiphon, Chlorogloeopsis, Camptylonema, Gloeotrichia, Nostochopsis, Rivularia, Schytonematopsis, Westiellopsis, Wollea* and *Chlorogloea* genera are efficient nitrogen fixers (Venkataraman, 1993).

They are more prevalent in tropical and subtropical regions, as compared with the temperature belts. Vaishampayam*etal*. (2001) stated that filament of *Anabaena* and *Nostoc* species are the most commonly found nitrogen-fixing organisms in rice fields, occurring as free-floating water blooms, forming a microbial mat. Many other rice field cyanobacteria include: *Nostoccommune* forming balls like structures of mucilage,*Scytonema* species showing characteristic false branching and heterocysts, *Calothrix* species showing characteristic terminalheterocysts; *Nodularia* species with vegetative cells andheterocysts; *Gloeotrichia*species showing distinctive ball like the circular assembly of filaments;and*Lyngbya* species having uniqueyellow-brown colouration of the mucilage sheath due to the presence of scytonemin, a UV absorbing compound.

2.3 State Perspective on Fly ash and Cyanobacteria

Akbar *et al*. (2016) described the silica present in the fly ash. Khandelwal and Shrivastava(2014) studied growth performance of some perennial terrestrial angiosperms, growing in non-polluted and polluted area around the Kota Thermal Power Plant and showed the effect of fly ash on leguminoseae plants.

Tiwari *et al*. (2005) examined in arid, water stress regions of Rajasthan namely,Achrol, Jaiselmer, Manwar and Pokharan. Common cyanobacterial

genera like *Phormidium, Oscillatoria, Nostoc, Anabaena, Calothrix, Westiellopsis*and*Chlorogloeopis* were isolated from arid zones samples.

Research Gap

The available literature showed that there is not so much work performed on the growth of wheat plants grown with fly ash mixed with cyanobacteria as biofertilizer. There are some reports on use of nitrogen fixing bacteria in some crops in combination with fly ash, which have obtained good results. Some reports are there only on use of cyanobacteria, but there is not much work on use of fly ash mixed with cyanobacteria on wheat plant, most of the work is focused on Rice. Hence the present study is carried out with a vision to fill the existing research gap. Bio-fertilizers, such as cyanobacteria can provide a suitable supplement to chemical fertilizers, and 'organic farming' can become a reality in the future.

CHAPTER 3
MATERIALS AND METHODS

The present study is based on appropriate research methodology in which the plan of research was designed, method of sampling and source of data were selected as per the requirement of the study. The present study was conducted in 2017-2019, to analyze the effect of fly ash and cyanobacteria on wheat crops. The procedural plan, design and structure of investigation for the present study are experimental. It is based on cause- and- effect relationship as the effects are measured for the known cause (Kumar, 2011).

3.1 Research Design

The True Experimental research design is used in the present study as it intends to find the concentration of Carbohydrate, Starch, Protein, Phenol and photosynthetic pigment Chlorophyll in wheat depends on the category chosen, i.e., Normal Soil, Soil with Fly ash, Soil with Fly ash + *Anabaena*, Soil with Fly ash + *Nostoc*, Soil with Fly ash + *Anabaena* + *Nostoc* 60%, Soil with Fly ash + *Nostoc* + *Anabaena* 40%.

3.2 Site of Sampling

The Organic seeds notified and certified variety Raj-1482 of the wheat sample bought from Rajasthan State Seeds Corporation Limited, Sriganganagar. The soil sample was collected from Murba 22 Chak Thakrawali, Sriganganagar. The fly ash sample was collected from Suratgarh Thermal Power Station.

3.3 Sampling

Random sampling technique was used for collecting samples.

3.4 Source of Data

The information for the study is collected afresh and for the first time throughexperiments and observations, so the source and kind of data is primary data (Kumar,2011).

3.5 Experimental Material

In the present research work experimental material is Wheat.

Taxonomy of Wheat (Linnaeus, 1753)

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae

Species: *Triticumaestivum*

Variety: Raj-1482

Figure: 3.1 Wheat Plant

Triticum aestivum

Wheat (*Triticumaestivum* L.) is the important cereal crop and the most important staple food of India. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). Triticeae is one of the tribes containing more than 15 genera and 300 species including wheat and barley.

Wheat belongs to the tribe Triticeae (= Hordeae) in the grass family Poaceae (Gramineae) (Briggle and Reitz, 1963) in which the one to several flowered spikelets are sessile and alternate on opposite sides of the rachis forming a true spike. The plant is a monocotyledonous plant which consists of a root and shootsystem. Two types of roots are discovered, the fundamental roots and the nodal roots (adventitious or crown roots), which emerge from the lower nodes of the shoot. The shoot consists of a series of repeating units or phytomers, each conceivably having a node, a leaf, lengthened internodes, and a bud in the axil of the leaf (Briggle and Reitz, 1963).

Each leaf includes the sheath, folding over the subtending leaf and a lamina. At the sheath and lamina intersection, there is a membranous structure, the ligule and a couple of little projections, the auricles. The base of the leaves in the stem is thickened to shape a hard bunch or pulvinus. The extended distal internodes increment long from the basal to the most distal (Briggle and Reitz, 1963).

Wheat is a Rabi crop in which seeds are planted from October to November and harvested in March to April. In India, a winter crop is developed in the rabi season with a temperature between 10 - l5°C and rain between 5 - 15 cm. Wheat needs about 10°C of temperature at the hour of planting, 15° C for plant development and 20° to 25° C for the development of the grains. About 12.5 cm of rain in winter is

a boon for wheat development. Light soil, sandy topsoil and earth soil are appropriate for wheat development (http://www.farmer.gov.in).

3.6 Required Materials & Equipments

Spade, polythene bags, strings, beakers, glass rod, distilled water, analytic balances, narrow mouth polyethene bottles with stoppers, vials, funnels, pipette, reciprocating electric shaker, Whatman's filter paper (No. 41&1/42), Digestion Vessels-250 ml, vapor recovery device, drying ovens, Temperature measurement device (IR sensor, thermocouple, thermistor), Centrifuge and centrifuge tubes, heating source (blockdigestor, microwave, etc.), funnel, graduated cylinder, volumetric flasks.

3.7 Soil Collection Method

The soil sample was collected from 22 Murba Thakrawali Sriganganagar by Quartering Method (http://www.agritech.tnau.ac.in). The particular soil collected was somewhat sandy loam to clay in texture, reddish brown in colour. Soil was collected during October 2017. After collection, samples were dried. Soil collected from the field (garden) was sterilized. The sterilized soil was cooled to room temperature and disposed in to the sterile pots.

The physicochemical analysis of the soil and fly ash were determined. The samples were brought to the laboratory for the analysis of parameters like pH, Electrical Conductivity, Organic Carbon, Total Nitrogen, Sulphur, Iron, Sodium, Potassium, Boron, Cadmium, Lead, Zinc, Nickel, Copper and Manganese.

3.8 Fly ashCollection Method

Fly ash samples were collected from Suratgarh Thermal Power Station. Fly ash from STPS was derived from sub-bituminous black coals. A representative bulk sample of freshly precipitated (unweathered) Fly ash was taken from the hopper of power station. The entire sample was taken at once, to reduce scope of any type of

change in Fly ash composition. After collection, the dry ash was thoroughly mixed and stored in plastic lined containers at room temperature before use. Ash collected from Electro Static Precipitator was relatively finer in texture; lower in pH and richer in nutrients comparatively ash from dumping site. The collected fly ash samples send in polythene bags properly tied with tagged for fly ash analysis in Fly ash Test Laboratory, CEG Test House and Research Centre Pvt. Ltd.

3.9 Methods for Physicochemical Analysis of Soil and Fly ash

The physicochemical parameters were selected for testing on the basis of their importance in influencing plant growth and their role as promoting or limiting factors.

pH

The pH value is a measure of the hydrogen ion activity of soil-water system and expresses the activity and alkalinity of the soil. pH is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of soil. The pH of a solution has been defined as the negative logarithm of the hydrogen ion activity, which in the dilute solution can be expressed as a concentration in gram mole per litre. The pH was determined in the soil-water suspension of ratio 1:2. The Electronic pH meter method (http://www.pharmaguideline.com) was used. The instrument commonly used in this method is a glass electrode pH meter with calomel reference Electrode introducing salt bridge.

A glass surface in contact with hydrogen ions of the solution under test gained an electrical potential, relying upon H^+ ions' concentration. A measure of the electrical potential is so, give H^+ ion concentration or pH of the solution.

Soil water suspensions (1:2) - 40g of soil was mixed into a 250 mL flask and 80 mL of distilled water was added in it. Then the mixture was shaken on the reciprocating shaker for one hour with stopper on the flask.

Firstly, the soil suspension sample was taken and the soil was warmed up for 15min. A known standard buffer solution in a beaker with pH 7.0 and pH 9.2, adjusted for the instrument and after then pH was measured.

Electrical Conductivity

Soil possesses at least a small amount of various soluble salts. These may be acidic, neutral or basic. They may arise from different sources (rocks, groundwater). Soluble salts present in soil dissociate into their respective cations and anions when coming in soil solution. These cations and anions bear current and impart conductivity (Tavakkoli*et al*., 2010). So, the measurement of EC can be directly connected to the soluble salt concentration. The number of soluble salts in a sample is estimated from the EC of aqueous soil extracts.

A simple Wheatstone bridge circuit is used to measure EC by the null method. 25 g of soil sample was dissolved in 50 ml distilled water in a 100 ml beaker and shaken properly for fifteen minutes on a mechanical shaker. It was kept on the stand for half an hour. The conductivity bridge was calibrated with a standard KCl solution, and cell constant was determined. The electrical conductivity of soil solution was measured in supernatant liquid by the Conductivity Bridge (ds/m) and the reading was noted.

Organic Carbon

Wet digestion or Walkley& Black (1934) method involves rapid titration procedure to estimate the organic carbon contentofsoil.Principleorganicmatterisoxidizedwithamixtureof$K_2Cr_2O_7$isbacktitrated withFerrousAmmoniumSulphate(FAS).Organiccarboninthesoilisoxidized toCO_2.0.5g of powdered and sieved (2mm) soil was weighed into a 500 ml conical

flask.10 ml of 1N $K_2Cr_2O_7$ solution was added and shaken to mix.20 ml of conc. H_2SO_4 was added from the side of the flask.The contents of the flask are kept undisturbed for 30 min. 3g of NaF or 10 ml of H_3PO_4& 100 ml of distilled water was added and shaken vigorously.10 drops of diphenylamine indicator is added which turns the solution violet. The solution was titratedagainst 0.5N FAS solution until the colour changes from violet to bright green and the volume of solution used is noted.A blank titration in a similar manner without the soil was carried out.

Percentage of Organic carbon in the soil = $\frac{(X—Y)\ /2\ \times 0.003\times 100}{S}$

% of organic carbon = (X-Y)/0.5

Sg = wt. of the sample,

Xg = vol. of FAS used in blank

Yg = vol. of FAS used to oxidize SOC

N = Normality of FAS

(X–F) = Volume of 1N $K_2Cr_2O_7$ used for the oxidation of carbon/2

0.003gSOC = 1ml of 1N $K_2Cr_2O_7$

Total Nitrogen

In the Kjelteb Auto Analyzer method (Kjeldahl, J. 1883) NH_4^+.N (liberated by distillation of the digest with strong alkali) is absorbed in unstandardized H_3BO_3 by titration against standard strong acid (HCl).

Digestion

The sample is digested in H_2SO_4 to convert organic N to NH_4^+.N Digestion block digester with tractor auto temperature controller was used in digestion. 1 to 2g of mineral soil low in N (60mesh) was transferred into a digestion tube and 10 ml of concentrated H_2SO_4 was added to it, mixed by swirling, heated at 200°C in digestion

block. Then one Kjeltab was added, again heated for 15-20 minutes untilKjeltab was dissolved (300°C).Then the temperature was raised to 375°C and heated until sample turned turquoise (45min).

Then the digestion tubes were removed from block, allowed to cool for 5 minutes. About 50 ml of water was added and mixed well until the example is in solution. Then followed instruments for the Kjelteb Auto analyzer and the alkali pump was set up to deliver 30 ml of 40% NaOH, then titrated with 0.01M NaOH and readings were calculated.

Sulphur

Soil is shaken with 0.15 % $CaCl_2$ solution. Chloride ions displace adsorbed sulphate during extraction. Calcium ions generally suppress the extraction of soil organic matter and hence it eliminates the contamination caused by extractable organic S. The filtrate is analyzed for S by the Turbidimetric method of Chesnin and Yien, (1950) in which the turbidity produced due to precipitation of sulphate, as barium sulphate is measured on a spectrophotometer at a wavelength of 420 nm using a blue filter. Gum acacia solution is added to stabilize the turbidity so that the precipitate of barium sulphate does not settle down.

10 g air-dried soil sample was weighed and transferred in a 150 ml conical flask and 50 ml of 0.15 % $CaCl_2$ solution was added and shaken for 30 minutes on a shaker. After then the solution was filtered through Whatman no. 42 filter paper and 20 ml of the filtrate pipetted out in 25 ml volumetric flask. One gram of 30-60 mesh $BaCl_2$ was added and shaken for 1 minute. Thereafter 1 ml of 0.25% gum acacia solution was added. Finally made up the volume by adding distilled water and was shaken for 1 minute and within 5 to 30 minutes after the development of turbidity was taken the reading on Spectrophotometer.

ppm of S in soil = Y x 6.25 (dilution factor)

Kg of S/ha = ppm x 2.24

ppm of S from standard curve against A value = Y

Absorbance reading = A

Potassium

The Ammonium acetate method (http://www.jenway.com) was used to determine available potassium.

1g of soil sample was weighted in a 100 ml conical flask, 25 ml of the neutral 1N ammonium acetate solution was added, shaken for five minutes and the solution was filtered through Whatman No. 1 filter paper. The concentration of K in the filtrate was measured using a flame photometer.

Preparation of standard curve for potassium - Suitable volumes of standard K solution diluted to get 100 ml of a working standard containing 10, 15, 20, 25, 30 and 40 mg K/L. Reading of the flame photometer was recorded for each of the working standards of K after adjusting blank to zero. A standard curve was drawn by plotting the reading against K concentrations.

Calculation-

Available K (Kg ha^{-1}) = C x 25/5x10^6 / 10^4 x 2.24 = C x 11.2

Where C stands for the concentration of potassium

Metallic Ions (Zn, Cu, Mn, Cd, Pb, Ni, & B)

USEPA 3050B Method **(**https://www.epa.gov)

In the present work, this method was used to determine for available metallic ions (Zn, Cu, Mn, Cd, Pb, Ni, & B).

The method is commonly used to determine the available micronutrients in soil sample & fly ash. This UPEPA 3050B method was tested by digestion procedures for the preparation of fly ash and soil samples for examining by flame atomic absorption spectrometry (FLAA). Samples prepared by this method may be analyzed by ICP-AES.

Method

For the digestion of samples, a 1-2 gram (wet weighted) or 1 gram (dry weighted) sample was digested with repeated addition of nitric acid (HNO_3) and hydrogen peroxide (H_2O). For GFAA or ICP-MS analysis, the resultant digestate was reduced in volume then diluted to a final volume of 100 ml. For ICP-AES or FLAA analyses, hydrochloric acid (HCl) was added to the initial digested and the sample was refluxed. In an optional step to increase some metals' solubility, this digestate was filtered. The filter paper and residues were rinsed; with hot HCl and then hot reagent water. Filter paper and residue were returned to the digestion flask, refluxed with additional HCl and then filtered again. The digestate was then diluted to a final volume of 100 ml. If required, a separate sample aliquot shall be dried for an entire per cent solids determination.

The sample mixed thoroughly to achieve homogeneity and sieved. All equipment used for homogenization should be cleaned to minimize the potential of cross-contamination. 0.01 g sample weighed and transferred to a 1-2 g sample (wet weighted) or 1 g sample (dry weighted) to a digestion vessel for each digestion procedure.

For the digestion of samples for examination by GFAA, added 10 mL of 1:1 HNO_3, the sample was mixed and covered with a watch glass or vapor recovery

device. The sample was heated to 95EC ± 5EC and refluxed for 10 to 15 minutes without boiling.

The sample was allowed to cool then 5 mL of concentrated HNO_3 was added; the cover was replaced and refluxed for 30 minutes.If brown fumes are generated, indicating oxidation of the sample by HNO_3, this step repeated (addition of 5 mL of conc. HNO_3) over 3: 3 and over, until no brown fumes were given off by the sample indicating the complete reaction with HNO_3 vapor recovery, the system either allowed the solution to evaporate to approximately 5 mL without boiling or heat at 95EC ± 5EC without boiling for two hours.

The sample was heated to 95EC ± 5EC and refluxed for 5 minutes at 95EC ± 5EC without boiling.The sample was allowed to cool for 5 minutes, 5 mL of concentrated HNO_3 was added; the sample was heated to 95EC ± 5EC, and refluxed for 5 minutes at 95EC ± three 5EC.After the step in Section has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O.

The vessel covered with a watch glass or vapors 2: 2 recovery device, now the covered vessel heated and the peroxide reaction started. Heated until effervescence subsides and cools the vessel.Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle.The sample is now ready for analysis by GFAA Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

Centrifugation

Centrifugation at 2,000- 3,000 rpm for 10 minutes is usually sufficient to clear the supernatant. The diluted digestate solution contains approximately 5% (v/v) HNO_3. For analysis, aliquots of appropriate volume taken and any required reagent or matrix modifier added.

For the analysis of samples for FLAA or ICP-AES, 10 ml concentration HCl added to the sample digest and covered with a watch glass or vapour recovery device. For direct energy coupling devices, such as a microwave, digested samples for analysis by FLAA by adding 5 ml HCl and 10 ml H_2O to the sample digested and heated to 95^0C ± 5EC, reflux at 95 ^{0}C ± 5EC without boiling for 5 minutes.Filtered the digestate through Whatman No. 41 filter paper and filtrate collected in a 100 mL volumetric flask. Volume made up and analyzed by the FLAA Section.

2.5 mL concentration HNO_3 was added and 10 mL concentrated HCl to a 1-2 g sample (wet weighted) or 1 g sample (dry weighted) and covered with a watch glass.The sample was placed on the heating source and refluxed for 15 minutes. Filtered the digestate through Whatman No. 41 filter paper and filtrate was collected in a 100 ml volumetric flask.After then washed the filter paper, while still in the funnel, with no more than 5 mL of hot (~95EC) HCl, after then with 20 mL of hot (~95EC) reagent water and collected washings in the same 100 mL volumetric flask.The filter removed and residue from the funnel and placed them back in the vessel. After that, 5 mL of concentration was added.

HCl placed in the vessel back on the heating source and heated at 95EC ± 5EC until the filter paper dissolves.The vessel was removed from the heating source and washed the covered and sides with reagent water. The residue was filtered and the filtrate was collected in the same 100 ml volumetric flask. Filtrate allowed to cool, then diluted to volume.High concentrations of metal salts with temperature-sensitive solubility can result in precipitates formation upon the cooling of the primary. If precipitation occurs in the flask upon cooling, volume was not diluted.

If precipitate forms on the bottom of a flask, badded up to 10 ml of concentrated HCl to dissolve the precipitate. After the residue are dissolved, diluted to

volume with reagent water, and analyzed by FLAA or ICP-AES. The concentrations determined are to be reported based on the actual weight of the sample.

3.10 Collection and CultureMethod for Cyanobacteria

The mother cultures of cyanobacteria (*Anabaena and Nostoc*) was collected from ICAR, New Delhi and then Mass production of cyanobacteria was performed on suitable culture medium i.e., BG-11 media (Allen and Stanier, 1968; Stanier*et al*., 1971).This medium supports growth of photoautotrophic blue green algae (*Nostoc* and *Anabaena*).

Table: 3.1 BG 11 Media Preparation for Culture of Cyanobacteria

S. No.	Ingredients	g/l
1.	Sodium Nitrate	1.500
2.	Di-potassium hydrogen phosphate	0.0314
3.	Magnesium sulphate	0.036
4.	Calcium chloride di-hydrate	0.0367
5.	Sodium carbonate	0.020
6.	Disodium magnesium EDTA	0.001
7.	Citric Acid	0.0056
8.	Ferric ammonium citrate	0.006
9.	Final pH after sterilization (at 25^0C)	7.1

1.627 grams prescribed ingredients are suspended in 1000 ml distilled water. The medium was heated to dissolve the medium. The pH was then adjusted to 7.1 by addition of 1M NaOH or HCl. Dispensed in flasks and sterilized by autoclaving at 121^0C for 15 minutes. The medium was cooled to room temperature.

3.11 Methods for Biochemical Analysis of Wheat

Quantitative Tests of Wheat (*Triticum aestivum*)

Wheat samples were taken for spectrophotometric studies. Whole plant was taken for sample analysis. Fresh plant material was used for analysis.Carbohydrate, Starch, Protein, Phenol and Chlorophyll content (Chlorophyll a, Chlorophyll b, and total) were analyzed. Fully expended fresh leaves plants were sampled (Stephen, 2000) randomly from each replicate pot for various biochemical analysis for further estimation of photosynthetic pigments, carbohydrate, starch, protein, phenol contents.

Estimation of Carbohydrates by Dubois Method (Dubois *et al*., 1956)

The total carbohydrate content includes all three types of carbohydrates: Sugar, Starch and Fiber. Sugars are easily digested into glucose or blood sugar.

In a hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has an absorption maximum at 490 nm.

100 mg of the sample was weighed into a boiling tube. It was hydrolyzed by keeping it in a boiling water bath for 3 hours with 5 mL of 2.5 N-HCl and cooled to room temperature. It neutralized with solid sodium carbonate until the effervescence ceases.

The volume was made up to 100 mL and Centrifuged. 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard pipetted out into a series of test tubes. 0.1 and 0.2 mL of the sample solution was pipetted out in two separate test tubes.The volume was made up of each tube to 1mL with water and a blank with 1mL of water was set. 1mL of phenol solution was added to each tube. After that, 5 mL of 96% sulphuric acid was added to each tube and shaken well.After 10 min, the tubes' content was shaken and placed it in a water bath at 25-30°C for 20 min. The colour was read at 490 nm. The

amount of total carbohydrate present in the sample solution was calculated using the standard graph.

Dubois Method is employed for establishing the relationship between concentration (glucose in this case) and Optical Density (OD) of this solution. Here, the concentration of a standard glucose solution is used with varying concentration to get the relationship.This relationship can then calculated for the unknown concentration of carbohydrates; once the OD of that solution is known.

The approximate relation that can be used is:

$$y = 0.0299x + 0.0259$$

Where, y: OD

x: concentration of carbohydrate solution

Absorbance corresponds to 0.1mL of the test = 'x' mg of glucose

Estimation of Starch by Anthrone Reagent Method (Hansen and Moller, 1975)

Starch is an important polysaccharide. It is the storage form of carbohydrate in plants abundantly found in roots, tubers, stems, fruits, and cereals.In wheat most of the carbs present in the form of starch .Starch, which is composed of several glucose molecules, is a mixture of two types of components, namely amylose and amylopectin.

The sample is treated with 80% alcohol to remove sugars and then starch is extracted with perchloric acid. In a hot acidic medium, starch is hydrolyzed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green coloured product with anthrone.

0.1 to 0.5 g of the sample was homogenized in hot 80% ethanol to remove sugars. Then it was centrifuged and the residue was retained. This residue was

repeatedly washed by hot 80% ethanol till the washing did not give colour with anthrone reagent.

The residue dried well over a water bath.To the residue, 5.0 mL of water and 6.5 mL of 52% perchloric acid were added. Extracted at 0°C for 20 minute. After then, they were centrifuged and the supernatant was saved and the extraction was repeated using fresh perchloric acid. It was centrifuged and the supernatant was pooled and made up to 100 mL. 0.1 ml of the supernatant was pipetted out and the volume made up to 1 mL with water.

The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1mL in each tube with water and 4 mL of anthrone reagent added to each tube, then heated, then for eight minutes in a boiling water bath, after that cooled rapidly and the intensity of green to dark green colour was read at 630 nm.

As seen the Anthrone-Sulphuric Acid method is employed for establishing the relationship between concentration (glucose in this case) and Optical Density (OD) of this solution. Here, the concentration of a standard glucose solution is used with varying concentrations to get the relationship.

This relation can then be used to calculate unknown concentration of Starch once the OD of that solution is known.

The approximate relation that can be used is:

$$y = 0.0294x + 0.0445$$

Where, y: OD

x: concentration of the starch solution

The glucose content in the sample was determined using the standard graph. Multiply the value by a factor of 0.9 to arrive at the starch content.

Estimation of Protein by Lowry Method (Lowry *et al*., 1951)

A protein is a naturally occurring, extremely complex substance that consists of amino acid residues joined by peptide bonds. Proteins are present in all living organisms and include many essential biological compounds such as enzymes, hormones and antibodies.

It is the most commonly used method for the determination of proteins in cell-free extracts because of its high sensitivity and quantities as low as 20 picogram Proteins can be measured. The peptide bond in polypeptide chain reacts with copper sulphate in an alkaline medium to a blue coloured complex.

In addition, tyrosine and tryptophan residues of proteins cause a reduction of the phosphomolybdate and phosphotungstate components of the Folin-ciocalteu reagent to give bluish products, which contribute towards enhancing the sensitivity of this method.

It is, however, important to remember that several compounds like EDTA, Tris. Carbohydrates, N, K, Mg ions, thiol reagents, phenols etc. interfere with the colour development and it should be ensured that such substances are not present in sample preparations.

Sample extract: - 1 g sample was macerated in pestle mortar in 5 ml of phosphate buffer and transferred to centrifuge tubes. The centrifuged homogenated at 8000 rpm for 20 min. The supernatant was collected and repeated the extraction of 4-5 times. The supernatants combined and made the volume to 50 ml with phosphate buffer. 1 ml of the above extract was taken and 1 ml of 20% TCA was added. It was kept for half an hour and centrifuged at 8000 rpm for 20 min. The pellet was washed with acetone twice and again centrifuged. The supernatant was discarded. The pellet was dissolved in 5 ml of 0.1 N NaOH and mixed well till it gets dissolved.

1 ml of the above solution was taken and 5 ml of freshly prepared alkaline copper sulphate reagent was added. After then it was mixed correctly and after 10 min, 0.5 ml of Follin's reagents was added. The content was mixed instantaneously and dissolved. The colour developed for 30 min.

The absorbance was recorded at 660 nm after setting the instrument with reagent blank, containing 1 ml of 0.1 N NaOH instead of the sample aliquot.

In another set of tubes, suitable aliquots of BSA solution dissolved (in a range of 10 pg/ml). The total volume to 1 ml with 0.1 N NaOH and developed the colour and a standard curve of absorbance at 660 nm versus BSA was created.

Lowry's method is employed for establishing the relationship between concentration (BSA) and Optical Density (OD) of this solution.

This relationship can then be used to calculate the unknown concentration of Protein once the OD of that solution is known.

The approximate relation that can be used is:

$$y = 0.0145x + 0.0425$$

Where, y: OD

x: concentration of Protein solution

From the standard curve the amount of protein was determined in the sample tubes and the amount of protein was calculated per µg/g of the sample.

Estimation of Phenol (Malik & Singh, 1980)

Analysis of phenol with the folin-ciocalteu reagent is based on the reaction between phenol and an oxidizing agent phosphomolybdate, which results in the formation of a blue complex. The intensity of the colour is measured in a spectrophotometer.

1 ml of the extract was taken into a graduated test tube.1 ml of folin- ciocalteu reagent added, followed by 2 ml of Na_2CO_3 solution.After that, the test tube was shaken and placed in a boiling water bath for exactly 1 min and cooled under running tap water. The blue solution to 25 ml with water was diluted and its absorbance of 650 nm was measured in the spectrophotometer.

Method of Mallick & Singh is employed for establishing the relationship between concentration (Catechol) and Optical Density (OD) of this solution. Here, the concentration of a standard Catechol solution is used with varying concentrations to get the relationship.

This relationship can be used for calculating unknown concentration of Phenol; once the OD of that solution is known.

The approximate relation that can be used is:

$$y = 0.0301x + 0.006$$

Where, y: OD

x: concentration of Catechol

When sediment occurs, the solution was filtered and centrifuged before measuring its absorbance and the phenol was calculated in the sample from a standard curve prepared with catechol.

Estimation of Chlorophyll (Arnon, 1949)

The Chlorophyll content was estimated according to the method of Arnon (1949). About 1 g of leaf sample was cut into small pieces and homogenized in a pre-cooled mortar and pestle using 80% (V/V) acetone. A pinch of calcium carbonate was added while grinding. The extract was centrifuged at 3000 rpm for 15 min and made up to 25 ml, with 80% (V/V) acetone. Chlorophyll estimation was done by reading optical density at 645 nm and 663 nm on the spectrophotometer.

The amounts of different pigments were determined by the equation of Arnon (1949). The levels of Chlorophyll 'a' and Chlorophyll 'b' were determined using the equation given below:

Chlorophyll 'a' (μ/g/ml) = (12.7 x O.D. at 663 nm) – (2.69 x O.D. at 645 nm)

Chlorophyll 'b' (μ/g/ml) = (22.9 x O.D. at 645 nm) - (4.08 x O.D. at 663 nm)

Total Chlorophyll (μ/g/ml) = (20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm)

The Chlorophyll content was expressed as mg Chlorophyll in per gram of sample.

SCHEMATIC PRESENTATION OF THE STUDY

Study on use of fly ash mixed with cyanobacteria as Biofertilizer in Wheat

Collection of soil from Murba 22 Chak Thakrawali Sriganganagar

⇩

Process of sample collection by quartering method

⇩

Physicochemical analysis of Soil

Collection of fly ash from Suratgarh Thermal Power Station

⇩

Collection of fly ash

⇩

Physicochemical analysis of fly ash

Collection of Cyanobacteria (*Nostoc*&*Anabaena*) from ICAR New Delhi

⇩

Preparation of BG -11 Culture Media & transfer of sample in it

⇩

In-Vitro Culture of Cyanobacteria on BG11media

⇩

Biofertilization Preparation by mixing Soil + Fly ash + Cyanobacteria

⇩

Optimization of concentration of fly ash and Cyanobacteria mixed with soil (as Biofertilizer) for Wheat Growth

⇩

First Experiment: Wheat Plant Pot Culture with Soil + fly ash (0% - 20%

⇩

Second Experiment: Wheat Plant Pot Culture with 12% fly ash + *Anabaena* (10% - 100%)

⇩

Third Experiment: Wheat Plant Pot Culture with 12% fly ash + *Nostoc* (10% - 100%)

⇩

Fourth Experiment: Wheat Plant Pot Culture with 12% fly ash + *Anabaena* 40% + *Nostoc* (10% - 100%)

⇩

Fifth Experiment: Wheat Plant Pot Culture with12% fly ash+ *Anabaena* (10-100 %) + *Nostoc* 60%

⇩

Biochemical Analysis of Wheat Plants for Carbohydrate, Starch, Protein, Phenol, Chl a, Chl b, Total Chlorophyll contents

⇩

Data Collection

⇩

Data Analysis

⇩

Results

CHAPTER 4
BIOFERTILIZER PREPARATION & POT CULTURE

4.1 Collection of Soil

Collection of Soil Samples

Soil sampling is the most challenging task, as a few grams of soil sample represent a given area. Thus, the soil sample is taken such that the collected sample reflects the true fertility of the soil of the area. The soil samples were collected from suitable fields from Murba 22chak Thakrawali, Sriganganagar from separate sites. The composite sample was prepared from these sets. Recently fertilized plots, channels, marshy tracts and areas near trees, wells, cattle dung and compost piles or other non-representative locations were avoided during the sampling (Fig.4.1 a-d).

Sampling Tools

The equipments like spade/khurpi, auger (tube and screw type), plastic bucket, plastic bag, scale and waterproof marker were used for soil sampling.

Sampling Depth

In most of the field crops, the root growth is confined to 10-20 cm depth and hence the sampling was done up to 15-20 cm for field crops.

Time of Soil Sampling

Soil samples were collected well before sowing of the targeted crops so that the soil can be tested in time.

Methods of Soil Sampling

The soil samples were taken in a zigzag manner to cover the entire field. At least 25 sub-samples were taken randomly and mixed to make a representative sample from a uniform field. The sampling spot from where the sample was collected was

cleaned with a spade. Then a 'V' shaped cut was made below the plow layer (0-15 cm) and then a uniform 1.5 cm thick slice of soil was taken out with a spade.

Collected soil samples were thoroughly mixed on a clean polythene sheet and the bulk was reduced by the quartering so that about 1kg of composite sample was retained and kept in a clean polythene bag. The sample bags were cleaned and fixed properly to avoid any mix-ups during processing.

Process of sample size quartering method

The sample was divided into four parts by drawing a '+' sign through it and the soil of the opposite corners was discarded. Remaining soil was mixed and divided into four parts and again it was separated from corners and then mixed (www.agritech.tnau.ac.in) (Figure 4.1 c, d).

Sample Soil Preparation

The sample was spread out on a plastic or a thick brown paper in the shade for drying as the wet soil samples collected from the field cannot be stored as changes occur with the time in storage condition. The soil was air-dried at 20-25 ^{o}C and 20-60% relative humidity (Jackson, 1958). Coarse concretions, stones, pieces of roots, leaves and other under-composed organic residues were taken away. Large lumps of moist soil were broken by hand. After air-drying, soil samples were crushed gently with a wooden mortar &pestle and sieved through a 2 mm sieve.The particles or material larger than 2 mm were discarded.

Sample Storage

The collected soil samples were packed in polythene bags. These bags were properly tied, tagged with the sample label and were stored till they were analyzed.

Physicochemical Analysis of Soil

The analysis of soil was conducted for the detection of parameters like pH,

electrical conductivity, organic carbon, potassium, copper and manganese at CEG Test House & Research Centre, Jaipur, Rajasthan.

The testing of some heavy metals in the soil like cadmium, lead, zinc and nickel was performed at Soil Testing Laboratory, Department of Agriculture, Government of Rajasthan, Hanumangarh.

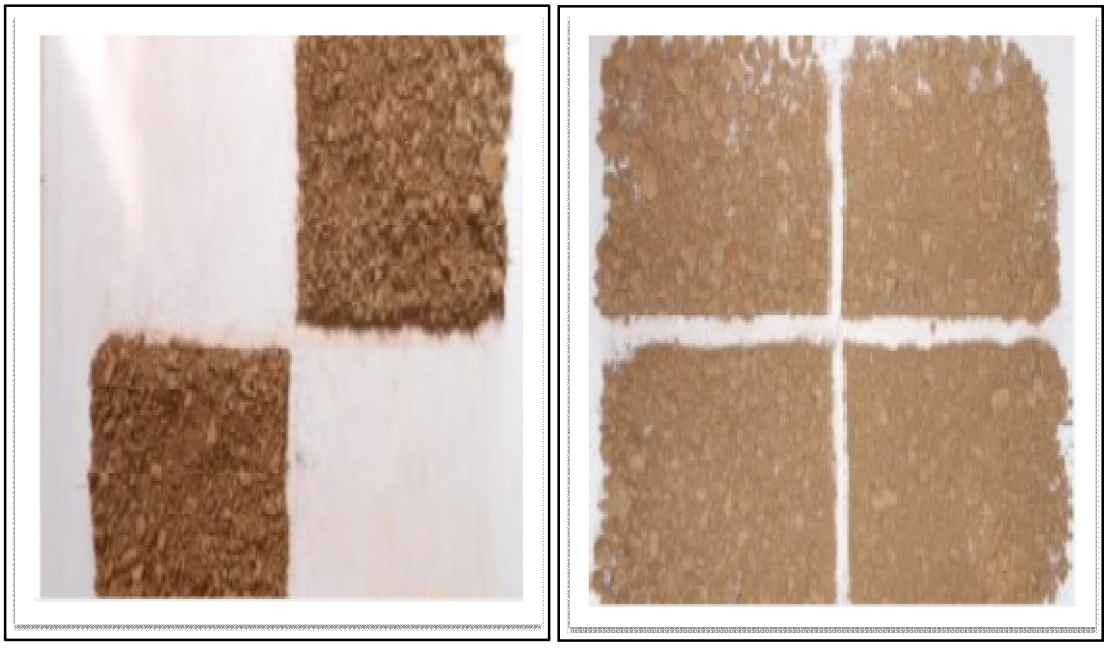
The soil was analyzed to detect the macro and microelement present in it (Table 4.1).

Table 4.1: Physicochemical Analysis of Soil

S. No.	Parameters (units)	Test Results
1.	pH (units)	8.31
2.	Electrical Conductivity (dS/m)	0.42
3.	Organic Carbon (%)	0.25
4.	Total Nitrogen (mg /kg)	0.04
5.	Sulphur (mg /kg)	9.98
6.	Potassium (mg /kg)	335.5
7.	Boron (mg /kg)	0.35
8.	# Cadmium (mg/kg)	*ND (DL 1.0)
9.	# Lead (mg/kg)	5.64
10.	# Zinc (mg/kg)	15.89
11.	# Nickel (mg/kg)	2.67
12.	#Copper (mg/kg)	0.37
13.	#Manganese (mg /kg)	2.54

*ND-Not Detected*DL-Detection Limit# Heavy metal Parameter 8-13

(a): Soil collection using spade **(b): Removal of foreign particles from soil sample**



(c): Quartering Method **(d): Two opposite quarters are discarded and & then it is mixed**

Figure 4.1: Collection of Soil

4.2 Collection of Fly ash

In Thermal Power Plants, coal is used as a fuel for generating electricity. Fly ash is a by-product material being generated by thermal power plants from the combustion of pulverized coal.After burning coal, 40% of total coal consumption is converted into fly ash, which is disposed-off from the thermal power plant.

Suratgarh Super Thermal Power Station is the oldest and first Super Thermal Plant of Rajasthan. It has an installed capacity of 1500 MW, which is the highest in the state. It is located 27 km from Suratgarh. The place has an extremely hot and cold climate and the temperature varies between 1ºC to 50 ºC (Fig 4.2 a, b, c).

Mainly the four types of ash are generated in thermal power plants, (www.coalhandlingplants.com)

1. Economizer ash (1%)
2. Air preheater ash (1%)
3. Bottom ash (10-20%)
4. Fly ash (80-90%)

Economizer ash (1%) and Air pre heater ash (1%) are fuel gases. These gases are produced when coal is combusted in the boiler.The fuel gases after passing around boiler tubes and super heater tubes in the furnace pass through an economizer and finally through the air pre heater before being exhausted to the atmosphere via ESP and chimney.

The ash generated below the furnace of the thermal power plant is called the bottom ash. The value of the bottom ash generated is around 20% of the total ash. Bottom ash is mostly coarse in nature hence it needs to be crushed before being transported to the ash handling system.

Around, 80% of ash generated in thermal power plants is fly ash. It is in the form of very fine particles collected via an economizer hopper, air-preheater hopper and electrostatic precipitator (ESP) (Fig 4.2 d & e).

Ash Holding System in Thermal Power Plants

Ash holding system in Thermal Power Plants are used to cool down the ash to manageable temperature, transferred to disposal area or storage which is further utilized in other industries.Ash holding system is generally divided into three types: fly ash holding system, bottom ash holding system and ash slurry disposal system.

Fly ash is captured and removed from the flue gases by economizer, air preheater and electrostatic precipitator (ESP) located at the furnace's outlet and before the induced draft. The fly ash is pneumatically transported from the collection hopper of an economizer, air preheater and electrostatic precipitator (ESP) to storage for subsequent transport (www.coalhandlingplants.com) (Fig. 4.2 e).

Sample Storage

The fly ash samples were collected from the Suratgarh Thermal Power Plant and packed in polythene bags properly and tagged with the sample label till their analysis.

Physicochemical Analysis of Fly ash

The physical and chemical analysis of fly ash was carried out at CEG Test House and Research Center, Jaipur, Rajasthan by USEPA 3050B method to analyze the macro and microelements present in it. Physicochemical analysis of fly ash comprises of detection of parameters like pH, electrical conductivity, organic carbon, total nitrogen, sulphur, sodium, potassium, boron, copper, manganese and heavy metals like cadmium, lead, zinc and nickel.

Table 4.2: Physicochemical Analysis of Fly ash

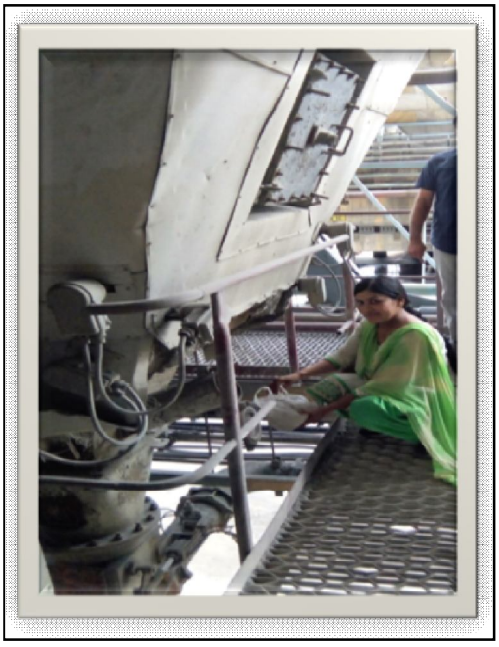
S. No.	Parameters (units)	Test Results
1.	pH (units)	6.31
2.	Electrical Conductivity (ds/m)	.71
3.	Organic Carbon (%)	0.12
4.	Total Nitrogen(mg/kg)	0.03
5.	Sulphur (S)(mg/kg)	1.63
6.	Potassium (K)(mg/kg)	0.048
7.	Boron (B)(mg/kg)	35.6
8.	# Cadmium (Cd)(mg/kg)	ND (DL 1.0)
9.	# Lead (Pb)(mg/kg)	9.51
10.	# Zinc (Zn)(mg/kg)	109.59
11.	# Nickel (Ni)(mg/kg)	38.34
12.	# Copper (Cu)(mg/kg)	37.37
13.	# Manganese (Mn)(mg/kg)	216.56

*ND-Not Detected

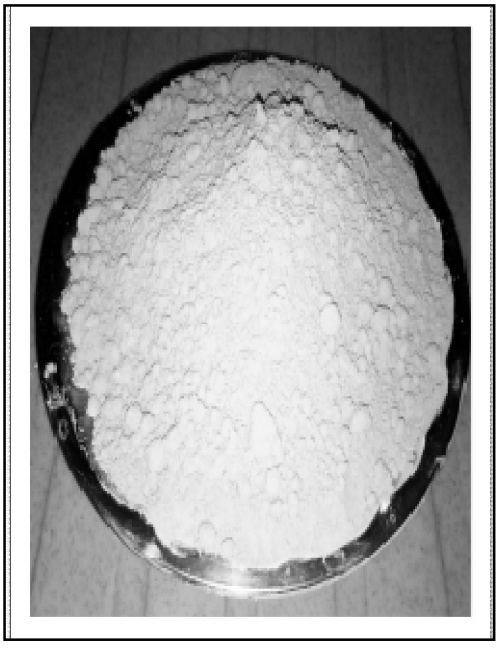
*DL-Detection Limit

Heavy metal Parameter 8-13

(a): Suratgarh Super Thermal Power Station



(b): Collection of Fly ash



(c): Fly ash

Figure 4.2: Collection of Fly ash from Suratgarh Thermal Power Station

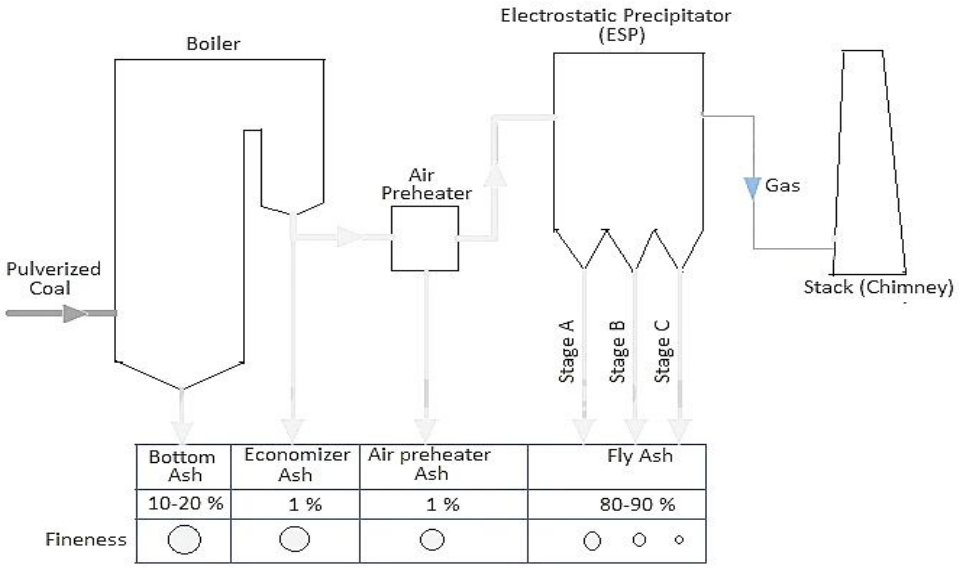


Figure 4.2 d: Types of Ash Generated in Thermal Power Plant
(www.coalhandlingplants.com)

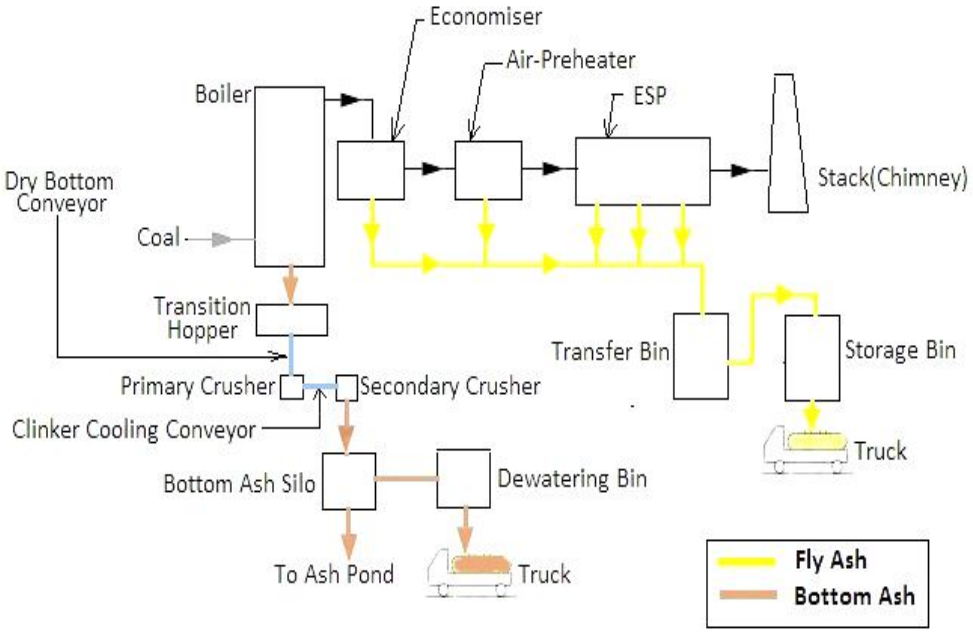


Figure 4.2 e: Working of Fly ash Holding System in Thermal Plant
(www.coalhandlingplants.com)

4.3 Collection of Cyanobacteria and its *In-vitro* Culturing

Collection of Cyanobacteria (*Nostoc* and *Anabaena)*

The pure mother cultures of cyanobacteria, *Anabaena* and *Nostoc,* were collected from ICAR New Delhi and then their sub culturing was performed on BG-11 media (Fig 4.3 A (a-d).

***In-vitro* culturing of Cyanobacteria**

The collected pure culture samples were enriched initially in BG-11 medium in the conical flask at 24+2℃ under light intensity (3200 lux) and a photoperiod of 16:8 for ten days.The enriched culture samples were then spread on agar plates and incubated under the same conditions. After the incubation period time, the freshly grown culture was picked out and transferred to BG-11 for subculture. These culture flasks were shaken manually 3-4 times a day.These were further cultured in a liquid medium for the next process. Thus mass production of cyanobacteria was produced through the *In-Vitro* method (Fig.4.3B (e-h), (4.3C (i-k).

4.4 Biofertilizer Preparation

After the collection of soil samples, fly ash and culturing of cyanobacteria as per the requirement of the present research work, they were mixed in different proportions and a mixture was obtained to study suitable biofertilizerfor the growth of wheat plants.

Dry samples of soil and Fly ash were mixed accordingly i.e. control (0% fly ash), 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20% fly ash by weight. Range of fly ash set upto 20% as review of literature states that high amount of fly ash adversely affects the plant growth and good results are found on lower concentrations

(Singh and Singh, 1986). Fly ash was mixed with soil to make a homogeneous mixture.

The optimum concentration of fly ash and cyanobacteria in biofertilizer for the growth of the wheat plant was determined in further experiments (I-V).The experiments were conducted using different concentrations of fly ash mixed with cyanobacteria. The suitable combination was determined by the biochemical analysis of wheat plant in the potculture.

(a): ICAR New Delhi **(b): National Center for B Algae at ICAR, New Delhi**

(c): Mother culture of *Anabaena* **(d): Mother culture of *Nostoc***

Figure 4.3.A Collection &*In-vitro* Culturing of Cyanobacteria

(*Anabaena* &*Nostoc*)

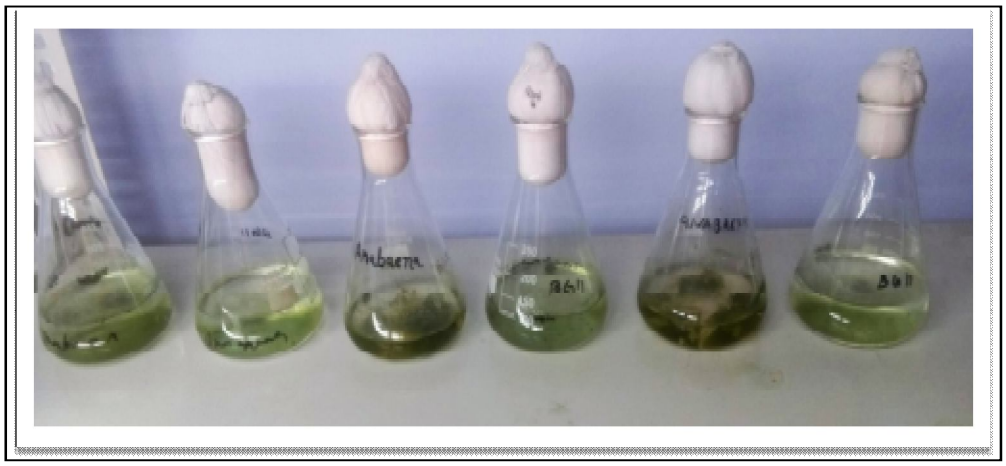
(e) Culture Media BG-11

(f): Transferring the Mother Cultures of *Anabaena* &*Nostoc* in BG-11 Media

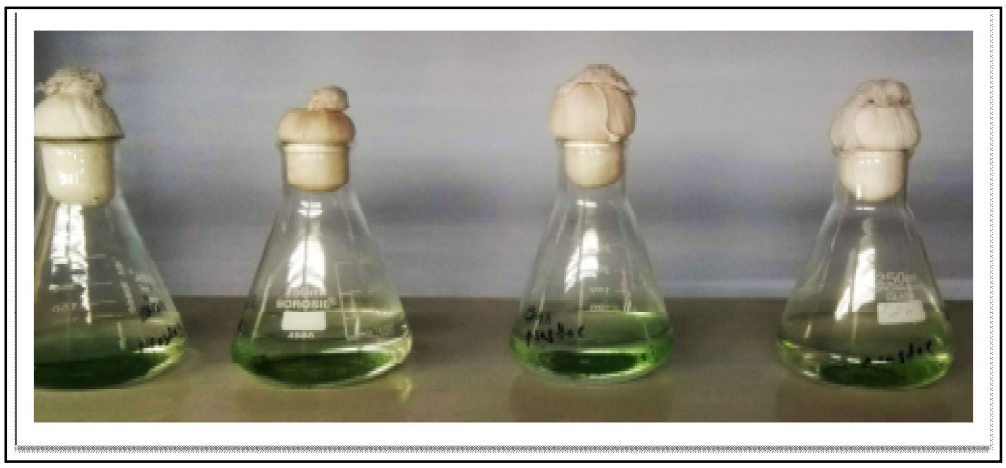
(g) *In-vitro* culture of *Anabaena*

(h) *In-vitro* culture of *Nostoc*

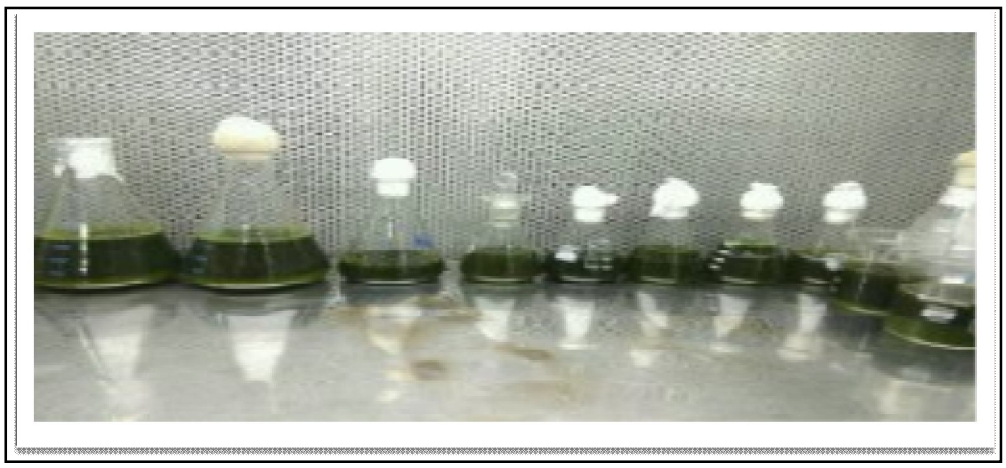
Figure 4.3.BCollection &*In-vitro* Culturing of Cyanobacteria (*Anabaena* &*Nostoc*)



(i): Cultures of *Anabaena*



(j): Cultures of *Nostoc*



(k): Scaling up of *In-vitro* culture of cyanobacteria

Figure 4.3.C Collection&*In-vitro* Culturing of Cyanobacteria (*Anabaena* &*Nostoc*)

4.5 Pot Culture Experiments

A series of pot culture experiments of wheat was set up using fly ash and cyanobacteria mixed as a biofertilizer.As wheat is a Rabi crop, so the pot culture experiment was conducted from November to January during Rabi season of the years 2017-19.After proper sterilization of wheat seeds with Mercuric chloride (Rebecca, 2012), these seeds were thoroughly washed with sterile distilled water and used for sowing in the pot.

A set of 5 experiments was performed to study the optimum concentration of Biofertilizer (fly ash mixed with cyanobacteria) and their effect on growth of wheat plant.Each set of pot culture experiment was performed in replica of five. So, two hundred and fifty-five pots were used to setup these experiments.

Table 4.3: Design of Pot Culture Experiments

S. No.	Experiments (combinations)	Number of Pots
1.	Experiment I (Soil + Fly ash (0% - 20%)	10 × 5 +5 (control) = 55
2.	Experiment II (Soil + 12% Fly ash + *Anabaena* (10% - 100%)	10 × 5 = 50
3.	Experiment III (Soil + 12% Fly ash + *Nostoc* (10% - 100%)	10 × 5 = 50
4.	Experiment IV (Soil + 12%Fly ash + *Nostoc* 60% + *Anabaena* (10% - 100%)	10 × 5 = 50
5.	Experiment V (Soil+ 12%Fly ash + *Anabaena* 40% + *Nostoc* (10% - 100%)	10 × 5 = 50

Experiment I

The first experiment was conducted to find out the effects of different concentrations of fly ash on wheat plant. Fifty-five pots were used for this experiment.Different concentrations of fly ash (control 0% , 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20%) with soil were maintained respectively in the experimental pots (Fig. 4.4 a, b). For each concentration, five replicate pots were prepared.To each concentration in pot, seeds of wheat were sown. In order to avoid the crowding effect, the seeds were planted almost equal distance in-between. Each pot was irrigated with normal water every day (Fig.4.4 c- e).

After performing experiments, wheat samples collected from pots of different concentrations of fly ash (control 0%, 2%, 4%, 6%, 8%, 10%,12%, 14%, 16%, 18% and20%) and then biochemically analyzed for Carbohydrate, Starch, Protein, Phenol and Chlorophyll contents.A combination of 12% of fly ash and soil was found good for the growth of wheat plants. In further experiments the 12% of fly ash was used with different combinations of cyanobacteria.

Experiment II

In second experiment, fifty pots with 12% Fly ash + soil were set, as 12% fly ash found optimum in experiment I. To each concentration the seeds of wheat were sown in the pots.After 5 days of sowing, different concentrations of *Anabaena* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) was added and these were maintained respectively in each experimental pot.Each pot was irrigated with normal water; it was also helpful for maintaining moisture for the growth of *Anabaena* (Figure 4.5 a-c).

After performing experiments, wheat samples were collected from pots (12% Fly ash + *Anabaena* (10% - 100%) which were then biochemically analyzed for Carbohydrate, Starch, Protein, Phenol and Chlorophyll contents

Experiment III

In this experiment, again fifty pots with 12% fly ash + soil were prepared and wheat seeds were sown.In Experiment III, different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) were maintained with 12% fly ash respectively in the each experimental pot (Figure 4.6 a-c).Everyday normal water was irrigated to each pot that was also helpful for maintaining moisture for the growth of *Nostoc.*

After the experiment, the samples of wheat collected from pots (with 12% fly ash + *Nostoc* (10% - 100%) and analyzed for biochemical constituents i.e. Carbohydrate, Starch, Protein, Phenol and Chlorophyll contents.

Experiment IV

For this experiment, fifty pots were used with 12% fly ash + soil. Seeds of wheat were planted. After sowing (5 days), different concentrations of *Anabaena* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) with control *Nostoc* 60% were maintained in each experimental pot respectively as in experiment III 60% *Nostoc* sample was found optimum.For each concentration, five replicate pots were kept. Normal water was irrigated to each pot every day, which was also helpful for maintaining moisture to the growth of Cyanobacteria (Figure 4.7 a-c).

After performing the experiment, samples of wheat were collected from pots (12% Fly ash + *Nostoc* 60% + *Anabaena* (10% - 100%) and were analyzed to study their Carbohydrate, Starch, Protein, Phenol and Chlorophyll contents.

Experiment V

For this experiment, fifty pots were used with 12% fly ash + soil. Seeds of wheat were planted.After sowing (5 days), different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) with Anabaena 40% (used as control) were maintained respectively in each experimental pot as in experiment II 40% *Anabaena* samplewas found optimum.For each concentration, five replicate pots were there. Each pot was irrigated everyday with normal water that was also helpful for maintaining moisture to the growth of *Anabaena* and *Nostoc.*

After performing the experiment, samples of wheat collected from pots (12% Fly ash + *Anabaena* 40% + *Nostoc* (10% - 100%) and analyzed for their biochemical constituents i.e. Carbohydrate, Starch, Protein, Phenol and Chlorophyll contents (Figure 4.8 a-c).

(a): 55 Pots with Normal Soil (5) +Soil mixed with fly ash 2%- 20% (50)

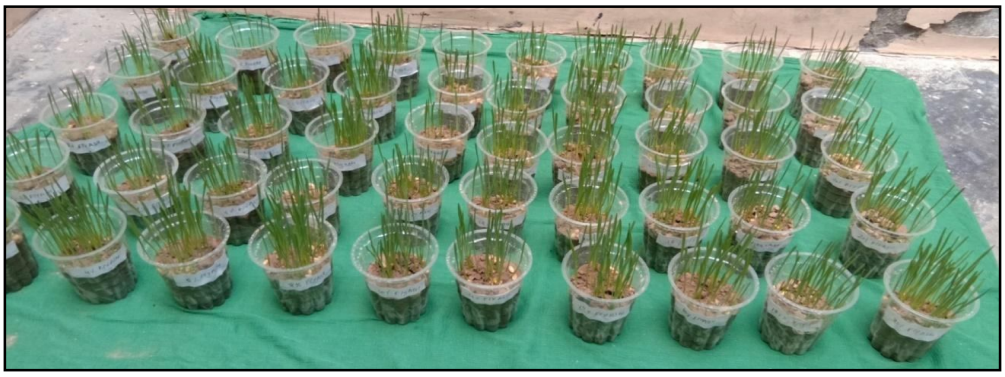
(b): Sowing of wheat seeds in different concentrations of fly ash

(control 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%)

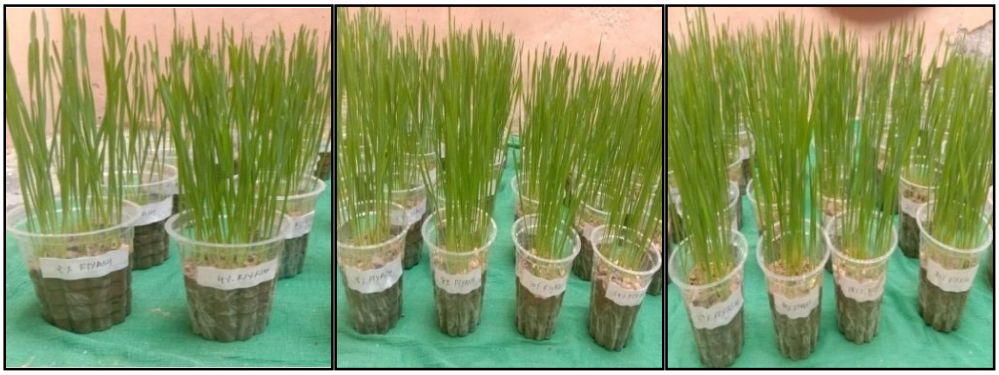
Figure 4.4A: Experiment I

(Soil + different concentrations of fly ash 0%- 20 %)

(c): After 5 Days of sowing wheat in different concentrations of fly ash (0%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%)



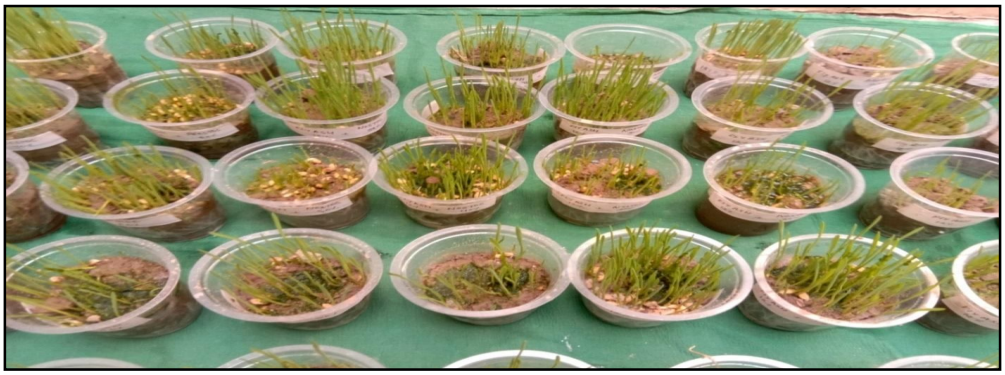
(d): After 10 Days of sowing wheat in different concentrations of fly ash (0%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%)



(e): After 15 Days of sowing wheat in different concentrations of fly ash (0%, 4%, 6%, 8%10%, 12%, 14%, 16%, 18%, 20%)

Figure 4.4 B: Experiment I

(Soil + different concentrations of fly ash 0% - 20 %)



(a): After 5 days of sowing wheat in different concentrations of *Anabaena* (10%, 20%, 30%, 40%, 50%, 60%,70%, 80%, 90% & 100%)

(b): After 10 days of sowing wheat in different concentrations of *Anabaena* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% & 100%)

(c): After 15 days of sowing wheat in different concentrations of *Anabaena*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% &100%)

Figure 4.5: Experiment II

(Soil + 12% Fly ash + different concentrations of *Anabaena* 10% - 100%)

(a): After 5 days of sowing wheat in different concentrations of *Nostoc*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% & 100%)

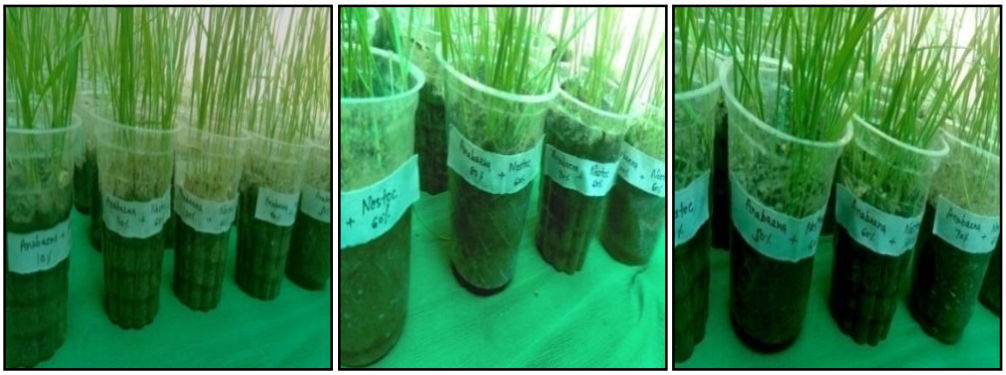
(b): After 10 days of sowing wheat in different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%& 100%)

(c): After 15 days of sowing wheat with different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% & 100%)

Figure 4.6: Experiment III

(Soil + 12% Fly ash + different concentrations of *Nostoc* 10% - 100%)

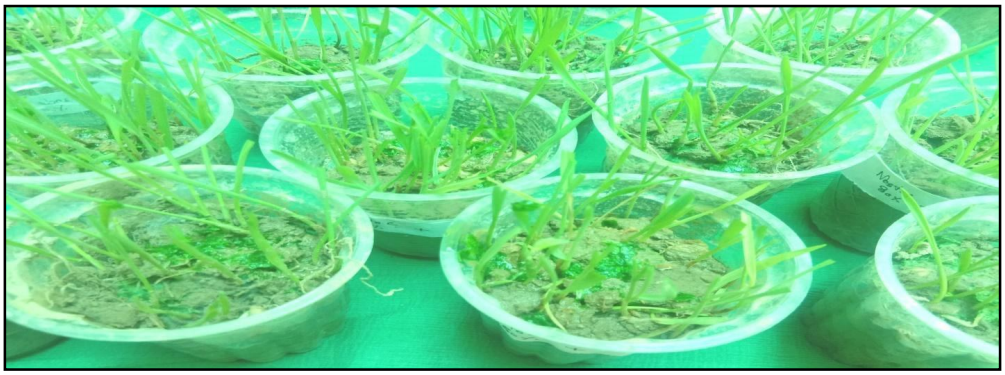
(a): After 5 days of sowing wheat in different concentrations of *Anabaena*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,100%) + *Nostoc* 60%



(b): After 10 days of sowing wheat in different concentrations of *Anabaena*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) + *Nostoc* 60%

(c): After 15 days of sowing wheat in different concentrations of *Anabaena*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) + *Nostoc* 60%

Figure 4.7: Experiment IV
(Soil with 12% Fly ash + different concentrations *Anabaena* 10% - 100% + *Nostoc* 60%)



(a): After 5 days of sowing wheat in different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,100%) + *Anabaena* 40%

(b): After 10 days of sowing wheat in different concentrations of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) + *Anabaena* 40%

(c): After 15 days of sowing wheat in different concentrations of *Nostoc*(10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) + *Anabaena* 40%

Figure 4.8: Experiment V
(Soil with 12% fly ash + different concentrations of *Nostoc* 10% - 100% + *Anabaena* 40%)

CHAPTER 5
BIOCHEMICAL ANALYSIS OF WHEAT

In present study, after the sowing of wheat seeds in different combinations of biofertilizers, the seeds germinate into plantlets. The samples of wheat were collected and their quantitative analysis was performed. Collected wheat samples of all the five experiments were analyzed for their biochemical constituents i.e. Carbohydrate, Starch, Protein, Phenol & Chlorophyll (a, b and total).In the first experiment, the control was normal soil. Later, in the subsequent experiments, this control (normal soil) was changed to the soil mixed with 12% fly ash (as it showed maximum concentrations for the photosynthetic pigments and biomolecules). This changed Control was then used with varying proportions of cyanobacteria to performed further experiments of pot cultures. Then collected samples from these were used to estimate the concentration of photosynthetic pigments and biomolecules.

For each experiment all the biochemical tests were performed and Carbohydrate, Starch, Protein, Phenol and Chlorophyll (a, b & total) pigments were analyzed. Here each experiment is discussed in reference of each parameter.

5.1 Experiment I

(Soil + different concentrations of Fly ash 2% - 20%)

The experiment I was performed with the wheat samples grown in different concentration of fly ash mixed with soil. The sample of the normal soil was taken as Control. The wheat samples were then sowed in the soil mixed with varying percentage of fly ash (2%-20%) and then the concentration of Carbohydrate, Starch, Protein, Phenol and Chlorophyll (a, b and total) pigments was measured in wheat plants.

Carbohydrate estimation

Estimation of Carbohydrates (Total Soluble Sugar) was performed by method suggested by Dubois *et al*., (1956). This method is employed for establishing the relationship between concentration (glucose) and Optical Density (OD). Here, the concentration of a standard glucose solution was used with varying concentration to get the relationship.The relation obtained from the standard glucose was used to estimate the unknown concentration. It was observed that the peak concentration of total carbohydrates was maximum in the sample of the 'soil + 12% fly ash' (Fig. 5.1 a). It is now important as this optimum concentration of fly ash was used as 'control' for further experiments.

Starch estimation

Estimation of Starch was performed by Anthrone-Sulphuric Acid (Hansen and Moller, 1975) method. This method is employed for establishing the relationship between concentration (glucose) and Optical Density (OD). Here, the concentration of a standard glucose solution was used with varying concentration to get the relationship. Then concentration of starch was measured. The relation obtained from the standard glucose is used to estimate the unknown concentration.It was observed that the peak concentration of total starch was in the sample of the 'soil + 12% fly ash combination (Fig. 5.1 b). It was used as 'control' for further experiments, as found optimum.

Protein estimation

Estimation of Protein was conducted by Lowry *et al*., (1951) method. This method is employed for establishing the relationship between concentration (BSA) and Optical Density (OD). Here, the concentration of a Standard BSA solution was used with varying concentration to get the relationship and then concentration was

measured.The relation obtained from the standard BSA was used to estimate the unknown concentration. The total protein was maximum in the sample of the 'Soil + 12% fly ash' (Fig. 5.1 c). As it is found to be good for protein and was used as control for further experiments.

Phenol estimation

Estimation of Phenol was performed by method of Malik & Singh (1980). This method is employed for establishing the relationship between concentration (catechol in this case) and Optical Density (OD). Here, the concentration of a standard catechol solution was used with varying concentration to get the relationship and then concentration of phenol was measured**.**The relation obtained from the standard catechol was used to estimate the unknown concentration. The maximum concentration of total phenol was observed in the sample of the 'Soil + 12% fly ash' (Fig.5.1 d). It showed highest phenol and was used as control for further experiments.

Chlorophyll estimation

Estimation of Chlorophyll was performed by Arnon, (1949) method with soil. This method is employed for establishing the relationship between concentration (acetone) and Optical Density (OD). Here, the optical density of pigments was observed at 645nm and 663nm. The amount of Chlorophyll a, Chlorophyll b and Total Chlorophyll were calculated with the help of equation of Arnon, (1949).The maximum concentration of Chlorophyll was observed in the sample of the 'Soil + 12% fly ash (Fig. 5.1e A, B, C). It showed highest Chlorophyll and was used as control for further experiments, as found optimum.

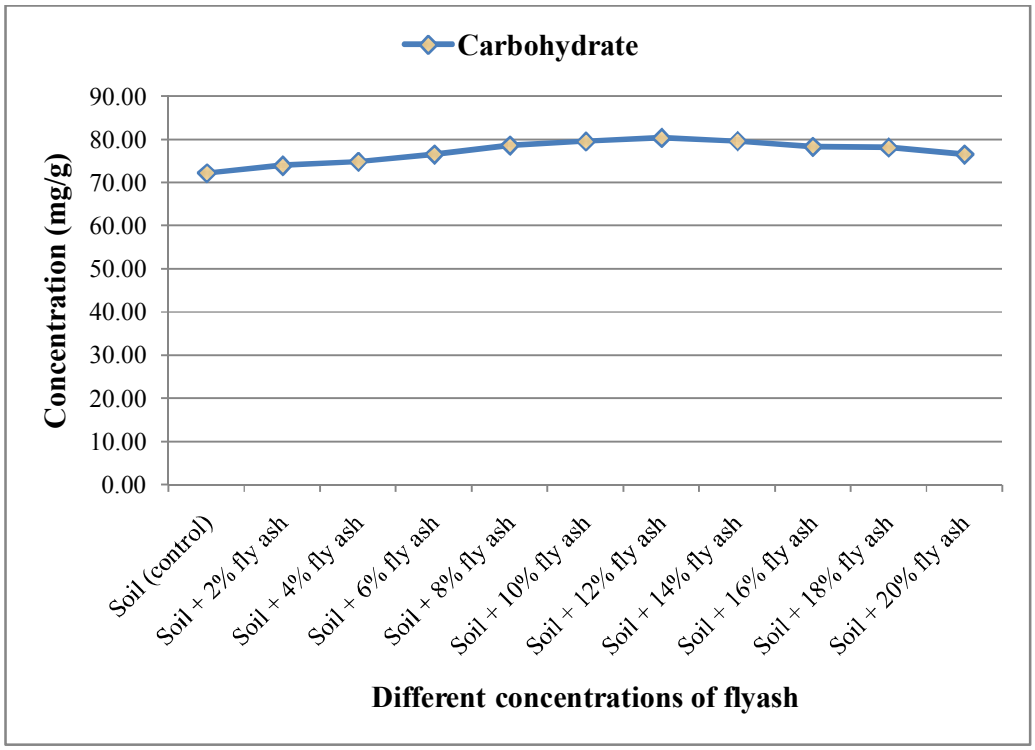


Figure: 5.1 (a) Estimation of Carbohydrates (Total Soluble Sugar) in wheat grown with different concentrations of fly ash (0%-20%) mixed with soil

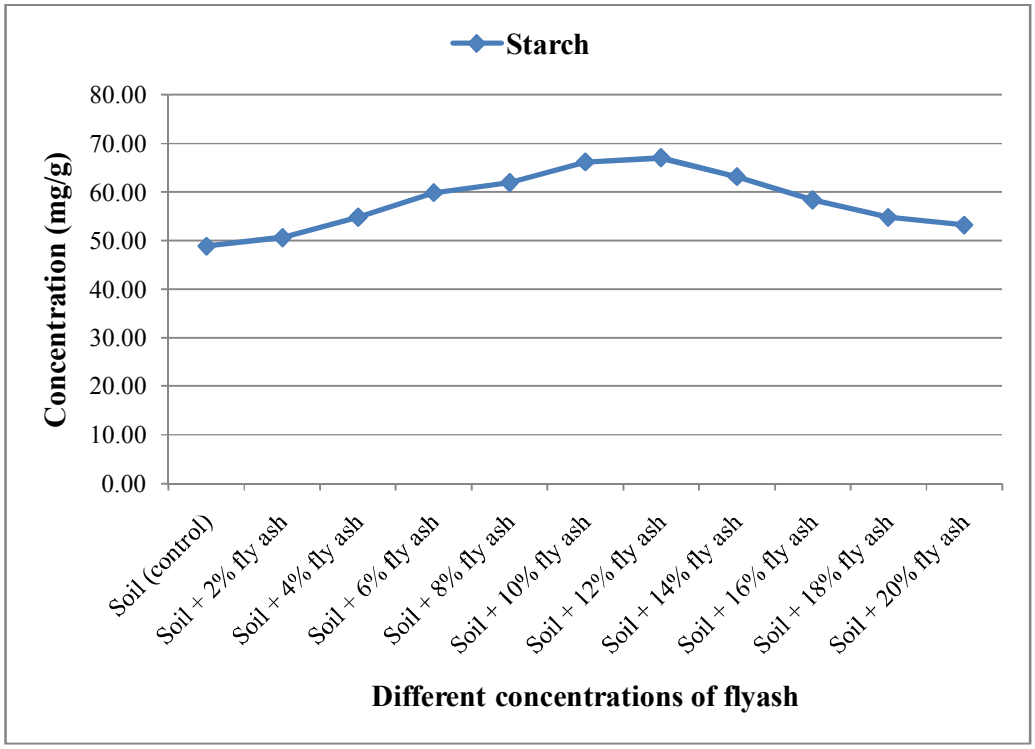


Figure: 5.1 (b) Estimation of Starch in wheat grown withdifferent concentrations of fly ash (0%-20%) mixed with soil

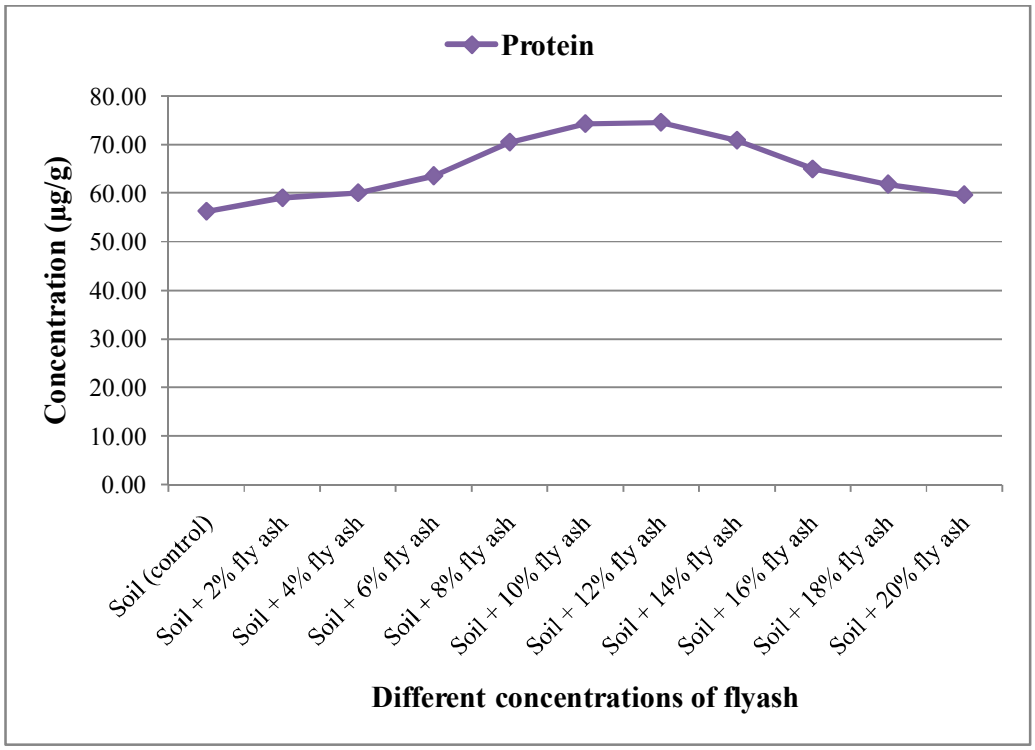


Figure: 5.1 (c) Estimation of Protein in wheat grownwith different concentrations of fly ash (0%-20%) mixed with soil

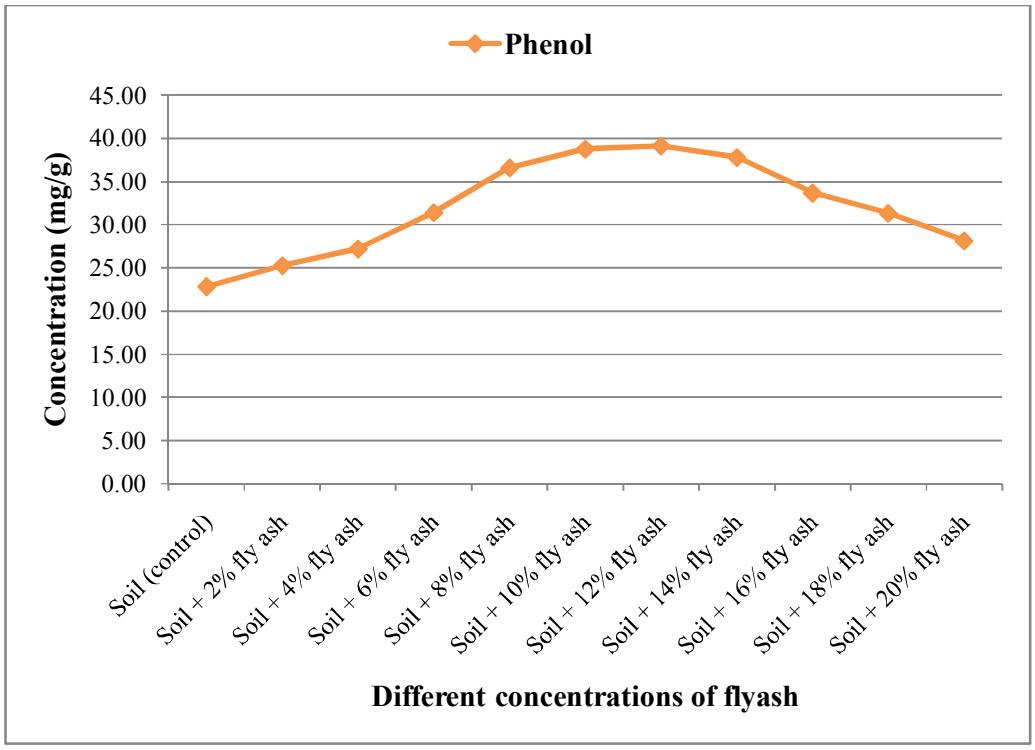


Figure: 5.1 (d) Estimation of Phenolin wheat grownwith different concentrations of fly ash (0%-20%) mixed with soil

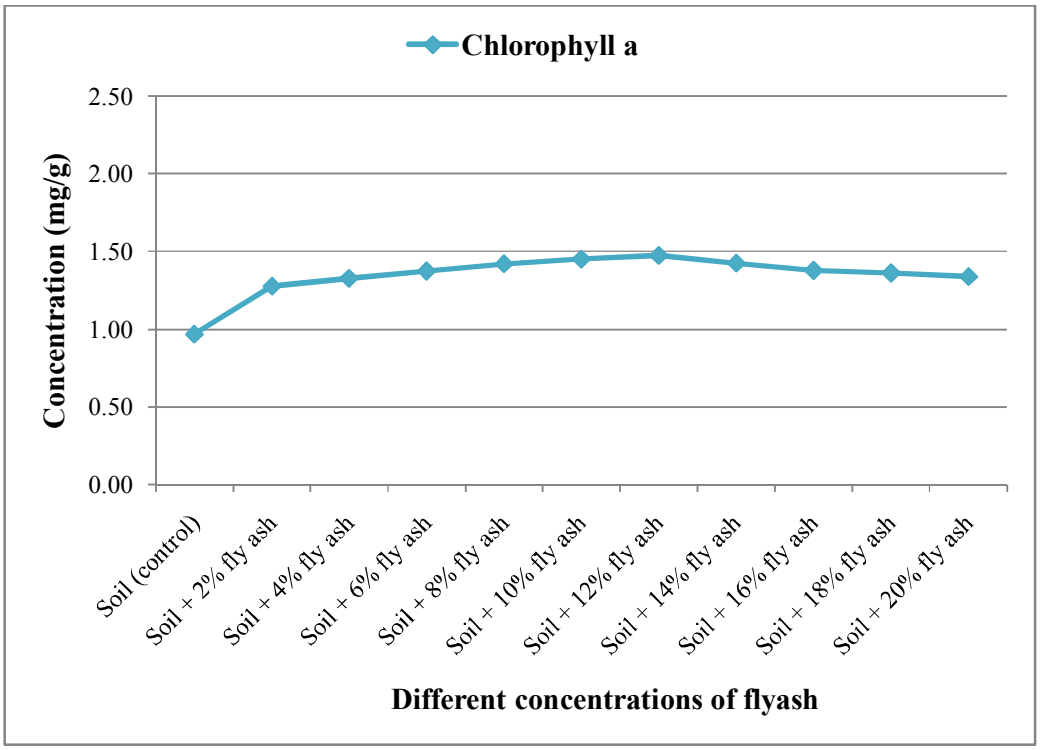


Figure: 5.1e (A) Estimation of Chlorophyll ain wheat grownwith different concentrations of fly ash (0%-20%) mixed with soil

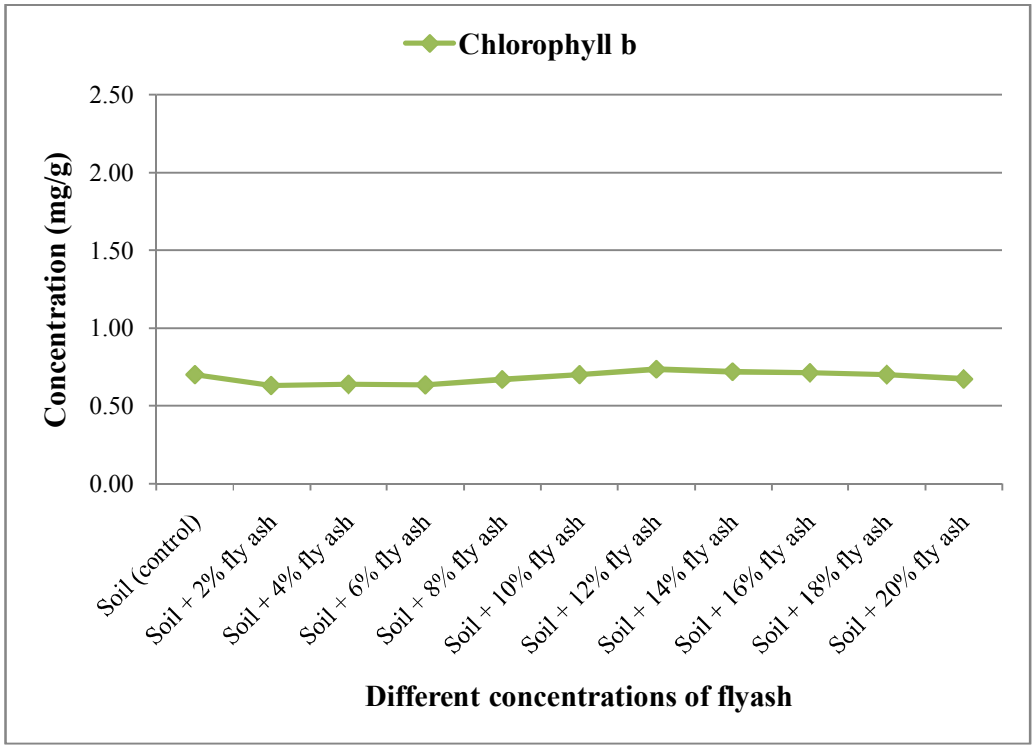


Figure: 5.1e (B) Estimation of Chlorophyll bin wheat grownwith different concentrations of fly ash (0%-20%) mixed with soil

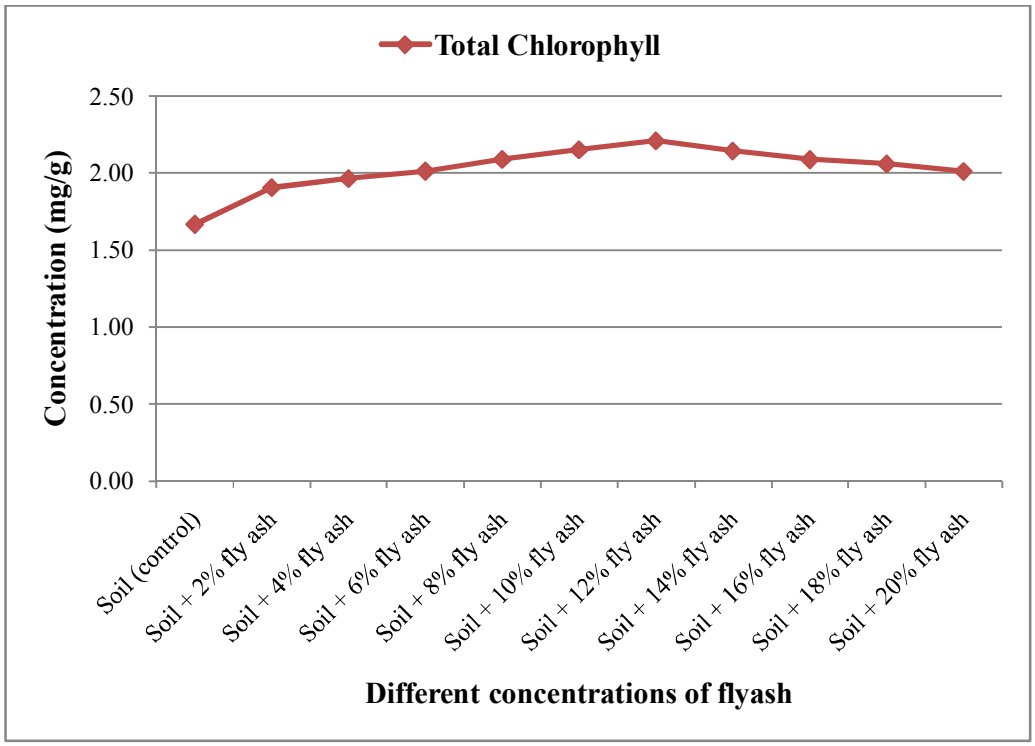


Figure: 5.1e (C) Estimation of Total Chlorophyll in wheat grownwith different concentrations of fly ash (0%-20%) mixed with soil

5.2 Experiment II

(Soil + 12%Flyash + 10% - 100% of *Anabaena)*

On the basis of experiment I, 'soil + 12% fly ash' was used as control in this experiment and mixed with varying percentages of *Anabaena (*10%-100%*)*.The analysis of biomolecules was done for wheat samples grown in soil and 12% fly ash with varying concentrations of *Anabaena* mixed in soil.

Carbohydrate

The estimation of Carbohydrates (Total Soluble Sugar) was performed by Dubois *et al*., (1956) method.The maximum concentration of carbohydrate was obtained in the samples grown with 40% *Anabaena concentration*(Fig. 5.2 a). It is important as optimum concentration of *Anabaena* is used as control in experiment V.

Starch

In this experimentestimation of Starch was performed with different concentration of *Anabaena* mixed in soil.The maximum concentration of starch was obtained in the samples of 40% *Anabaena* (Fig**.** 5.2 b).It is important as optimum concentration of *Anabaena* should be used as control in experiment V.

Protein

In this experiment estimation of Protein was conducted with different concentration of *Anabaena* mixed in soil.The maximum concentration of protein was obtained in the samples of 40% *Anabaena* (Fig. 5.2 c).It is important as optimum concentration of *Anabaena* should be used as control in experiment V.

Phenol

In this experiment estimation of Phenol was conductedwith different concentration of *Anabaena* mixed in soil.The maximum concentration of phenol was obtained in the samples of 40% *Anabaena* (Fig. 5.2 d).

It is important as optimum concentration of *Anabaena* should be used as control in experiment V.

Chlorophyll

In this experiment estimation of Chlorophyll (a, b & total) was conducted with different concentration of *Anabaena* mixed in soil.The maximum concentration of Chlorophyll (a. b & total) was obtained in the samples of 40% *Anabaena* (Fig.5.2e A, B, C). It is important as optimum concentration of *Anabaena* should be used as control in experiment V.

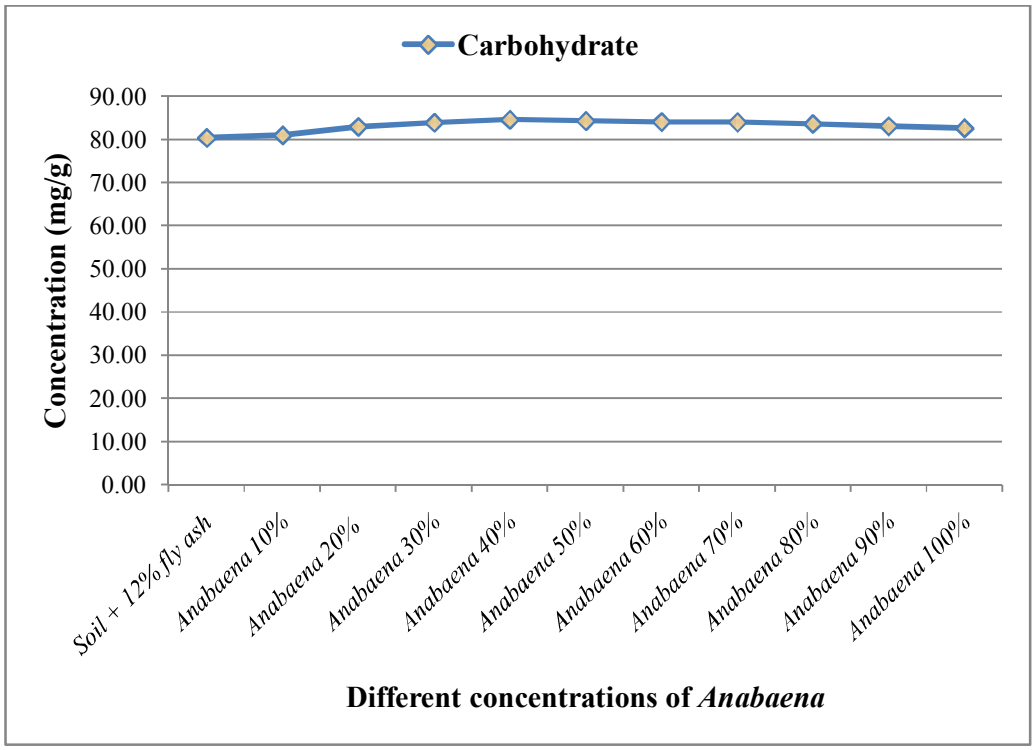


Figure: 5.2 (a) Estimation of Carbohydrates (total soluble sugar) in wheat grown with different concentrations of *Anabaena*(10-100%) mixed with 12%flyash soil

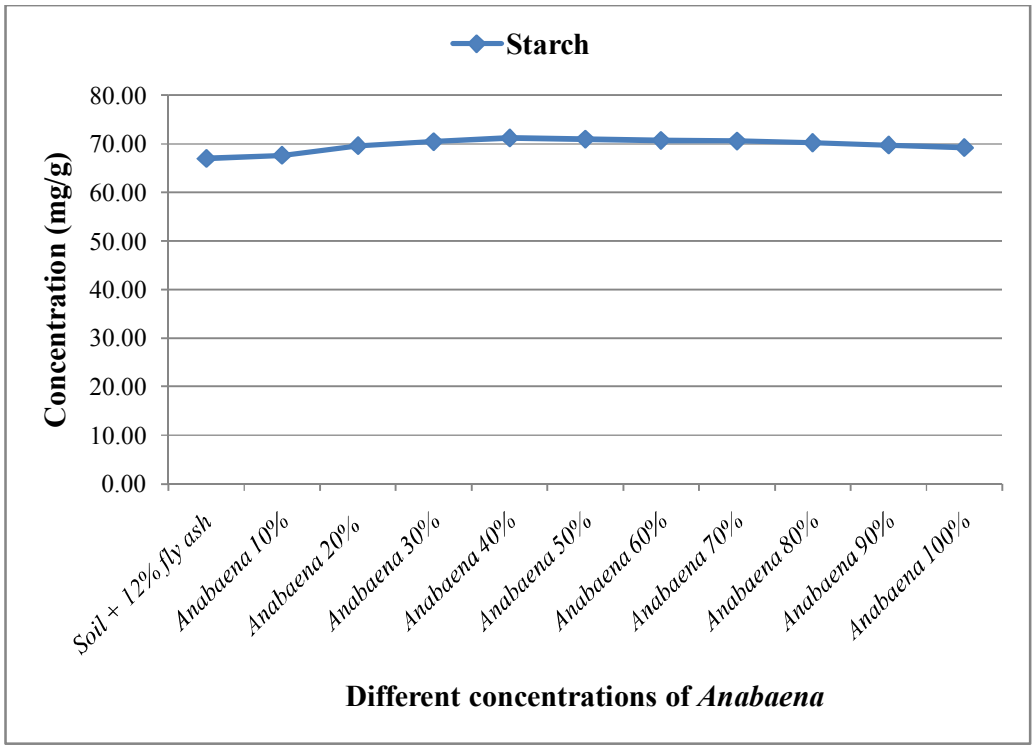


Figure: 5.2 (b) Estimation of Starch in wheat grown with different concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

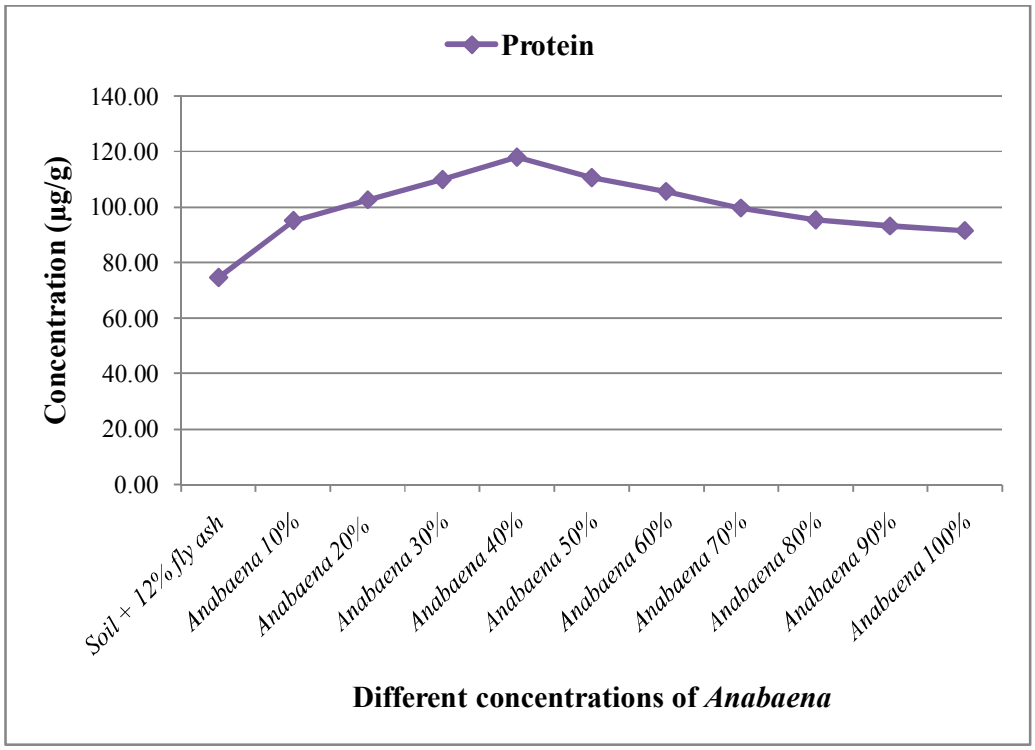


Figure 5.2 (c) Estimation of Protein in wheat grown with different concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

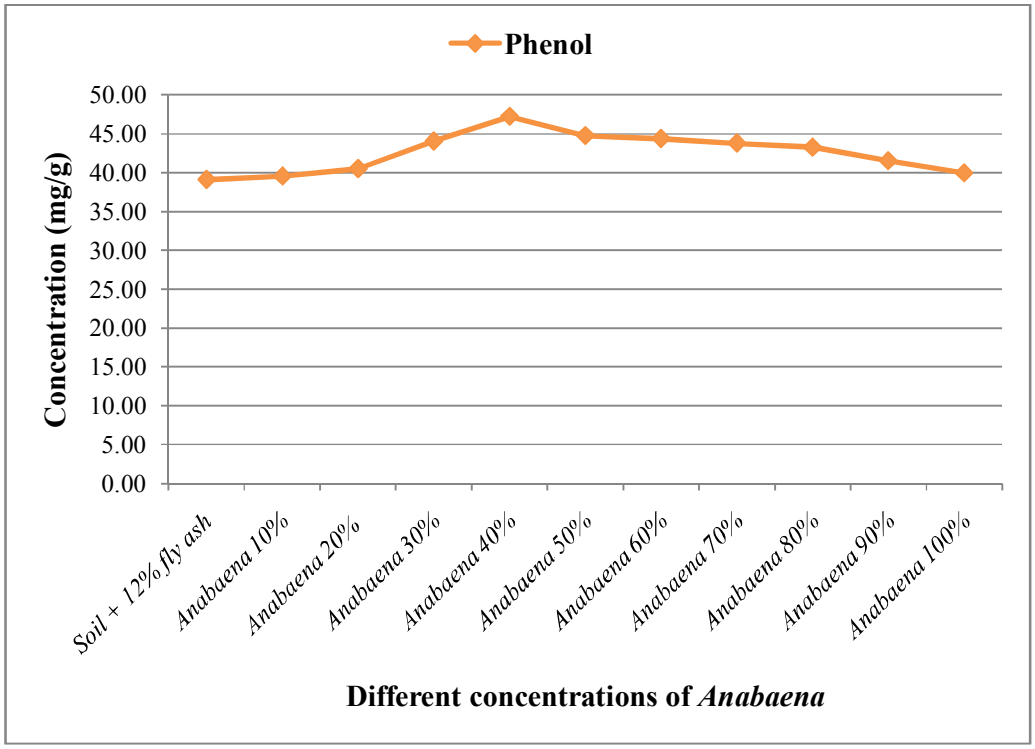


Figure: 5.2 (d) Estimation of Phenol in wheat grown with different concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

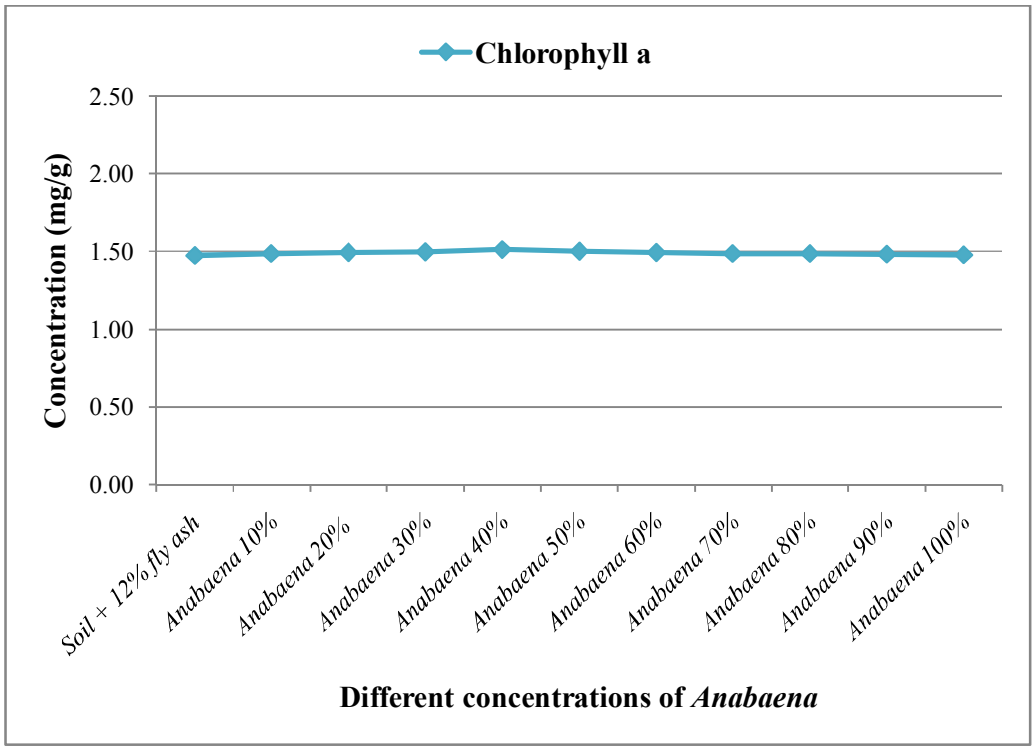


Figure: 5.2e (A) Estimation of Chlorophyll a in wheat grown withdifferent concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

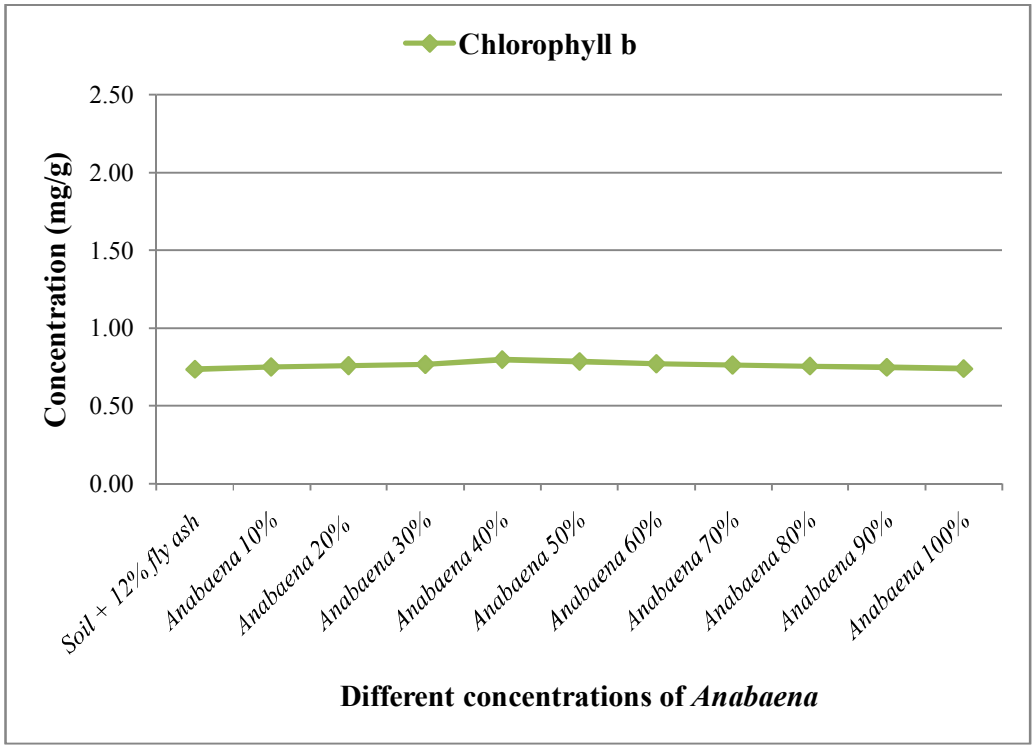


Figure: 5.2e (B) Estimation of Chlorophyll b in wheat grown withdifferent concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

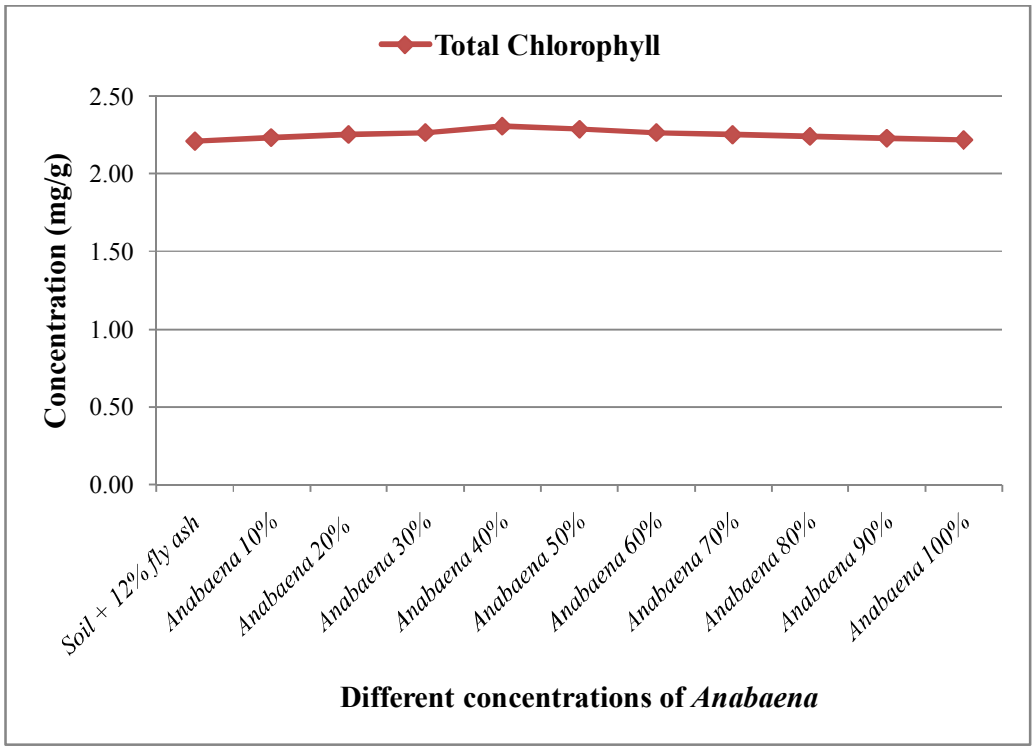


Figure: 5.2e (C) Estimation of Total Chlorophyll in wheat grown withdifferent concentrations of *Anabaena* (10-100%) mixed with 12% fly ash & soil

5.3 Experiment III

(Soil + 12% Fly ash + 10% - 100% of *Nostoc)*

In this experiment, for the next batch of samples the 'soil + 12% fly ash' was used as control and mixed with varying percentages of *Nostoc concentration* (10%-100%).

Carbohydrate

Estimation of Carbohydrates (Total Soluble Sugar) was performed with different concentration of *Nostoc* mixed with soil.It was observed that the maximum concentration of carbohydrate was obtained in the sample where *Nostoc* 60% *was* used (Fig.5.3 a)**.** It was used as standard for experiment IV**.**

Starch

Estimation of Starch was performed with different concentration of *Nostoc* mixed with soil.It was observed that the maximum concentration of starch was obtained in the sample where *Nostoc 60%* was used (Fig.5.3 b)**.** It was used as standard for experiment IV**.**

Protein

Estimation of Protein was performed with different concentration of *Nostoc* mixed with soil.It was observed that the maximum concentration of protein was obtained in the sample where *Nostoc 60%* was used (Fig.5.3 c) and it was used as standard for experiment IV**.**

Phenol

Estimation of Phenol was performed with different concentration of *Nostoc* mixed with soil.It was observed that the maximum concentration of phenol was obtained in the sample where *Nostoc 60%* was used (Fig.5.3 d)**.** It was used as standard for experiment IV**.**

Chlorophyll

Estimation of Chlorophyll was performed with different concentration of *Nostoc* mixed with soil.It was observed that the maximum concentration of Chlorophyll (a, b & total) was obtained in the sample of *Nostoc 60%* was used (Fig.5.3e A, B, C)**.** It was used as standard for experiment IV.

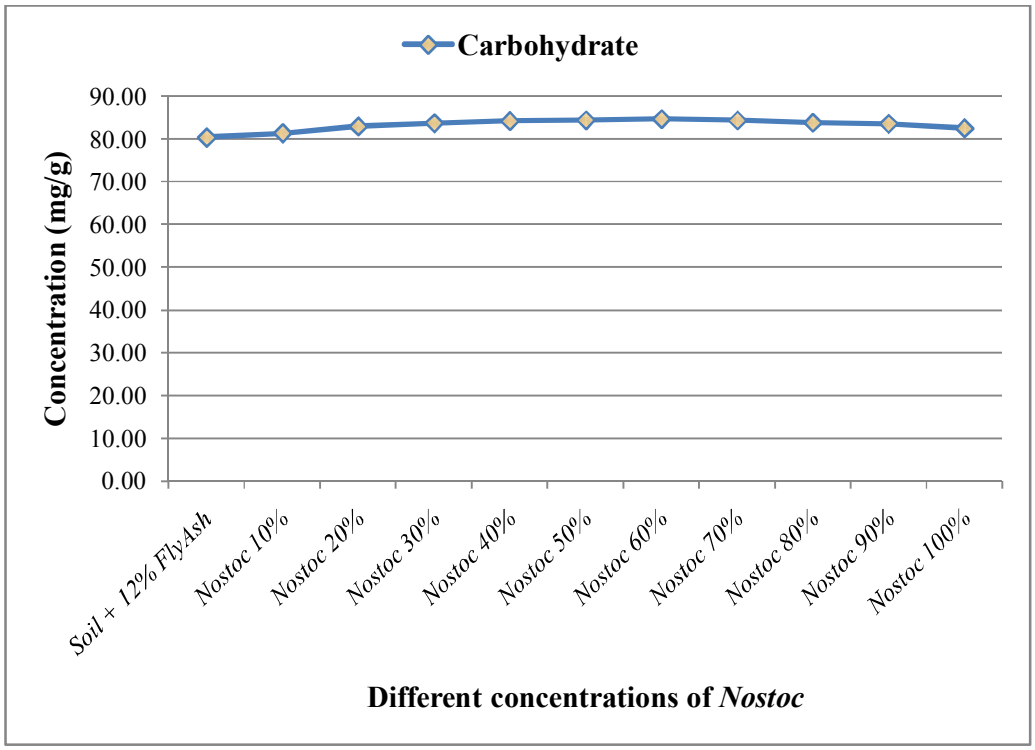


Figure: 5.3 (a) Estimation of Carbohydrates (total soluble sugar) in wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash in soil

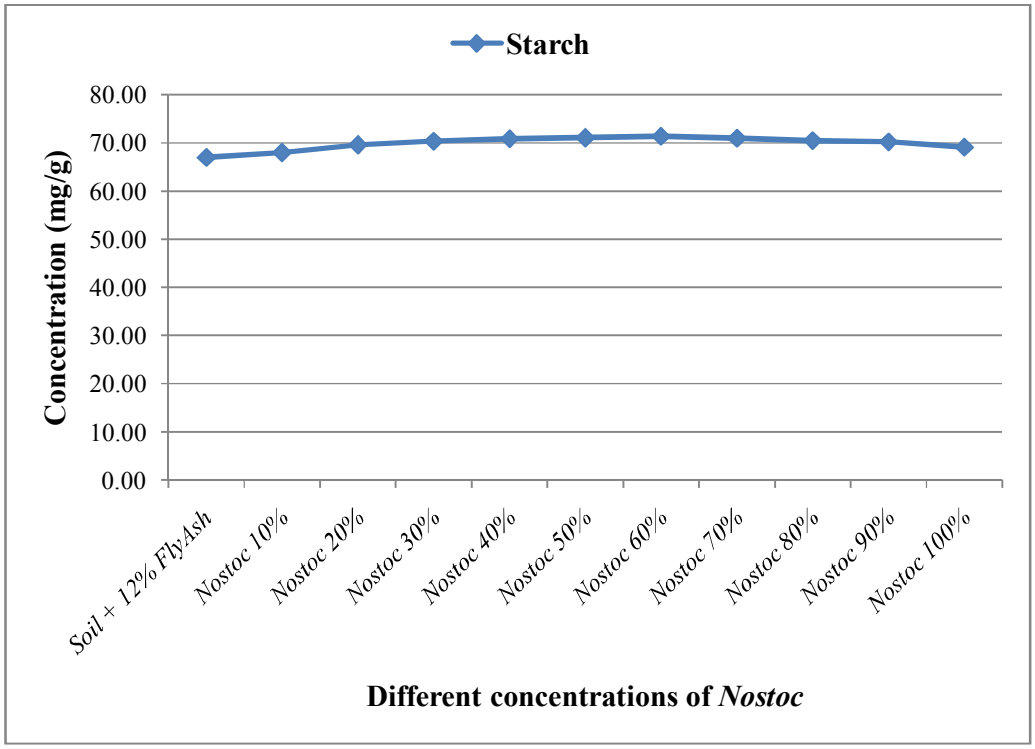


Figure: 5.3 (b) Estimation of Starch in wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash in soil

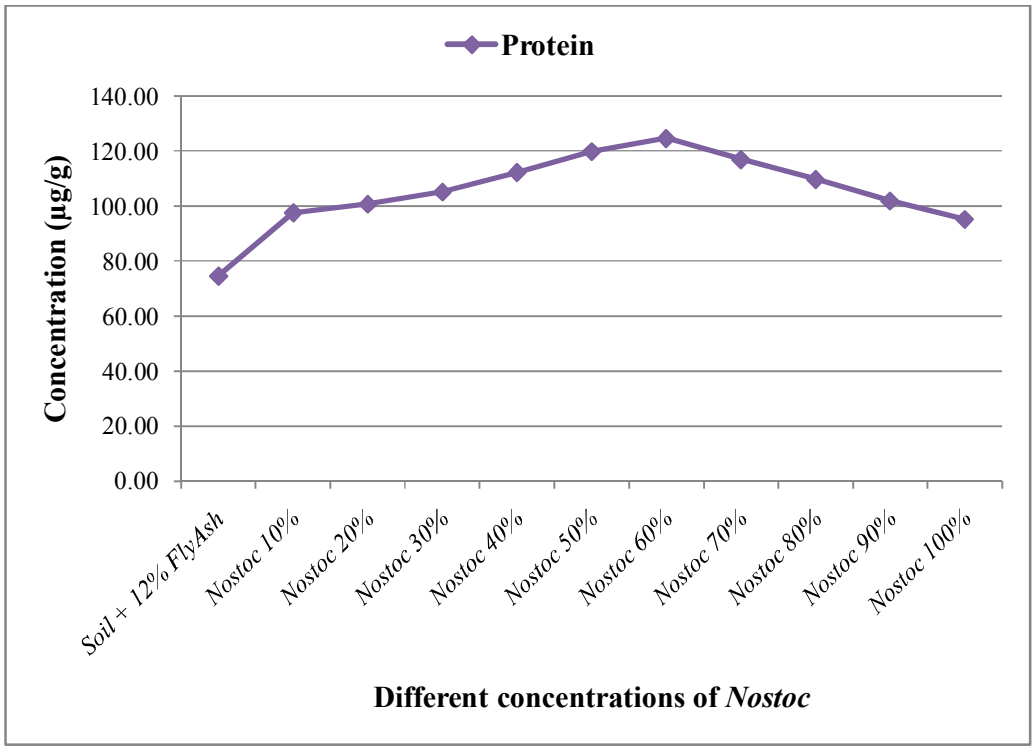


Figure 5.3 (c) Estimation of Protein in wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash in soil

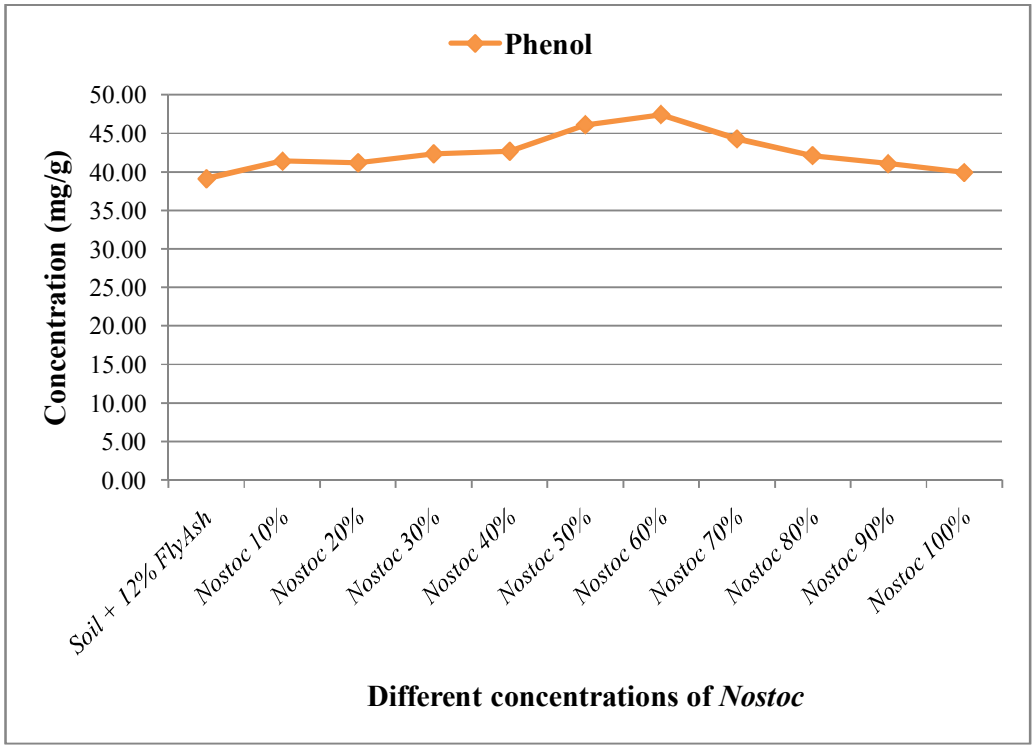


Figure: 5.3 (d) Estimation of Phenol in wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash in soil

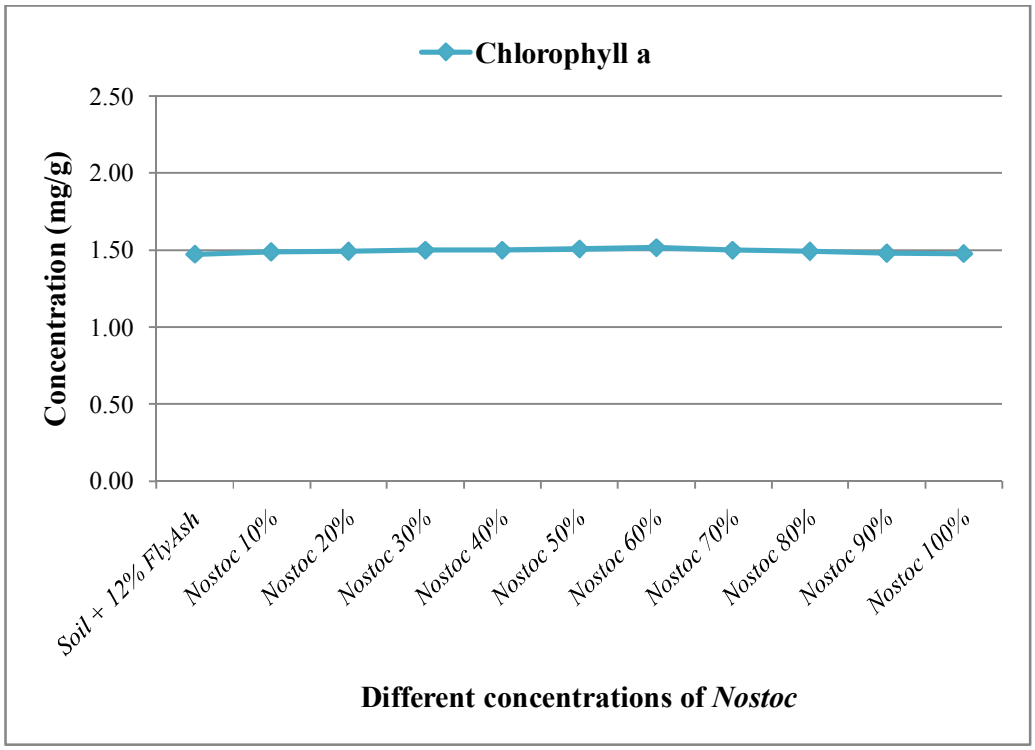


Figure: 5.3e (A) Estimation of Chlorophyll ain wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash & soil

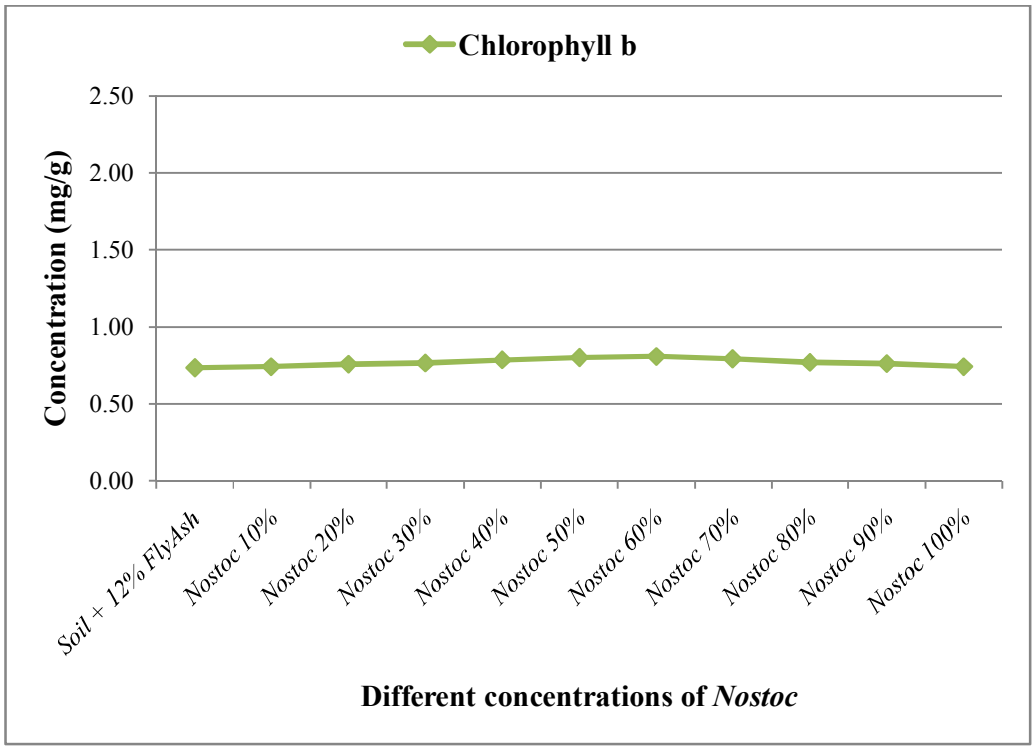


Figure: 5.3e (B) Estimation of Chlorophyll bin wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash & soil

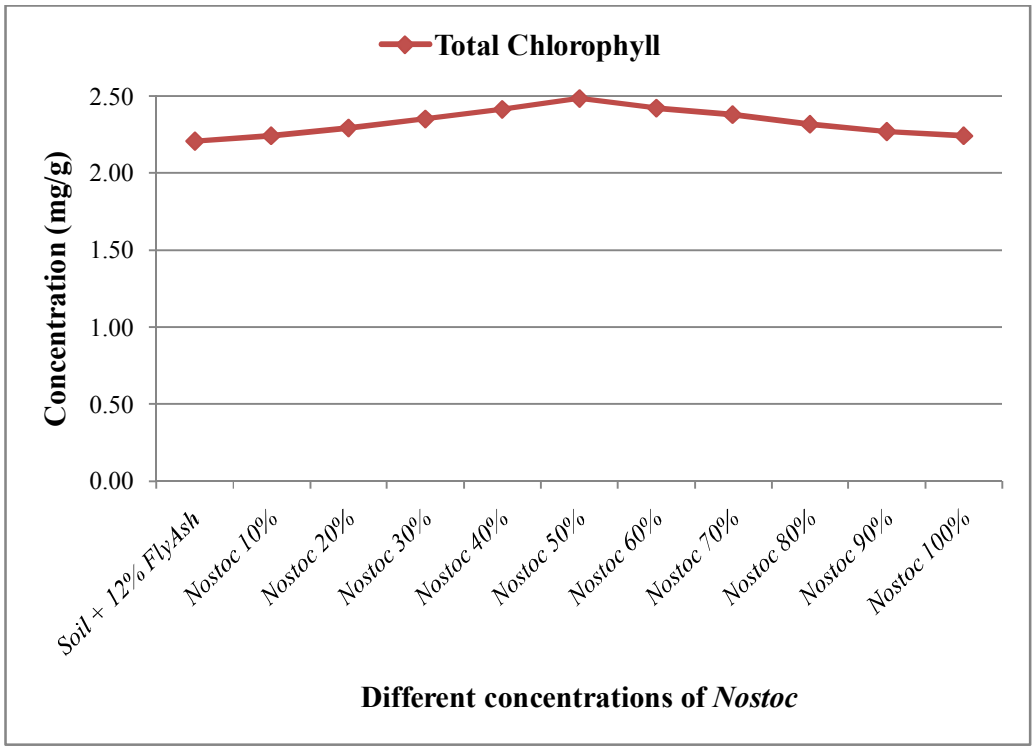


Figure: 5.3e (C) Estimation of Total Chlorophyll in wheat grown with different concentrations of *Nostoc* (10-100%) mixed with 12% fly ash & soil

5.4 Experiment IV

(Soil + 12 % Fly ash + *Nostoc* 60% + *Anabaena* (10% - 100%)

The samples of '*Nostoc* 60% + soil with 12% fly ash' was used as control and mixed with varying percentages of *Anabaena (*10% - 100%). In the experiment III it was found that the maximum concentration of biomolecule was obtained in *Nostoc* 60%. *So in* this experiment *Nostoc 60%*+ Soil with 12% Fly ash was used as a control.

Carbohydrate

Estimation of Carbohydrates (Total Soluble Sugar) was performed for wheat samples grown in different concentration of *Nostoc* 60% +*Anabaena* (10%-100%) mixed with 12% fly ash in soil.It was observed that the maximum concentration of

carbohydrate is obtained in the sample of *Anabaena 50%* with *Nostoc* 60% + Soil with 12% fly ash **(**Fig. 5.4 a).

Starch

Estimation of starch was performed with different concentration of *Nostoc* 60% + *Anabaena* (10% - 100%) mixed with 12% fly ash in soil.It was observed that the maximum concentration of starch is obtained in the sample of *Anabaena50% withNostoc* 60% + Soil with 12% fly ash **(**Fig. 5.4 b).

Protein

Estimation of protein was performed with different concentration of *Nostoc* 60% +*Anabaena* (10% - 100%) mixed with 12% fly ash in soil.It was observed that the maximum concentration of protein is obtained in the sample with *Anabaena* 50% + *Nostoc* 60% + Soil with 12% fly ash **(**Fig. 5.4 c).

Phenol

Estimation of phenol was performed with different concentration of *Nostoc* 60% + *Anabaena* (10% - 100%) mixed with 12% fly ash in soil.It can be observed that the maximum concentration of phenol is obtained in the sample with *Nostoc* 60% + *Anabaena* 50% + soil with 12% fly ash **(**Fig. 5.4 d).

Chlorophyll

Estimation of Chlorophyll was performed with different concentration of *Nostoc* 60% + *Anabaena* (10% - 100%) mixed with 12% fly ash in soil.It was observed that the maximum concentration of Chlorophyll (a, b & total) is obtained in the sample with *Anabaena* 50% + *Nostoc* 60% + soil with 12% fly ash (Fig.5.4e A, B, C).

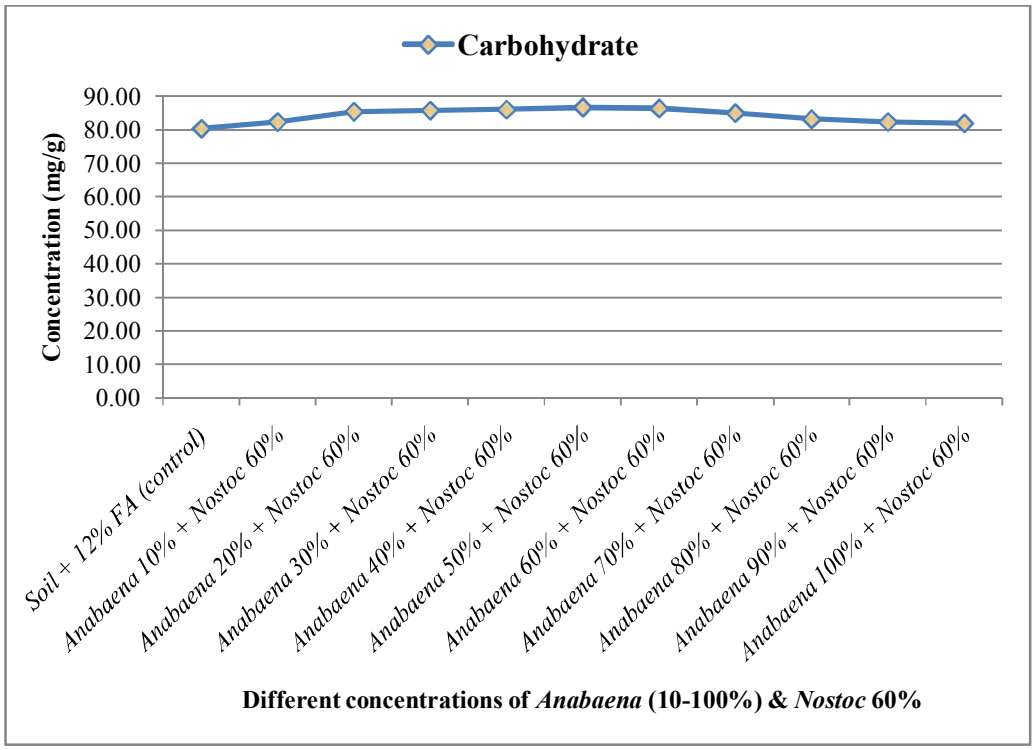


Figure: 5.4 (a) Estimation of Carbohydrates (total soluble sugar) in wheat grown with different concentrations of *Anabaena* (10-100%) &*Nostoc* 60% mixed with 12% fly ash & soil

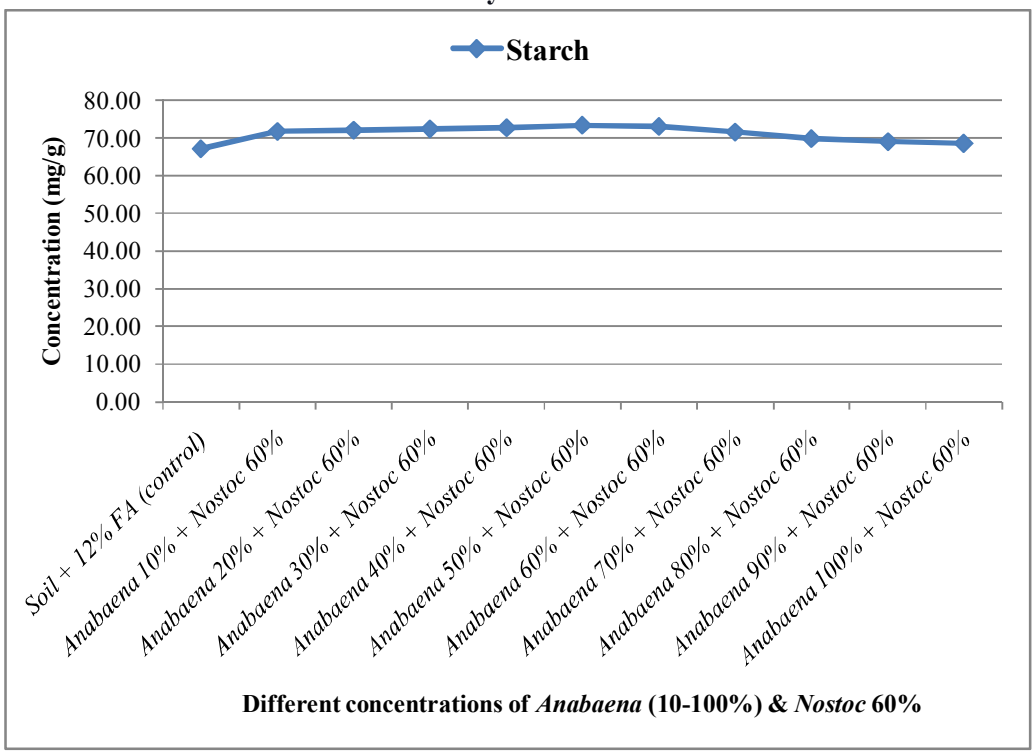


Figure: 5.4(b) Estimation of Starch in wheat grown with different concentrations of *Anabaena* (10-100%) &*Nostoc* 60% mixed with 12% fly ash & soil

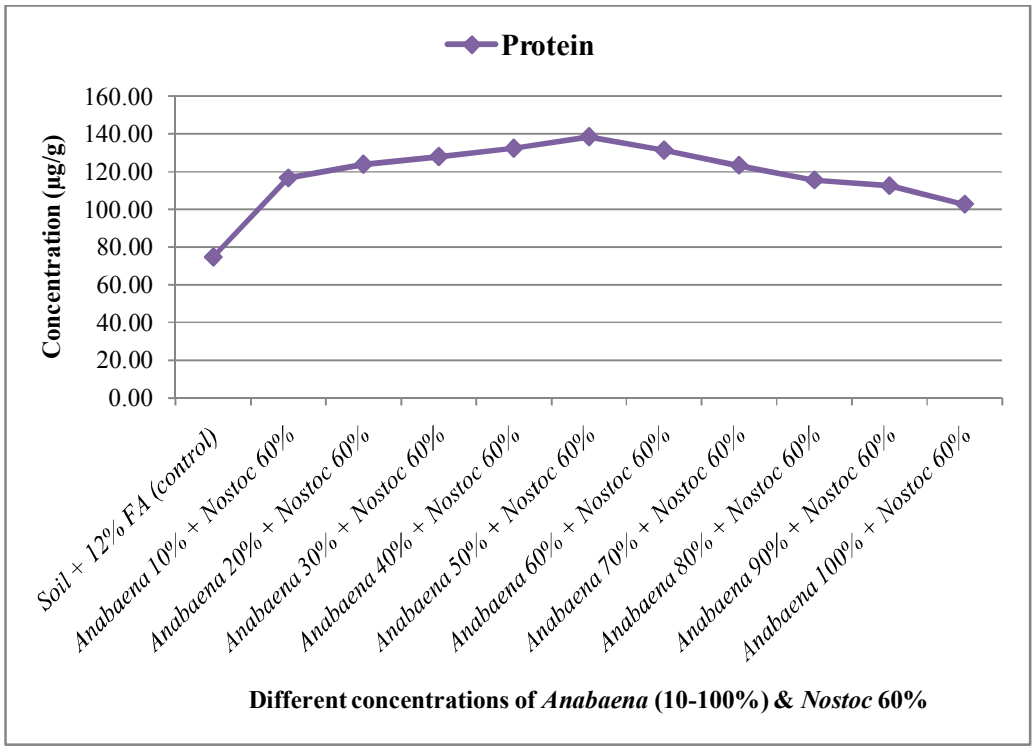


Figure 5.4 (c) Estimation of Protein in wheat grown with different concentrations of *Anabaena* (10-100%) &*Nostoc* 60% mixed with 12% fly ash & soil

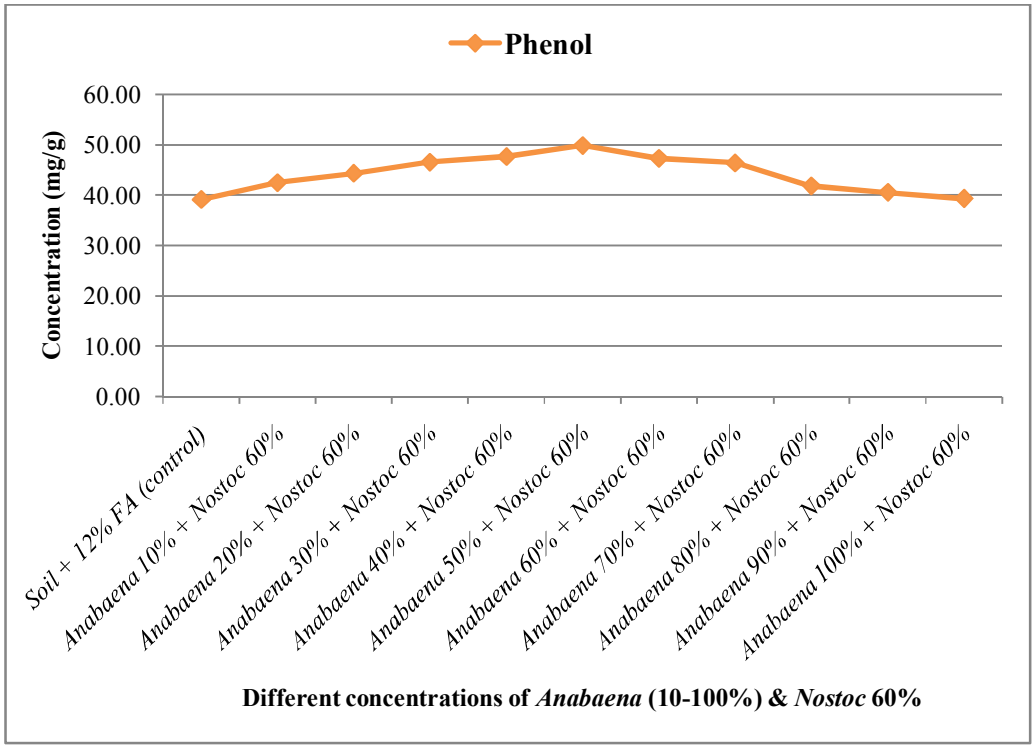


Figure: 5.4(d) Estimation of Phenol in wheat grown with different concentrations of *Anabaena* (10-100%) &*Nostoc* 60% mixed with 12% fly ash & soil

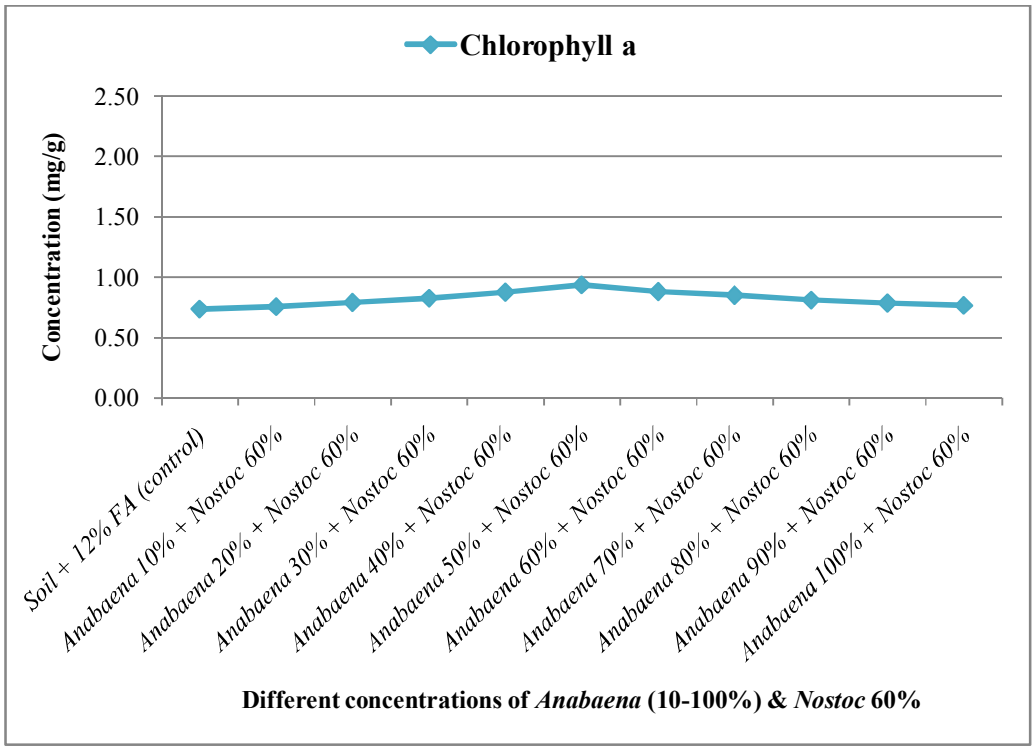


Figure: 5.4e (A) Estimation of Chlorophyll a in wheat grown with different concentrations of *Anabaena*(10-100%)&*Nostoc*60% mixed with 12% flyash&soil

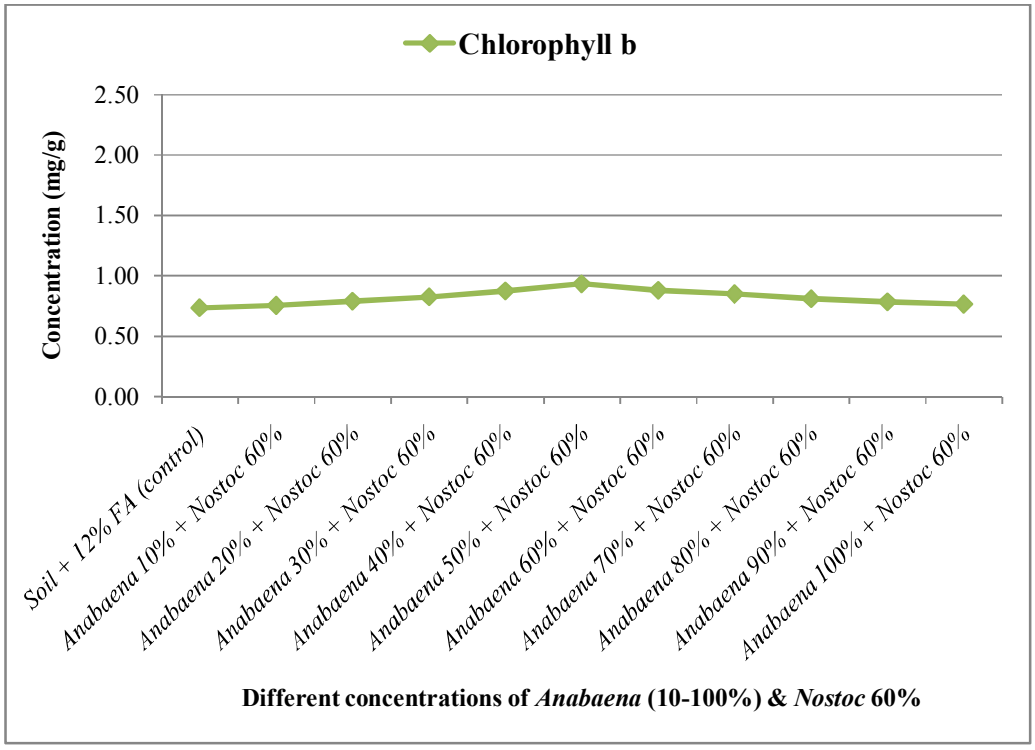


Figure: 5.4e (B) Estimation of Chlorophyll b in wheat grown with different concentrations of *Anabaena*(10-100%)&*Nostoc* 60% mixed with 12% flyash&soil

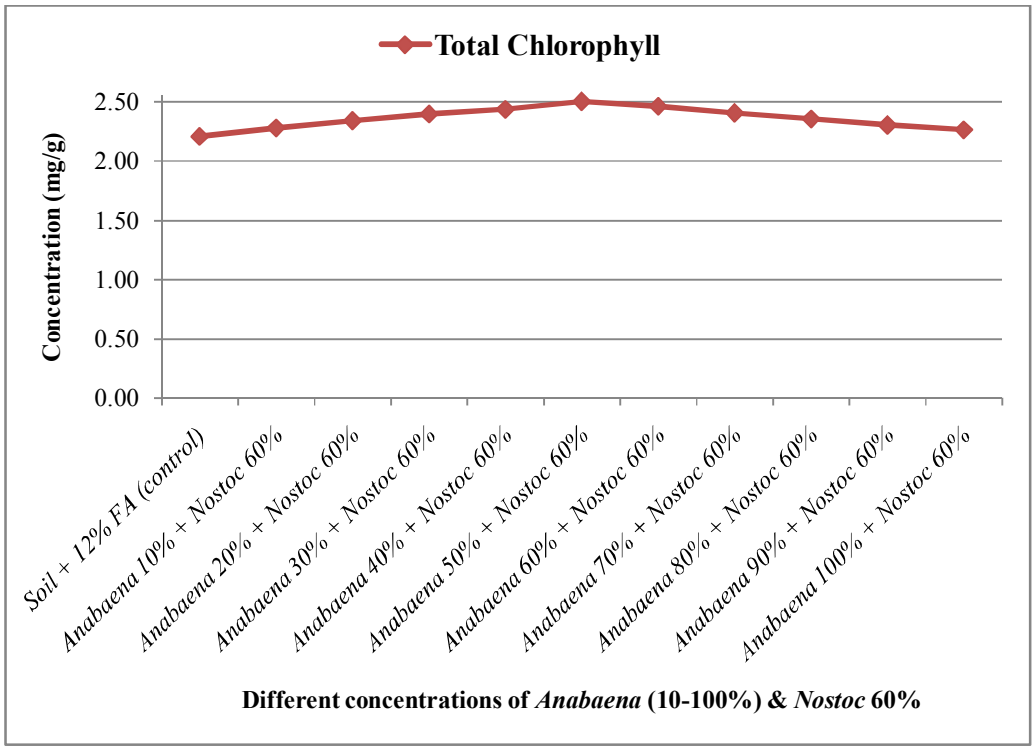


Figure: 5.4e (C) Estimation of Total Chlorophyll in wheat grown with different concentrations of *Anabaena* (10-100%) & *Nostoc* 60% mixed with 12% flyash&soil

5.5 Experiment V

(Soil + 12%Flyash+ *Anabaena* 40% + *Nostoc* (10% - 100%)

It was found in the experiment II that the maximum concentration of biomolecules was obtained in the *Anabaena 40%* (Fig.5.2a, b, c, d, e)**.** So it was used as control in this experiment.In this set, 40% concentration of *Anabaena* and the percentage of *Nostoc* was varied from10% - 100% in samples.*Anabaena 40%* + soil with 12% fly ash were used as a control for this batch. The samples are prepared from the 'soil with 12% fly ash (as control) + *Anabaena* 40% mixed with varying percentages of *Nostoc (*10% - 100%).

Carbohydrate

Estimation of carbohydrates (Total Soluble Sugar) was performed with *Anabaena 40% + Nostoc 10%* - 100% mixed with 12% fly ash in soil.The maximum concentration of carbohydrate was obtained in the sample of *Nostoc* 50% with *Anabaena* 40% + Soil with 12% fly ash (Fig. 5.5 a).

Starch

Estimation of starch was performed with different concentration of *Anabaena* 40% + *Nostoc 10%*- 100% mixed with 12% fly ash in soil.The maximum concentration of starch was obtained in the sample of *Nostoc 50%* with *Anabaena* 40% + soil with 12% fly ash (Fig. 5.5 b).

Protein

Estimation of protein was performed with *Anabaena 40% + Nostoc10%* - 100% mixed with 12% fly ash in soil.The maximum concentration of protein was obtained in the sample of *Nostoc 50% with Anabaena* 40% + soil with 12% fly ash (Fig. 5.5 c).

Phenol

Estimation of phenol was performed with *Anabaena 40% + Nostoc 10%*- 100% mixed with 12% fly ash in soil.The maximum concentration of phenol was obtained in the sample of *Nostoc 50%* with *Anabaena* 40% + soil with 12% fly ash **(**Fig. 5.5 d).

Chlorophyll

Estimation of Chlorophyll was performed with *Anabaena 40%+ Nostoc 10%* - 100% mixed with 12% fly ash in Soil.The maximum concentration of Chlorophyll (a, b, & total) was obtained in the sample of *Nostoc 50%* with *Anabaena* 40% + soil with 12% fly ash (Fig. 5.5e A, B, C).

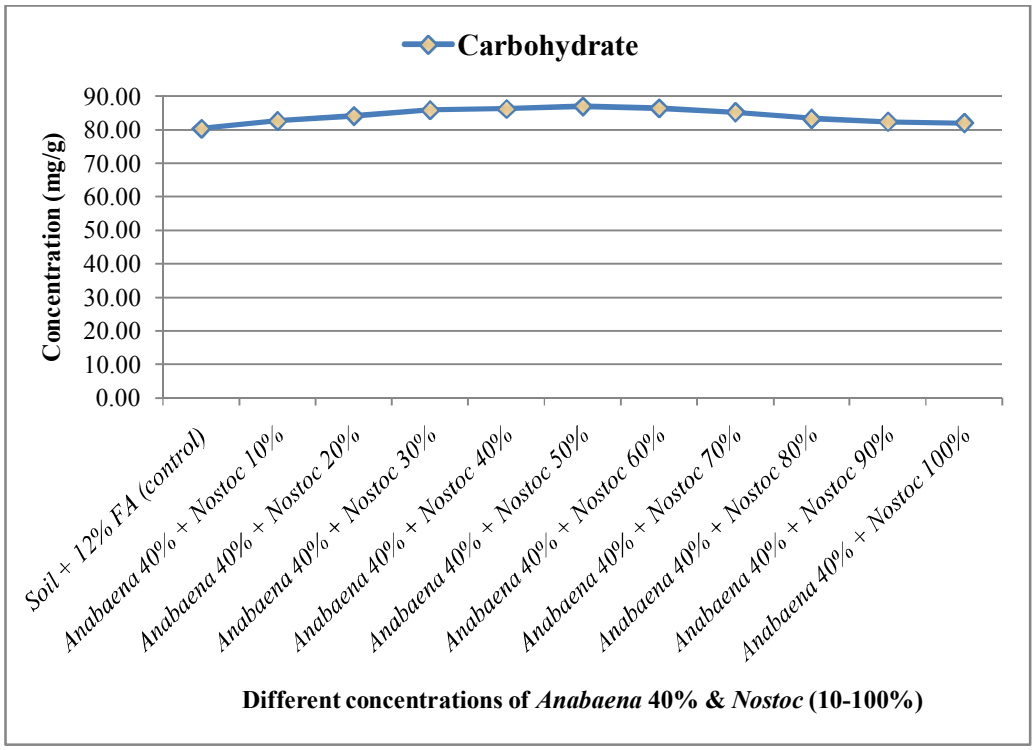


Figure: 5.5 (a) Estimation of Carbohydrates (total soluble sugar) in wheat grown with different concentrations of *Anabaena* 40% &*Nostoc* (10-100%) mixed with 12% fly ash & soil

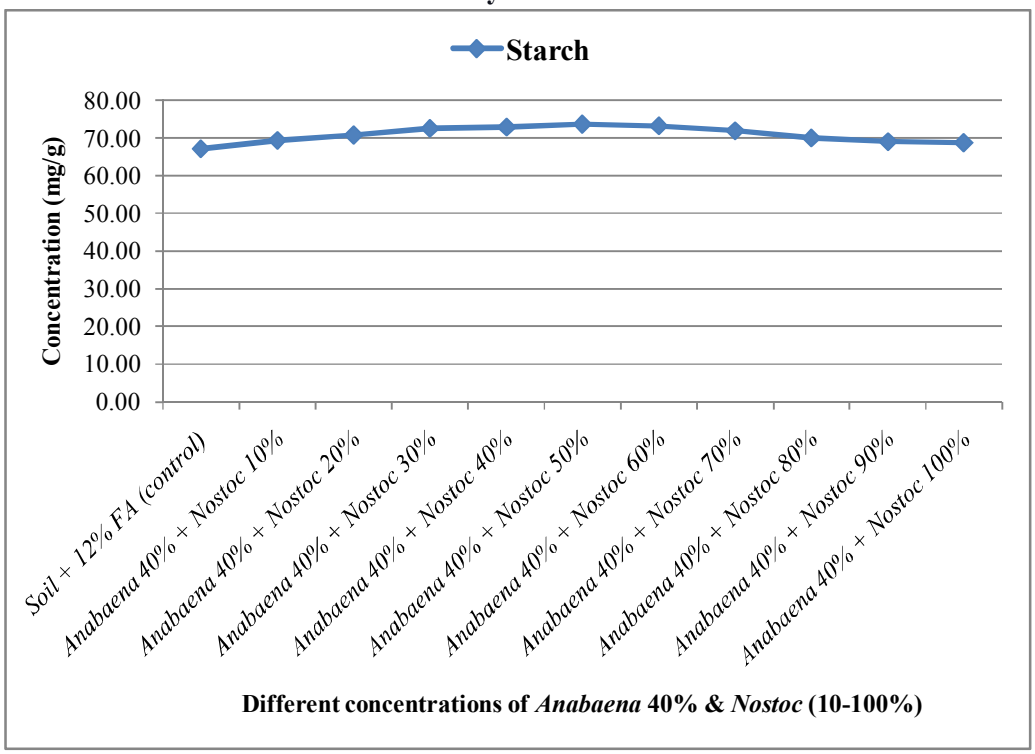


Figure: 5.5 (b) Estimation of Starch in wheat grown with different concentrations of *Anabaena* 40%&*Nostoc* (10-100%) mixed with 12% flyash&soil

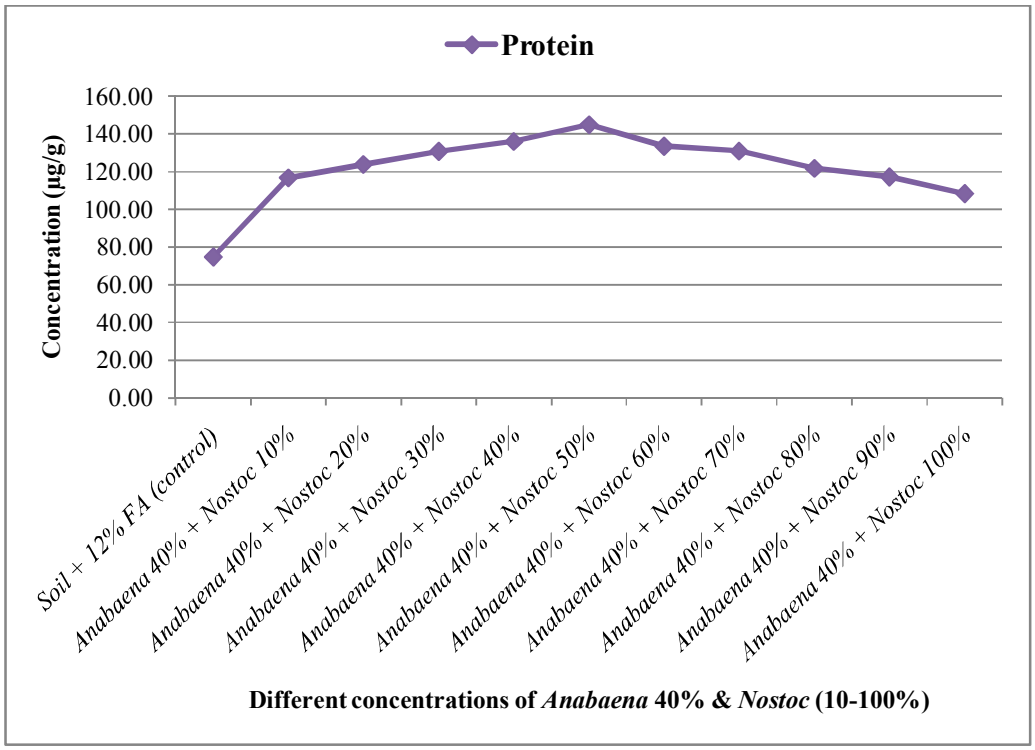


Figure 5.5 (c) Estimation of Protein in wheat grown with different concentrations of *Anabaena* 40% &*Nostoc* (10-100%) mixed with 12% fly ash & soil

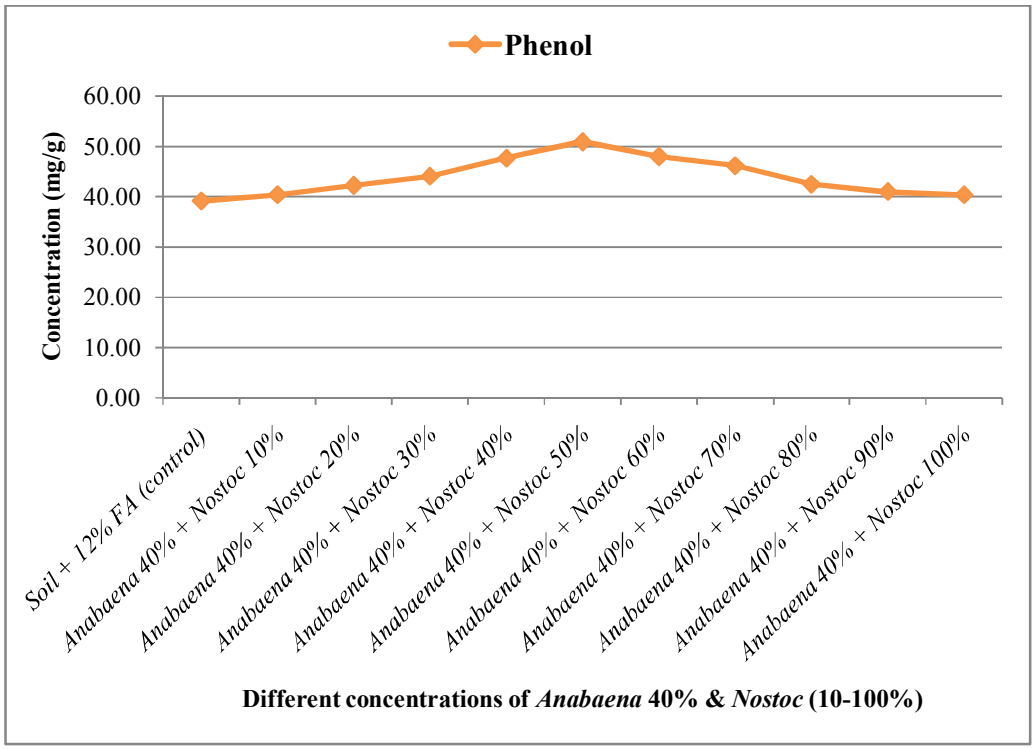


Figure: 5.5 (d) Estimation of Phenol in wheat grown with different concentrations of *Anabaena* 40%&*Nostoc* (10-100%) mixed with 12% flyash&soil

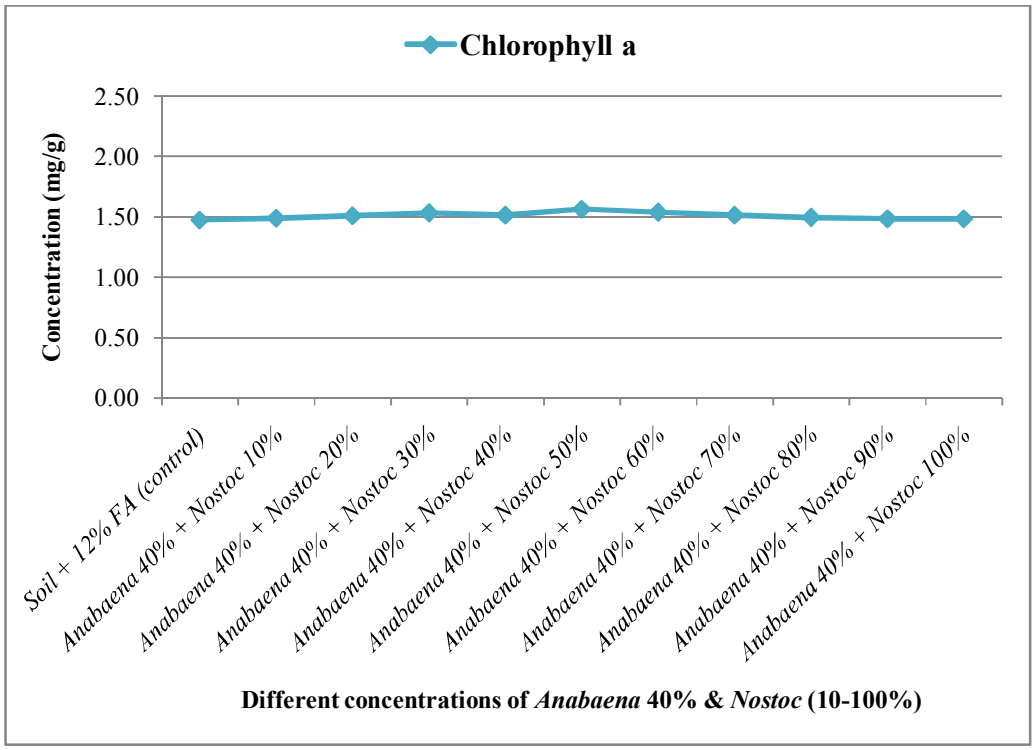


Figure: 5.5e (A) Estimation of Chlorophyll a in wheat grown with different concentrations of *Anabaena* 40%&*Nostoc*(10-100%) mixed with 12% flyash&soil

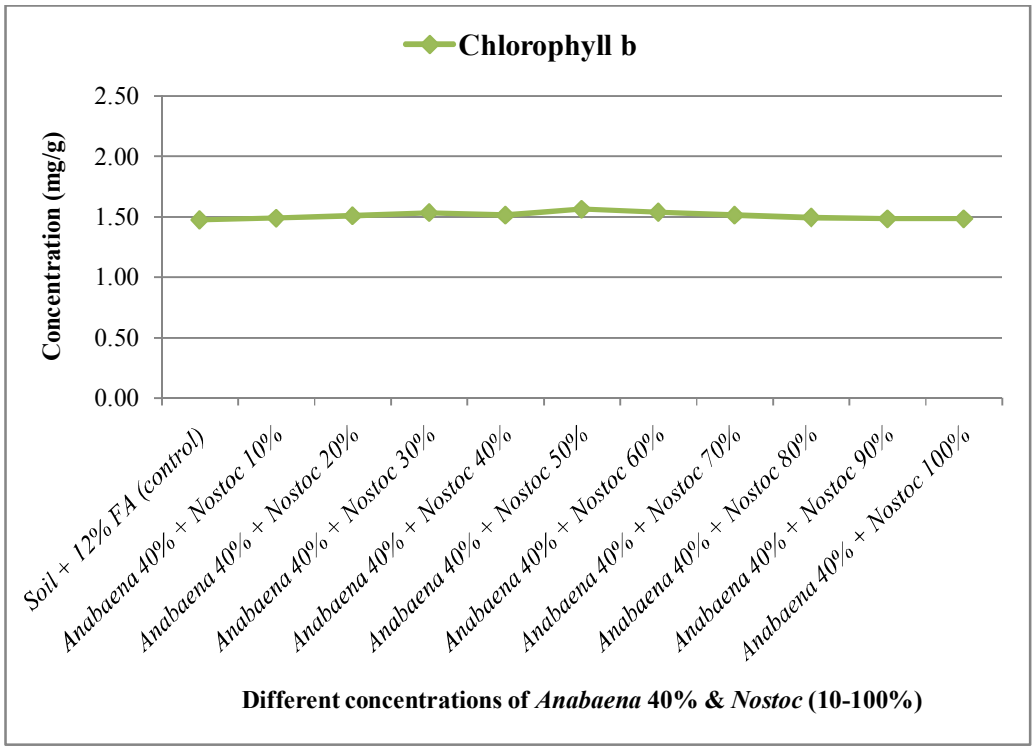


Figure: 5.5e (B) Estimation of Chlorophyll b in wheat grown with different concentrations of *Anabaena* 40%&*Nostoc* (10-100%) mixed with 12% flyash&soil

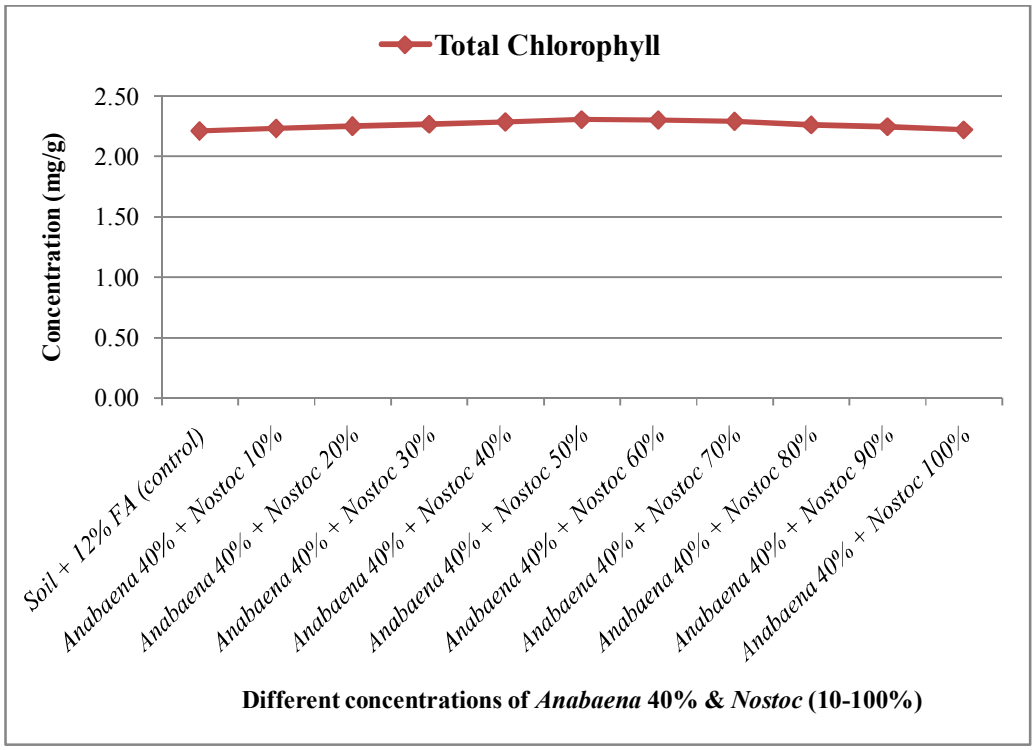


Figure: 5.5e (C) Estimation of Total Chlorophyll in wheat grown with different concentrations of *Anabaena* 40%&*Nostoc*(10-100%) mixed with 12% flyash&soil

CHAPTER 6
RESULTS AND DISCUSSION

Fly ash is a by-product material which is generated by thermal power plants by combustion of pulverized coal. Coal-based Thermal Power Plants have been a significant source of Power generation in India, where about 75% of the total power obtained is from coal-based Thermal Power Plants (http://www.tifac.org.in). Production of fly ash in India is currently 118 million metric tons, which is estimated to be 440 million metric tons by 2030 (Tripathi *et al*., 2015).

Fly ash, a residue of coal consumption, is primarily made up of oxides of Aluminum and Silicon, but is also enriched with several other essential (Zinc, Iron, Manganese, Boron and Molybdenum) and non-essential metals (Nickel, Chromium, Lead, Aluminum, Silicon). In fact, fly ash consists of all the elements present in soil except organic carbon and nitrogen (Kumar *et al*., 2000).

Thus fly ash improves the properties of soil. The result of the physicochemical analysis of fly ash indicated that fly ash has an alkaline pH, which may be due to occurrence of oxides of calcium and magnesium with acid-neutralizing properties (Mishra and Shukla, 1986 a &1986b; Taylor and Schuman, 1988). However the amount of nitrogen in fly ash is very low (Kumar *et al*., 2000). It is observed that the nitrogen content in the soil is higher than in fly ash as 0.61% and 0.56% respectively (Cafer *et al.,* 2015).But it can be used for soil by mixing nitrogen-containing elements in fly ash. Thus, by adding nitrogen-containing elements to the fly ash, the fly ash can be used for the growth of plants. However plants cannot utilize nitrogen in elemental form. It is useful for agriculture as fly ash has similar soil

content. Some cyanobacteria can contribute nitrogen and growth promoting substances to plants.

The study is conducted with the aim to analyze the appropriate amount of fly ash which can improve soil quality and increase plant growth without harmful effect. The bio-fertilizer prepared by mixing of different concentrations of fly ash and cyanobacteria was used to grow wheat. The effect of different concentrations of fly ash mixed with soil was studied on the biochemical analysis of wheat samples in different set of experiments. Biochemical compounds were recorded highest with 12% fly ash concentration in wheat. A trend was observed in present study that biochemical contents decreases with an increase in the concentration of fly ash above 12%. Hence this 12% concentration of fly ash level proved to be optimally useful for the growth of wheat plant.

In the experiment I, normal soil was used as a control for growing wheat and wheat samples were analyzed for their biochemical constituents. The concentration was observed for Carbohydrate (72.20 mg/g), Starch (48.87 mg/g), Protein (56.27 µg/g) and Phenol (22.83 mg/g) (Fig. 5.1 a, b, c, d & Fig. 6.1, 6.2, 6.3, 6.4). Under the same treatment, the concentration was observed for Chlorophyll a (0.968 mg/g), Chlorophyll b (0.699 mg/g) and Total Chlorophyll (1.66 mg/g) (Fig. 5.1e A, B, C & Fig 6.5 A, B, C).

Normal soil was treated as a control in experiment I and soil mixed with different concentration of fly ash 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20%. In this experiment maximum concentration was observed for Carbohydrate (80.40 mg/g), Starch (67.07 mg/g.), Protein (74.60 µg/g.) and Phenol (39.13 mg/g) (Fig. 5.1 a, b, c, d & Fig. 6.1, 6.2, 6.3, 6.4) on the 12% fly ash. Under the same

treatment, the concentration was observed for (i) Chlorophyll a (1.47 mg/g), (ii) Chlorophyll b (0.736 mg/g),) and Total Chlorophyll (2.20 mg/g) (Fig. 5.1e A, B, C & Fig 6.5A, B, C).

It is observed that the Carbohydrate concentration in wheat sample increases with an increase in fly ash percentage until it reaches to a maximum at 12% of fly ash (Fig. 6.1) and then decreases with increase in fly ash concentrations. A similar trend was observed for Starch, Protein, Phenol and Chlorophyllpigment concentration, they increases with an increase in the percentage of fly ash and reaches a maximum at 12% fly ash in wheat sample and then decreases after 12% fly ash concentration (Fig.6.2, 6.3, 6.4, 6.5 A, B. C).

In consequence, photosynthetic pigmentChlorophyll a, Chlorophyll b, total Chlorophyll and the biomolecules Carbohydrate, Starch, Protein and Phenol were found to be increased with normal soil + fly ash up to the 12% fly ash.In the present study, the use of normal soil + 12% fly ash concentration for wheat growth shows optimum results for biochemical constituents and pigments in wheat and so it was used as control for further experiments.

Several studies of soil revealed that fly ash, shows its influence on physical, chemical and biological properties and significantly affect plant growth.The plants like Alfalfa (*Medicago sativa*), Barley (*Hordeum vulgare*), Bermuda grass (*Cynodon dactylon*), white clover (*Trifolium repens*), Sunflower (*Helianthus sp.),* Groundnut (*Arachis hypogaea*), Aromatic grasses, i.e. (*Cymbopogon martini*) and Citronella (*Cymbopogonnardus*) have shown positive results of fly ash application (Weinstein, 1989 & Neelima *et al.*, 1995).The fly ash application leads to enhance growth and yield of crop plants in crops like alfalfa, barley, Bermuda grass and white clover (Hill

and Lamp, 1980; Elseewi *et al*., 1980 a &1980b; Weinstein, 1989). In fly ash amended soil, fly ash was attributed to the increased availability of major plant nutrients (Asokan *et al.*, 1995).

Other crops positively affected by the fly ash application were *Sesbania cannabina, Cajanus cajan*, maize, eggplant, mungbean (*Vigna radiata L.*) and ornamental plants oilseed crops such as *Mentha piperita*. Studies revealed that the use of fly ash in agriculture is because of its mineral content (Kalra *et al*., 1997 & Singh *et al*., 1997).

In the present study highest amount of carbohydrate content was observed with 12% fly ash. Similar results were obtained of carbohydrate in different plant species like in *Balanites aegyptica* by Vijayvergya and Vijay, (2006), in *Cassia obtusifolia* and *Cassia siamea* by Sharma *et al.,* (2006), in species of Araucaria by Unikrishnan *et al.,* (2007), in Sea weed by Sornalakshmi and Kumar, (2014), in *Terminalia catappa* by Nagesh *et al.,* (2007).They also showed increase in carbohydrate content after using fly ash mixed with soil. Their findings coincide with the results of the present study.

In the present work Protein concentration is increasing with the increase in concentration of fly ash but after 12% fly ash is not showing any increment. This is the similar with the finding of Singh, *et al.,* (2008) and Qurratula *et al*., (2014) that protein content in leaves of in *B. vulgaris* decreases significantly with increase under low concentration of fly ash from control to 25%.

Audichya, (1999); Borhade, *et al*., (1984); Chatrath*, et al.,* (1996); Ansari, *et al*.,*(*2011); Singh *et al.,* (2010) reported increase in protein contents of leguminous crop i.e. *Lensculinaris L, Vicia faba L, Cicer arietinum L, Phaseolus sp. L, Pisum*

sativum L, Glycine max, Vigna radiata L. They observed that optimum results from control up to 50% fly ash. There was a substantial increase in growth and physiological parameters.

Similar findings were made by Gupta *et al.,* (2007) on *Phaseolus vulgaris* and Niaz *et al*., (2008) on *Eclipta alba* in which protein content increased from control to 60% fly ash and decreased from 60% to100% fly ash.

Phenol concentration also increases with the increase in concentration of fly ash but it is not showing any increase after 12% of concentration in this study. Similarly, Khandelwal and Shrivastava, (2014) observed increase in phenol concentration up to 40% fly ash and a decline after that in *Trigonella foenum graecum*. Their result favors the findings of present work. Similar results were observed by Malik and Singh, (1980), Agarwal and Gupta, (1993), Niyaz and Singh 2006; Hisamuddin and Singh, 2007.

In the present study highest amount of Chlorophyll content (a, b & total) was observed with 12% fly ash, after this it was declining.

The present investigation indicates higher Chlorophyll a and b concentration in wheat plants could be due to the micronutrients available in fly ash than the control. Similar reports have been made by (Niyaz and Singh, 2006; Hisamuddin and Singh, 2007) on *Eclipta* plant. The higher Chlorophyll in fly ash containing soil is due to the presence of high N, K and Mg which are present in fly ash resulting in higher content of Chlorophyll a (Rai *et al*., 2002). As per finding of Canjura *et al*., (1991) the higher content of Chlorophyll b in fly ash containing fields is due to higher P content in fly ash amended soil.

Similar findings were obtained by Gupta *et al.,* (2007) on *Phaseolus vulgaris* and Niaz *et al., (*2008) on *Eclipta alba* in which Chlorophyll content increased from control to 60% fly ash and then decreased.However, excessive application of fly ash show decrease in terms of quality and growth as it was reported for *B. vulgaris* at rates 20% (Singh and Agrawal, 2007).

Asokan *et al.,* (1995) also found 20% fly ash suitable for soya bean. 20% fly ash also reported well for rice plant (Singh & Singh, 1986). Significant effects on rice were recorded with the application of fly ash up to 20%. However, fly ash addition higher than 20% decreases the output (Singh & Singh, 1986).

There have also been some workers whose results showed that fly ash exploited at the rate of 20% proved beneficial for many crops like soybean, cabbage, chickpea, cucumber, lentil, maize, potato, wheat, tomato, etc. (Khan and Khan, 1996; Mishra and Shukla, 1986b; Raghav and Khan, 2002; Singh, 1989 & Ram and Masto, 2010).

Doongar and Ghosh, (2013) also reported that nutrients uptake increases in 20% fly ash. They found positive results fly ash application on Alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), Bermuda grass (*Cynodondactylon*) and white clover (*Trifoliumrepens*), sunflower (*Helianthus sp*.), groundnut (*Arachishypogaea*), aromatic grasses, i.e. (*Cymbopogon martini*) and citronella (*Cymbopogonnardus*). Katiyar *et al*., 2012 reported that fly ash is proved beneficial for palak, mung bean and chili plants up to 25%.

It can be concluded that the application of fly ash as a component of biofertilizer improves soil properties and fertility. It enhances plant growth but at high rates of fly ash application may cause adverse effects.However, due to the high mutability in the nature and composition of fly ash (pH, major & micronutrients) and

soil (pH, texture, and fertility), a specific fly ash application rate cannot be recommended for all plants. Summarizing the impacts of fly ash on soil properties and soil fertility, which increase plant growth, it can be said that fly ash can be a valuable source of readily available plant micro and macro-nutrients.

The use of fly ash on acidic soils can improve their physical, chemical, and biological properties and to convert the problematic soils including wasteland into agricultural land or for re-vegetation purposes.

The present study showed that utilization of fly ash as a component in bio-fertilizer formulations is a safe and effective alternative, 12% of fly ash levels proved to be optimally useful for the growth of wheat plant. The use of this component increases to the cost of product by giving an advantage to soil and crops. The use of fly ash as a component in biofertilizer is effective to utilize problematic fly ash waste in a useful manner.

Now the further experiments were conducted by mixing cyanobacteria with fly ash in different concentrations where 12% fly ash was used as control sample. After the completion of experiments wheat samples were collected and analyzed for Carbohydrate, Starch, Protein, Phenol and Chlorophyll (a, b & total).

As experiment I stated that 12% fly ash gives optimum results ,so in the Experiment II control was then changed to Normal soil + 12% of the fly ash, with different concentration of *Anabaena* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%). In this experiment maximum concentration was observed for Carbohydrate (84.57 mg/g), Starch (71.23 mg/g), Protein (117.93 µg/g) and Phenol (47.23 mg/g) (Fig. 5.2 a, b, c, d) in the sample with 40% *Anabaena*. Under the same

treatment, the concentration was observed for (i) Chlorophyll a (1.51 mg/g), (ii) Chlorophyll b (0.796 mg/g) and Total Chlorophylls (2.30 mg/g) (Fig. 5.2e A, B, C).

The Experiment III was used performed with varying percentage of *Nostoc,* 12% of the fly ash used as a control with different concentration of *Nostoc* (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, & 100%). In this experiment maximum concentration was observed for Carbohydrate (84.73 mg/g), Starch (71.40 mg/g), Protein (124.67 µg/g) and Phenol (47.47 mg/g) (Fig.5.3 a, b, c, d) in the sample of 60% *Nostoc*. Under the same treatment, the concentration was observed for (i) Chlorophyll a 1.51mg/g (ii) Chlorophyll b 0.803 mg/g) and Total Chlorophylls (2.31 mg/g) (Fig. 5.3e A, B, C).

In the Experiment IV, 12% of the fly ash and 60% *Nostoc* (on the basis of experiment III) used as a control with different concentrations of *Anabaena* (10%, 20%,30%, 40%, 50%, 60%, 70%, 80%, 90%,100%). In this experiment maximum concentration was observed for Carbohydrate (86.70 mg/g), Starch (73.37 mg/g), Protein (138.47 µg/g) and Phenol (49.93 mg/g) (Fig.5.4 a, b, c) in the sample with 50% *Anabaena.* Under the same treatment, the concentration was observed for Chlorophyll a 1.54 mg/g, ii) Chlorophyll b 0.937 mg/g) and Total Chlorophyll 2.48 mg/g) (Fig. 5.4e A, B, C)

In the Experiment V, 12% of the fly ash and 40% *Anabaena* used as a control (on the basis of results of experiment II) with different concentration of *Nostoc* (10%, 20%,30%, 40%, 50%, 60%, 70%, 80%, 90%,100%). In this experiment maximum concentration was observed for Carbohydrate (87.03 mg/g), Starch (73.70 mg/g), Protein (145.00 µg/g) and Phenol (50.90 mg/g), (Fig.5.5 a, b, c, d) in the sample of 50% *Nostoc.* Under the same treatment the concentration was observed for

Chlorophyll a (1.56 mg/g), Chlorophyll b (0.939 mg/g) and Total Chlorophyll (2.50 mg/g) (Fig. 5.5e A, B, C).

The positive effect of Cyanobacteria (*Anabaena* and *Nostoc*) on the pigment (Chlorophyll a, b & Total Chlorophyll) and biomolecules (Carbohydrate, Protein, Phenol & Starch) of wheat plant were studied and their significant effects were observed.

The current study has shown that among the different percentage of *Anabaena* used (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), the maximum concentration was observed for Chlorophyll a,Chlorophyll b and Total Chlorophylls at 40% *Anabaena* (Fig. 6.5 A, B, C). Similarly, the concentration of Carbohydrate, Starch, Protein and Phenol of wheat plant was observed to a maximum at 40% *Anabaena*(Fig. 6.1, 6.2, 6.3, 6.4).

When *Nostoc* was used at different percentages (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) then maximum concentration observed for Chlorophyll a, Chlorophyll b and Total Chlorophylls were at 60% *Nostoc* (Fig. 6.5 A, B, C). Similarly, the concentration of Carbohydrate, Starch, Protein and Phenol of the wheat plant was observed to be maximum at 60% *Nostoc* (Fig. 6.1, 6.2, 6.3, 6.4).

This maximum concentration in the Experiment III was at 60% *Nostoc*. Then, the control was changed to this 60% *Nostoc.* The percentage of *Anabaena* was then varied (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%). In this experiment i.e. IV, maximum concentration for Chlorophyll a, Chlorophyll b and Total Chlorophyll was observed at 40% *Anabaena* and 60% *Nostoc* (6.5 A, B, C). Under the same treatment, the concentration was observed for Protein, Carbohydrate, and

Phenol (on 60% *Nostoc*) to be maximum with *Anabaena* 50%. So the best result for this experiment was observed with 50% *Anabaena* (Fig. 6.1, 6.2, 6.3, 6.4).

The optimum results were obtained in the Experiment II with 40% *Anabaena.* So in the experiment V, the control was 40% *Anabaena.* The percentage of *Nostoc* was then varied (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%). In this experiment, maximum concentration observed for Chlorophyll a, Chlorophyll b and Total Chlorophyll at 50% *Nostoc* (6.5 A, B, C). Under the same treatment, theconcentration for Carbohydrate, Starch, Protein and Phenol was highest at *Nostoc* 50%. So the best result was shown with 50% *Nostoc* (Fig. 6.1, 6.2, 6.3, 6.4).

Results of present study revealed increase in Carbohydrate, Starch, Protein, Phenol and Chlorophyll content of wheat plant. Increased content of phenol, protein and Chlorophyll in the leaves of Rice plant with cyanobacteria has also been recorded by Singh *et al.,* (2011).

According to the work of Pennell, (1992) and Bergan *et al.,* (1996) cyanobacteria form symbiotic relationships with plants and releases carbohydrate-rich arabino galactan proteins. These proteins are proposed to act as signaling molecules and play an important role in the regulation of plant growth and development.

Anabaena and *Calothrix sp.* were evaluated in a field experiment, for their role in improving the nutritional quality of wheat grains, in terms of protein content and important micronutrients (Fe, Cu, Zn and Mn) (Rana *et al.,* 2012). Hussain and Hasnain, (2011); Mazhar *et al.,* (2013) also observed significant enhancement in protein content of cyanobacterial inoculated plants.

In the present work an increase was observed in Chlorophyll of Experiment II - V, grown with cyanobacteria. Studies of Spiller and Gunasekaran, (1990); Nilsson, *et al*., (2005); Karthikeyan *et al*., (2007) stated that increased content of Chlorophyll in leaves could be caused by a higher amount of nitrogen assimilated by cyanobacteria from the atmosphere and delivered to plant tissues.

Similar results were obtained by Rai *et al.,* (2004) however they worked on*Prosopis juliflora* and stated that fly ash amended with BGA showed most luxuriant growth. These results are due to the ameliorating capacity of cyanobacteria in improving physicochemical properties, which supported the growth by using fly ash with cyanobacteria.

In some studies, all the plants grown in cyanobacteria amended fly ash showed maximum increase in all growth parameters as compared to control particularly Chlorophyll a, Chlorophyll b, total Chlorophyll, leaf protein (Rai *et al.,* 2004)

According to Tripathi *et al.,* (2008) integrated use of fly ash, cyanobacteria and nitrogen fertilizer is good for improved growth and mineral composition of the rice plants besides reducing the high demand of nitrogen fertilizers.

BGA proved to be best ameliorant for fly ash to support plant growth which may be due to the nitrogen fixed by cyanobacteria and supply of available phosphorus to the plant in soluble form and increased organic matter (Rai *et al.,* 2000)

The results found in this work are in line with the investigations of other researchers. In recent years cyanobacteria, have emerged as potential candidates for their application to develop environmentally friendly and sustainable agricultural practice (Singh *et al.,* (2016), Singh *et al*., (2017).The use of organic fertilizers, bio-

fertilizers and other microbial products are beneficial because it allows limiting chemical fertilizer application, which is harmful to the environment.

According to Ordog and Pulz, 1996; Masojidek and Prasil, 2010; Chojnacka *et al.,* 2010; Sahu *et al.,* 2012 the application of cyanobacteria can reduce chemical fertilizer to 50% and give better results to enhance wheat growth. The increased length of shoots and biomass of wheat could be caused by several physiological factors, including growth-promoting substances such as macronutrients (N, P, K, Ca, and Mg), microelements (S, Zn, Fe, Mn, Cu, Mo, Co) and several secondary metabolites that can be excreted by cyanobacteria.

Findings of present research work support the use of cyanobacteria with fly ash as biofertilizerfor wheat plant.

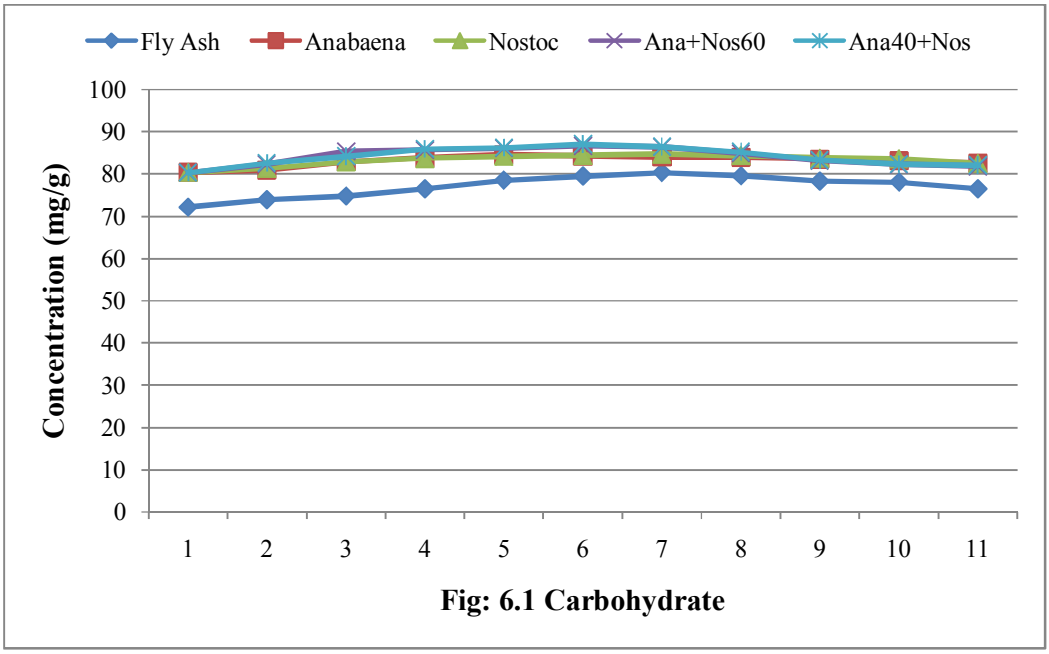


Fig: 6.1 Carbohydrate

Figure: 6.1 Effect of different concentrations of Fly ash, *Anabaena*, *Nostoc*, *Anabaena* (10%-100%) +*Nostoc 60*% &*Anabaena* 40% +*Nostoc* (10%-100%) mixed with soil on Carbohydrate (mg/g) status of the wheat

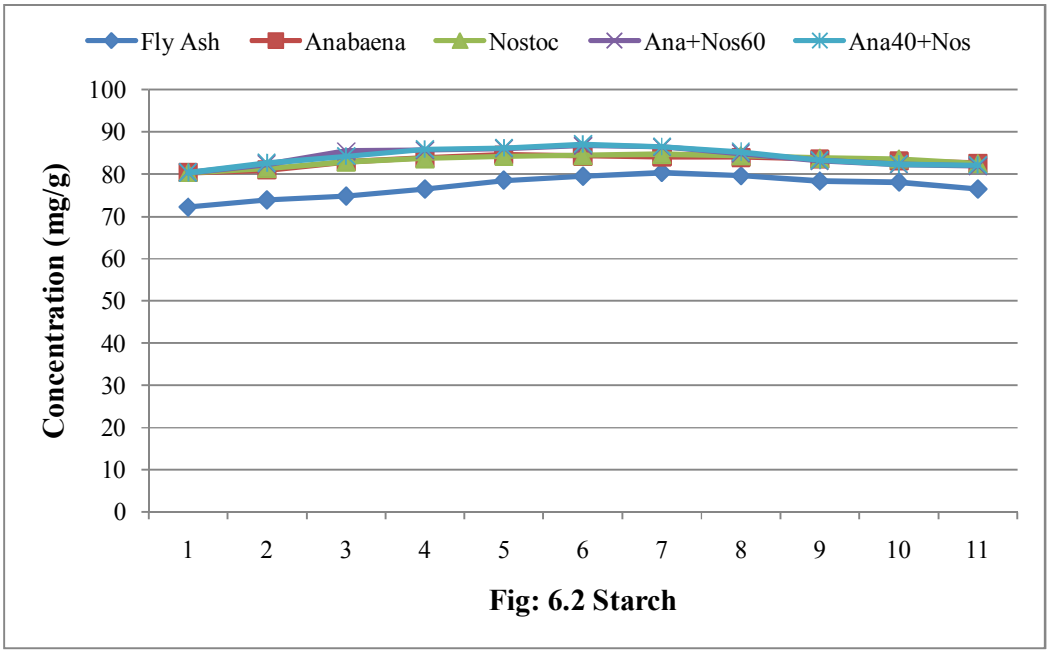


Fig: 6.2 Starch

Figure: 6.2 Effect of different concentration of Fly ash, *Anabaena*, *Nostoc*, *Anabaena* (10%-100%) +*Nostoc* 60%&*Anabaena* 40% +*Nostoc* (10% - 100%) mixed with soil on Starch (mg/g) status of the wheat

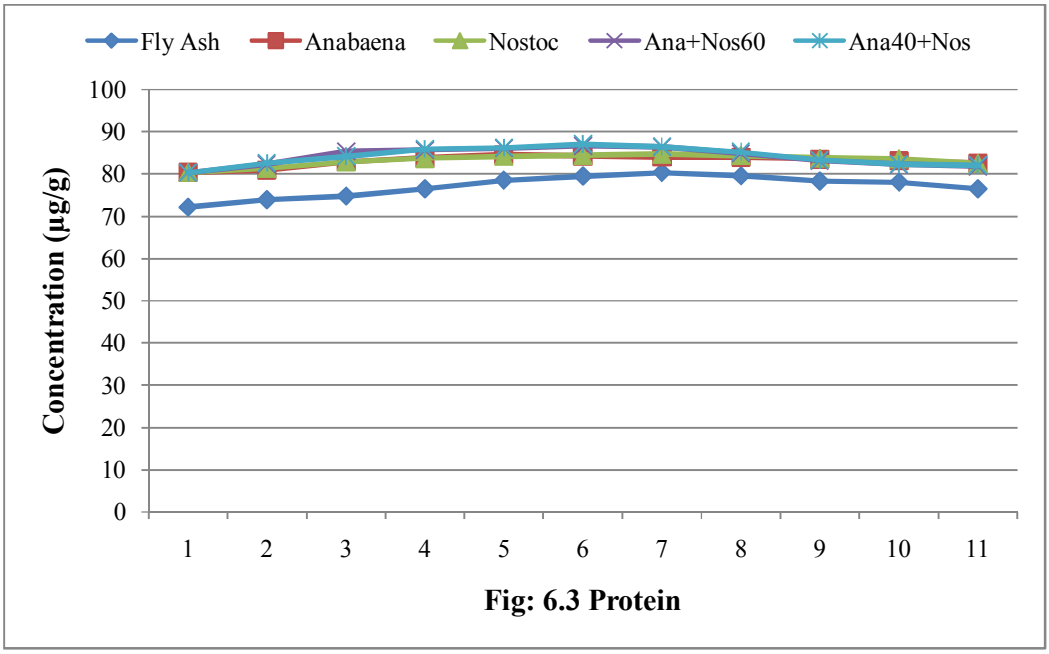


Fig: 6.3 Protein

Figure: 6.3 Effect of different concentrations of Fly ash,*Anabaena*, *Nostoc*, *Anabaena* (10%-100%) +*Nostoc* 60% &*Anabaena* 40% + *Nostoc* (10% -100%) mixed with soil on Protein (µg/g) status of the wheat

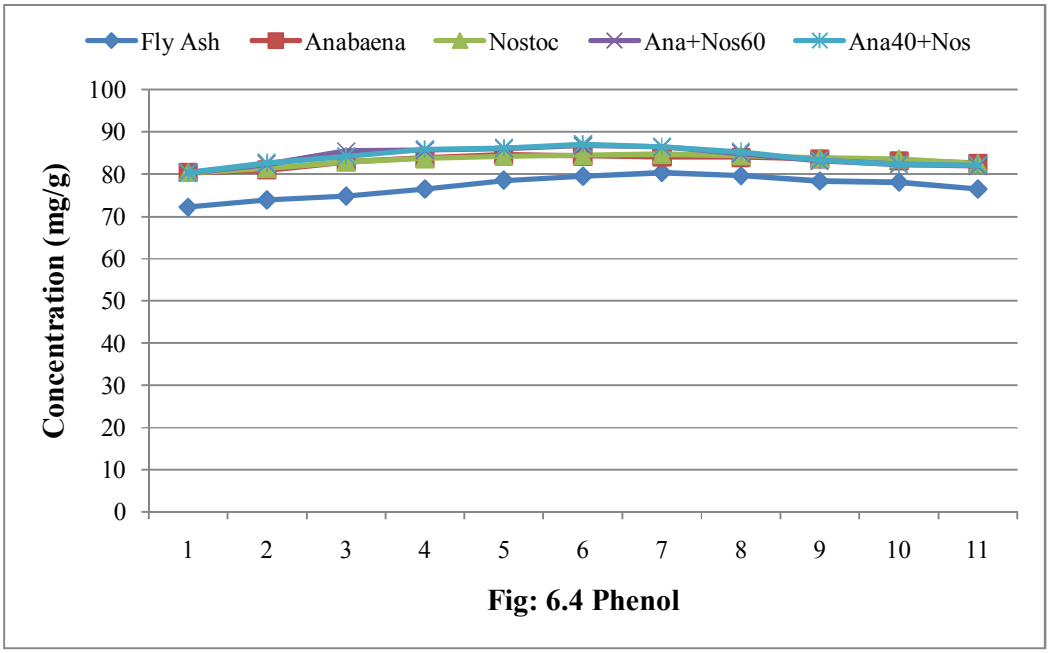


Fig: 6.4 Phenol

Figure: 6.4 Effect of different concentration of Fly ash, *Anabaena*, *Nostoc*, *Anabaena* (10% - 100%) + *Nostoc* 60% &*Anabaena* 40% + *Nostoc* (10% -100%) mixed with soil on Phenol (mg/g) status of the wheat

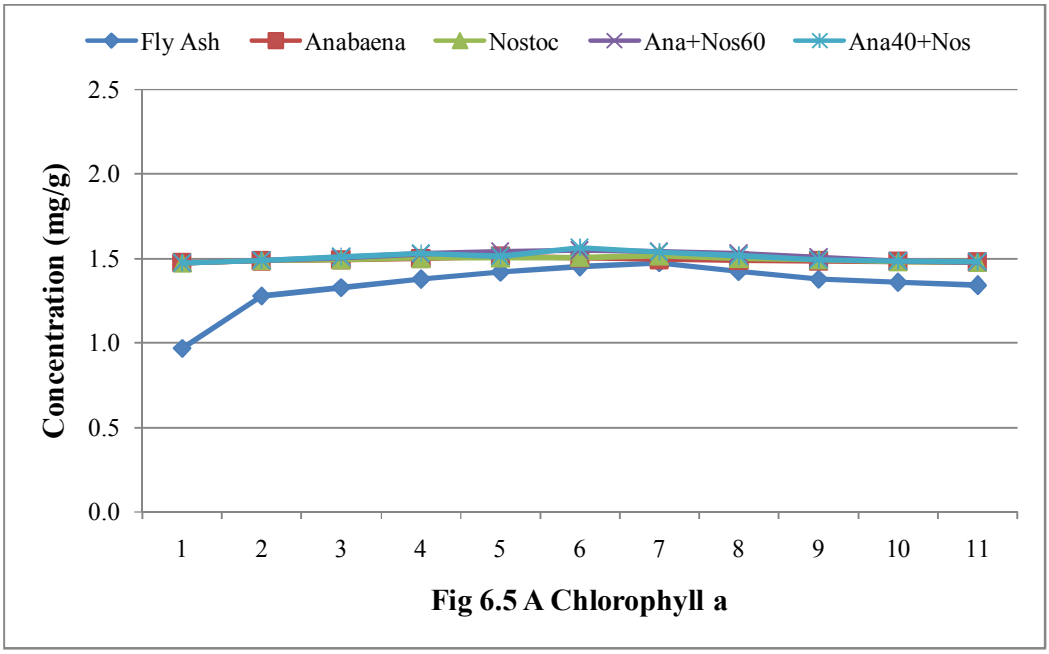


Fig 6.5 A Chlorophyll a

Figure: 6.5A Effect of different concentration of Fly ash, *Anabaena*, *Nostoc*, *Anabaena* (10% - 100%) + *Nostoc* 60% &*Anabaena* 40% + *Nostoc* (10% - 100%) mixed with soil on Chlorophyll a (mg/g) status of the wheat

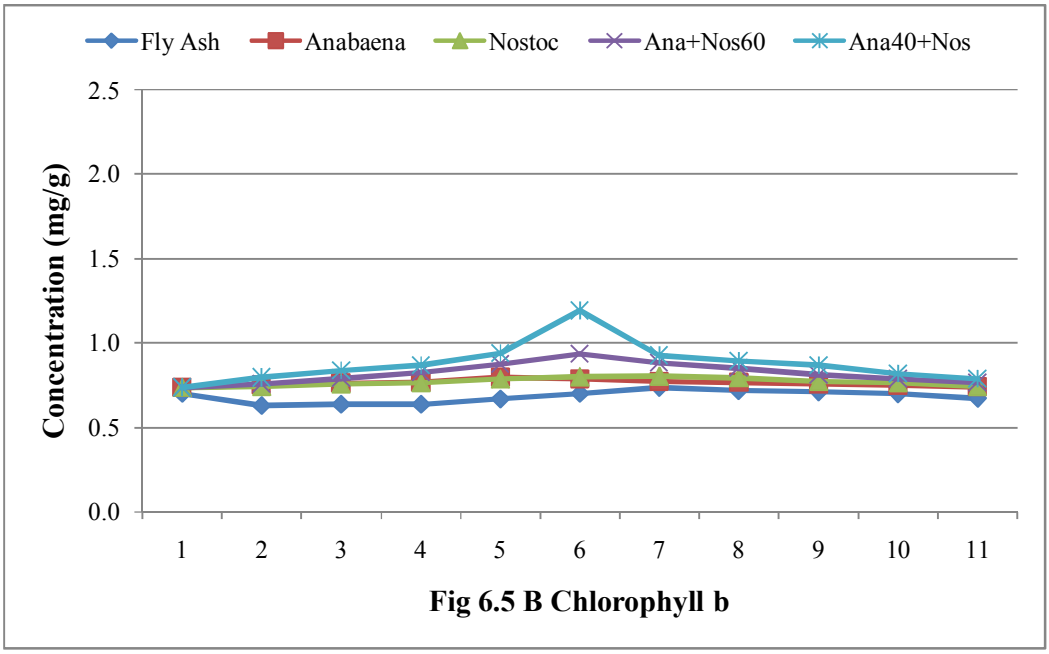


Fig 6.5 B Chlorophyll b

Figure: 6.5B Effect of different concentration of Fly ash, *Anabaena*, *Nostoc*, *Anabaena* (10%-100%) +*Nostoc* 60% &*Anabaena* 40% + *Nostoc* (10% - 100%) mixed with soil on Chlorophyll b (mg/g) status of the wheat

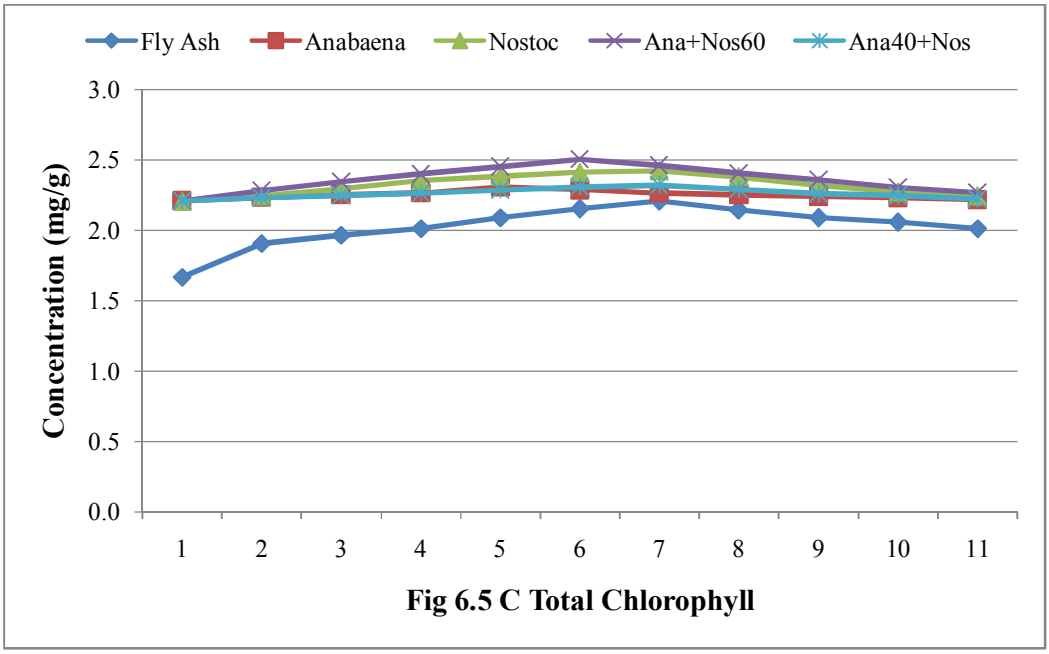


Figure: 6.5C Effect of different concentration of Fly ash, *Anabaena, Nostoc, Anabaena* (10%- 100%) +*Nostoc* 60% &*Anabaena* 40% + *Nostoc* (10%-100%) mixed with soil on Total Chlorophyll (mg/g) status of the wheat

Statistical Analysis

Overall result of the present study was analyzed statistically. All experiments were performed in 5 series and in five replicates for each treatment within each series. Experiments were carried out outdoor in pots. Every replicate under such conditions contained plants grown individually.

The effect was tested under field conditions. Within each series; each replicate was set up in a completely randomized block design. An effect of the fly ash with cyanobacteria was studied on biochemically. The obtained data, given as means from series and replicates, were processed applying analysis of variance (ANOVA).

One-way ANOVA (Analysis of One Variable) is used to study the hypothesis that concentration of Carbohydrate, Starch, Protein, Phenol, Chlorophyll a, Chlorophyll b & Total Chlorophyll depends on the category chosen, i.e., control, soil

with (2% - 20%) fly ash, soil with fly ash (12%) + *Anabaena* (10% - 100%), soil with fly ash(12%) + *Nostoc* (10% - 100%), soil with fly ash + *Anabaena* (10% - 100%) + *Nostoc* (60%), soil with fly ash + *Anabaena* (40%) + *Nostoc* (10% - 100%).

The estimations of Carbohydrate, Starch, Protein, Phenol, Chlorophyll a, Chlorophyll b & Total Chlorophyll for different groups are tabulated respectively in the Table: 6.1, 6.2, 6.3, 6.4 & 6.5.

ANOVA Single Factor tool was used to calculate the Fvalue.

One-way ANOVA is a hypothesis test that allows tocompare more groups' means.

As Fval>Fcrit: Significant Difference (with different groups)

It shows that there is a significance difference on the biochemical status of wheat, grown with fly ash mixed with cyanobacteria as biofertilizer. Here the p-value of significance of ANOVA is 0.05.

First of all the ANOVA (Analysis of One Variable) was performed to test the hypothesis that concentration of Carbohydrate depends on the category chosen, i.e., control, soil with fly ash, soil with fly ash + *Anabaena*, soil with fly ash + *Nostoc*, soil with fly ash + *Anabaena* + *Nostoc*(60%), soil with fly ash + *Anabaena* (40%)+ *Nostoc*.

Table 6.1a shows the effect of different concentrations of Fly ash*, Anabaena, Nostoc, Anabaena* (10% - 100%) + *Nostoc* (60%)& *Anabaena* (40%) + *Nostoc* (10% - 100%) mixed in soil on Carbohydrate (mg/g) status of the wheat.

The estimations of carbohydrates for different groups are tabulated in Table 6.1b ANOVA Single Factor tool was used to calculate the F-value (Table 6.1c).

The summary (Table 6.1b) indicate that the mean range from a low of 77.1543 for fly ash to high of 84.1666 for *Anabaena* + *Nostoc*(10 - 100%).

These sample means are different.

Here Fval = 24.1585

Fcri = 2.55717

As Fval > Fcrit: Significant Difference (with different groups)

Thus it represents that Carbohydrate content is showing significant increase.

Further the ANOVA (Analysis of One Variable) performed to test the hypothesis, that concentration of Starch depends on the category chosen, i.e., control, soil with (2% - 20%) fly ash, soil with fly ash (12%) + *Anabaena* (10% - 100%), soil with fly ash (12%) + *Nostoc* (10 - 100%), soil with fly ash + *Anabaena* (10% - 100%) + *Nostoc* (60%), soil with fly ash + *Anabaena* (40%) + *Nostoc* (10%-100%).

Table 6.2a shows the effect of different concentrations of fly ash, *Anabaena, Nostoc*, *Anabaena* (10% - 100%) + *Nostoc* (60%)&*Anabaena* (40%) + *Nostoc* (10% - 100%) mixed in soil on Starch (mg/g) status of the wheat crop.

Estimations of Starch for different groups are tabulated in Table 6.2b Single Factor tool was used to calculate the Fvalue (Table 6.2c).

The summary (Table 6.2 b) indicate that the mean range from a low of 58.0849 for fly ash to high of 71.0364 for *Anabaena* (10 - 100%) + *Nostoc*(60%).

These sample means are different.

Here Fval = 33.37570481

Fcri = 2.55717915

As Fval > Fcrit: Significant Difference (with different groups)

It shows significant effect of biofertilizer on Starch content of plant.

Further the ANOVA (Analysis of One Variable) performed to test the hypothesis, that concentration of Protein depends on the category chosen, i.e., control, soil with (2% - 20%) fly ash, soil with fly ash (12%) + *Anabaena* (10% - 100%), soil

with fly ash (12%) + *Nostoc* (10 - 100%), soil with fly ash + *Anabaena* (10% - 100%) + *Nostoc* (60%), soil with fly ash + *Anabaena* (40%)+ *Nostoc* (10% - 100%).

Table 6.3a shows the effect of different concentrations of fly ash, *Anabaena, Nostoc, Anabaena* (10% - 100%) + *Nostoc* 60% &*Anabaena* (40%) + *Nostoc* (10% - 100%) mixed in soil on Protein (µg/g) status of the wheat crop.

Estimations of Protein for different groups are tabulated in Table 6.3b ANOVA Single Factor tool was used to calculate the Fvalue (Table 6.3c).

The summary (Table 6.3b) indicate that the mean range from a low of 65.0848 for fly ash to high of 121.6787 for *Anabaena*(40%) + *Nostoc*(10 - 100%).

These sample means are different.

Here Fval = 26.92722722

Fcri = 2.55717915

Fval > Fcrit: Significant Difference (with different groups)

Analysis revealed significant effect on Protein content of plants grown with biofertilizer.

Further the ANOVA (Analysis of One Variable) performed to test the hypothesis, that concentration of Phenol depends on the category chosen, i.e., control, soil with (2% - 20%) fly ash, soil with fly ash (12%) + *Anabaena* (10% - 100%), soil with fly ash (12%) + *Nostoc* (10 - 100%), soil with fly ash + *Anabaena* (10%-100%) + *Nostoc* (60%), soil with fly ash + *Anabaena*(40%) + *Nostoc* (10% - 100%).

Table 6.4a shows the effect of different concentrations of fly ash, *Anabaena, Nostoc, Anabaena* (10%-100%) + *Nostoc* (60%)& *Anabaena* (40%) + *Nostoc* (10% - 100%) mixed in soil on Phenol (mg/g) status of the wheat crop.

The estimations of Phenol for different groups are tabulated in Table 6.4b ANOVA Single Factor tool was used to calculate the Fvalue (Table 6.4c).

The summary (Table 6.4b) indicate that the mean range from a low of 32.0121 for fly ash to high of 44.1757 for *Anabaena*(10 - 100%) + *Nostoc*(60%).

These sample means are different.

Here Fval=19.3817389

Fcri=2.55717915

As Fval > Fcrit: Significant Difference (with different groups)

Test is showing significant effect on Phenol of tested plants.

Further the ANOVA (Analysis of One Variable) performed to test the hypothesis, that concentration of Chlorophyll depends on the category chosen, i.e., control, soil with (2% - 20%) fly ash, soil with fly ash (12%) + *Anabaena* (10% - 100%), soil with fly ash (12%) + *Nostoc* (10% - 100%), soil with fly ash + *Anabaena* (10% - 100%) + *Nostoc* (60%), soil with fly ash + *Anabaena* (40%) + *Nostoc* (10% - 100%).

This would include ANOVA for Chlorophyll a, Chlorophyll b and Total Chlorophyll.

Table 6.5A (i) shows the effect of different concentrations of fly ash, *Anabaena, Nostoc, Anabaena* (10%-100%) + *Nostoc* 60% & *Anabaena* 40% + *Nostoc* (10% -100%) mixed in soil on Chlorophyll a (mg/g) status of the wheat crop.

The estimations of Chlorophyll a for different groups are tabulated in Table 6.5A(ii) ANOVA Single Factor tool was used to calculate the Fvalue (Table 6.5A(iii).

The summary (Table 6.5A(ii) indicate that the mean range from a low of 1.3451 for fly ash to high of 1.5112 for *Anabaena*(10 - 100%) + (*Nostoc* 60%).

These sample means are different.

Here Fval = 13.08869455

Fcri = 2.55717915

As Fval > Fcrit: Significant Difference (with different groups)

Test is showing significant on Chlorophyll a of tested plants.

Table 6.5B (i) shows the effect of different concentrations of fly ash, *Anabaena, Nostoc, Anabaena* (10%-100%) + *Nostoc* (60%) & *Anabaena,* (40%) + *Nostoc* (10% -100%) mixed in soil on Chlorophyll b (mg/g) status of the wheat crop.

The estimations of Chlorophyll b for different groups are tabulated in Table 6.5 B(ii) ANOVA Single Factor tool was used to calculate the Fvalue (Table 6.5B(iii).

The summary (Table 6.5B(ii) indicate that the mean range from a low of 0.682597091 for fly ash to high of 0.877712 for + *Anabaena* (10-100%) + *Nostoc* (60%).

These sample means are different.

Here Fval = 14.14442431

Fcri = 2.55717915

As Fval > Fcrit: Significant Difference (with different groups)

Test is showing significant on Chlorophyll b of tested plants.

Table 6.5C(i) shows the effect of different concentrations of fly ash, *Anabaena, Nostoc, Anabaena* (10% - 100%) + *Nostoc* (60%)& *Anabaena*(40%) + *Nostoc* (10% - 100%) mixed in soil on Total Chlorophyll (mg/g) status of the wheat crop.

The estimations of Total Chlorophyll for different groups are tabulated in Table 6.5C(ii) ANOVA Single Factor tool was used to calculate the Fvalue (Table 6.5C(iii).

The summary (Table 6.5C (ii) table indicate that the mean range from a low of 2.0272 for fly ash to high of 2.3856 for *Anabaena*(40%) + *Nostoc*(10 -100%). These sample means are different.

Here Fval = 13.08869455

Fcri = 2.55717915

As Fval > Fcrit: Significant Difference (with different groups)

Test is showing significant on Total Chlorophyll of tested plants.

Results of the present study also revealed important finding that the use of fly ash in experiment I showed comparatively lower range of biomolecules &pigments while in experiment II, III, IV, & V where cyanobacteria was mixed in fly ash showed higher range of biomolecules &pigments composition was observed (Fig. 6.1 - 6.5). It shows that mixing of cyanobacteria with fly ash is more beneficial than single use of fly ash.

On the basis of overall study, it can be concluded that when the wheat plants are grown in soil containing fly ash 12% mixedwith *Anabaena* (40%) and *Nostoc*(60%) that was used as biofertilizer, the concentration of biomolecules i.e. Carbohydrate, Starch, Protein, Phenol, Chlorophyll a, Chlorophyll b & Total Chlorophyll was optimum.

Finally the present study revealed that the fly ash mixed with cyanobacteria as biofertilizer in combination of: 12% fly ash + (40% - 50%)*Anabaena* + (50%-60%)*Nostoc* is best for the wheat plant.

Table 6.1a - Estimation of Carbohydrate with different Groups with ANOVA

Samples	Fly ash	*Anabaena*	*Nostoc*	*Anabaena +Nostoc*60	*Anabaena*40+*Nostoc*
1	72.200	80.400	80.400	80.400	80.400
2	73.933	80.933	81.367	82.367	82.633
3	74.833	82.967	82.933	85.400	84.167
4	76.533	83.867	83.700	85.767	85.900
5	78.600	84.567	84.233	86.067	86.200
6	79.533	84.300	84.433	86.700	87.033
7	80.400	84.067	84.733	86.400	86.500
8	79.633	83.967	84.367	84.933	85.267
9	78.367	83.600	83.833	83.167	83.300
10	78.133	83.067	83.600	82.300	82.400
11	76.533	82.600	82.533	81.900	82.033

Table 6.1b- ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	848.698	77.1543636	6.79376025
Anabaena	11	914.335	83.1213636	1.83668025
Nostoc	11	916.132	83.2847273	1.87471462
*Anabaena+Nostoc*60	11	925.401	84.1273636	4.67174085
*Anabaena*40+*Nostoc*	11	925.833	84.1666364	4.69746005

Table 6.1c

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	384.123973	4	96.0309932	24.1595232	2.55717915
Within Groups	198.74356	50	3.97487121		
Total	582.867533	54			

P = 0.05

Table 6.2a- Estimation of Starch with different Groups with ANOVA

Samples	Fly Ash	*Anabaena*	*Nostoc*	*Anabaena* +*Nostoc*60	*Anabaena*40+*Nostoc*
1	48.867	67.067	67.067	67.067	67.067
2	50.600	67.600	68.033	71.700	69.300
3	54.833	69.633	69.600	72.067	70.833
4	59.867	70.533	70.367	72.433	72.567
5	61.933	71.233	70.900	72.733	72.867
6	66.200	70.967	71.100	73.367	73.700
7	67.067	70.733	71.400	73.067	73.167
8	63.200	70.633	71.033	71.600	71.933
9	58.367	70.267	70.500	69.833	69.967
10	54.800	69.733	70.267	68.967	69.067
11	53.200	69.267	69.200	68.567	68.700

Table 6.2b - ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	638.934	58.0849091	37.6981629
Anabaena	11	767.666	69.7878182	1.83592576
Nostoc	11	769.467	69.9515455	1.87488187
Anabaena+*Nostoc*60	11	781.401	71.0364545	4.37887827
*Anabaena*40+*Nostoc*	11	779.168	70.8334545	4.69724007

Table 6.2c

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	1347.98034	4	336.995085	33.3757048	2.55717915
Within Groups	504.850889	50	10.0970178		
Total	1852.83123	54			

P = 0.05

Table 6.3a - Estimation of Protein with different Groups with ANOVA

Samples	Fly Ash	*Anabaena*	*Nostoc*	*Anabaena* +*Nostoc*60	*Anabaena*40+*Nostoc*
1	56.267	74.600	74.600	74.600	74.600
2	59.067	95.067	97.600	116.600	116.733
3	60.067	102.533	100.733	123.867	123.800
4	63.600	109.867	105.200	127.933	130.733
5	70.533	117.933	112.267	132.333	136.000
6	74.333	110.533	119.867	138.467	145.000
7	74.600	105.600	124.667	131.400	133.467
8	70.933	99.600	116.933	123.267	130.867
9	65.000	95.333	109.733	115.467	121.733
10	61.867	93.133	101.933	112.467	117.200
11	59.667	91.467	95.200	102.667	108.333

Table 6.3b - ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	715.934	65.0849091	42.1017007
Anabaena	11	1095.666	99.606	137.736592
Nostoc	11	1158.733	105.339364	192.677258
Anabaena+*Nostoc*60	11	1299.068	118.097091	312.226782
*Anabaena*40+*Nostoc*	11	1338.466	121.678727	349.788516

Table 6.3c

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	22285.6441	4	5571.41102	26.9272349	2.55717915
Within Groups	10345.3085	50	206.90617		
Total	32630.9526	54			

P = 0.05

Table 6.4a - Estimation of Phenol with different Groups with ANOVA

Samples	Fly Ash	*Anabaena*	*Nostoc*	*Anabaena* +*Nostoc*60	*Anabaena*40+*Nostoc*
1	22.833	39.133	39.133	39.133	39.133
2	25.267	39.567	41.400	42.533	40.367
3	27.200	40.533	41.200	44.400	42.233
4	31.400	44.067	42.367	46.600	44.033
5	36.600	47.233	42.700	47.700	47.633
6	38.767	44.767	46.133	49.933	50.900
7	39.133	44.367	47.467	47.367	47.967
8	37.800	43.800	44.300	46.433	46.133
9	33.667	43.267	42.133	41.867	42.433
10	31.333	41.533	41.067	40.600	40.967
11	28.133	39.967	39.933	39.367	40.333

Table 6.4b - ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	352.133	32.0120909	32.2415015
Anabaena	11	468.234	42.5667273	6.68705122
Nostoc	11	467.833	42.5302727	6.41717422
Anabaena+*Nostoc*60	11	485.933	44.1757273	13.6141142
*Anabaena*40+*Nostoc*	11	482.132	43.8301818	14.588947

Table 6.4c

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	1140.38547	4	285.096368	19.3814457	2.55717915
Within Groups	735.487881	50	14.7097576		
Total	1875.87335	54			

P = 0.05

Table 6.5A (i): - Estimation of Chlorophyll a with different Groups with ANOVA

Samples	Fly Ash	*Anabaena*	*Nostoc*	*Anabaena*+*Nostoc*60	*Anabaena*40+*Nostoc*
1	0.967	1.473	1.473	1.473	1.473
2	1.277	1.486	1.487	1.488	1.487
3	1.327	1.492	1.491	1.504	1.509
4	1.375	1.499	1.500	1.528	1.532
5	1.420	1.512	1.501	1.539	1.513
6	1.451	1.501	1.507	1.547	1.563
7	1.473	1.493	1.517	1.540	1.538
8	1.424	1.487	1.499	1.530	1.514
9	1.376	1.485	1.491	1.507	1.492
10	1.361	1.481	1.482	1.485	1.485
11	1.340	1.478	1.478	1.478	1.482

Table 6.5A (ii) - ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	14.796676	1.34515	0.01894
Anabaena	11	16.39352	1.49032	0.00012
Nostoc	11	16.431677	1.49378	0.00016
Anabaena+*Nostoc*60	11	16.623983	1.51127	0.00074
*Anabaena*40+*Nostoc*	11	16.594308	1.50857	0.00077

Table 6.5A (iii)

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Between Groups	0.21732485	4	0.054331212	13.08869455	2.55717915
Within Groups	0.207550158	50	0.00415003		
Total	0.424875007	54			

P = 0.05

Table 6.5B (i) - Estimation of Chlorophyll b with different Groups with ANOVA

Samples	Fly Ash	*Anabaena*	*Nostoc*	*Anabaena* +*Nostoc*60	*Anabaena*40+*Nostoc*
1	0.699	0.736	0.736	0.736	0.736
2	0.629	0.749	0.742	0.758	0.795
3	0.637	0.760	0.758	0.791	0.835
4	0.637	0.766	0.766	0.826	0.868
5	0.668	0.797	0.785	0.876	0.940
6	0.701	0.787	0.801	0.937	1.190
7	0.736	0.771	0.804	0.883	0.925
8	0.719	0.764	0.793	0.849	0.892
9	0.711	0.756	0.772	0.812	0.868
10	0.699	0.749	0.764	0.787	0.820
11	0.672	0.739	0.743	0.765	0.786

Table 6.5B (ii) - ANOVA: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fly ash	11	7.508568	0.68259709	0.00133137
Anabaena	11	8.374864	0.76135127	0.00034896
Nostoc	11	8.463774	0.769434	0.00056215
Ana+*Nos*60	11	9.019498	0.81995436	0.00376057
*Anabaena*40+*Nostoc*	11	9.654832	0.877712	0.01447583

Table 6.5B (iii)

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*Fval*	*F crit*
Between Groups	0.23172945	4	0.05793236	14.1444243	2.55717915
Within Groups	0.20478869	50	0.00409577		
Total	0.43651813	54			

P = 0.05

Table 6.5C (i): - Estimation of Total Chlorophyllwith different Groups with ANOVA

Total Chlorophyll					
Samples	**Fly Ash**	***Anabaena***	***Nostoc***	***Anabaena +Nostoc60***	***Anabaena40+Nostoc***
1	1.666568	2.209448	2.209448	2.209448	2.209448
2	1.905864	2.235232	2.244114	2.281662	2.229172
3	1.963788	2.252164	2.29491	2.343746	2.249342
4	2.012028	2.265056	2.353756	2.39977	2.265858
5	2.088548	2.308604	2.415484	2.703482	2.286444
6	2.151404	2.288464	2.483688	2.502996	2.307416
7	2.209448	2.263868	2.422346	2.463132	2.319892
8	2.143444	2.250976	2.379184	2.406306	2.291702
9	2.087124	2.241292	2.318318	2.359164	2.262264
10	2.059736	2.230004	2.271978	2.304684	2.245748
11	2.011376	2.216696	2.243342	2.267552	2.221538

Table 6.5C (ii) - ANOVA: Single Factor

SUMMARY				
Groups	***Count***	***Sum***	***Average***	***Variance***
Fly ash	11	22.299328	2.027211636	0.021984622
Anabaena	11	24.761804	2.251073091	0.000877327
Nostoc	11	25.636568	2.330597091	0.007658792
Anabaena+Nostoc60	11	26.241942	2.385631091	0.01862375
Anabaena40+Nostoc	11	24.888824	2.262620364	0.001280545

Table 6.5C (iii)

ANOVA					
Source of Variation	***SS***	***df***	***MS***	***F***	***F crit***
Between Groups	**0.82144246**	**4**	**0.205360615**	**20.3629618**	**2.55717915**
Within Groups	**0.504250357**	**50**	**0.010085007**		
Total	**1.325692816**	**54**			

P = 0.0

CHAPTER 7
SUMMARY

Coal-based thermal power plants have been a significant source of power generation in India, where about 75% of the total power obtained is from coal-based thermal power plants. Fly ash is a byproduct material which is generating from thermal power plants. The total fly ash production in India has been 118 million metric tons. The fly ash production is estimated to be 440 million metric tons by 2030.Disposal of high amount of fly ash from Thermal Power Plants absorbs huge amount of water, energy and land area by ash ponds.

Fly ash can be used as a soil fertilizer that may improve physical, chemical and biological properties of soil, because italso enriched with macro and micro nutrients. However, the amount of nitrogen in fly ash is very low.But it can be used by mixing nitrogen-containing elements in fly ash. Thus, by adding nitrogen-containing elements to the fly ash, it can be used for the growth of plants. In this context cyanobacteria was used to fulfill nitrogen deficiency in flyash.

Different concentrations of cyanobacteria i.e. *Anabaena*&*Nostoc* were mixed with fly ash. The both cyanobacterial genera are easy to grow in natural conditions, so chosen for the study, but most of the work with them conducted on Rice than other crops. Here wheat is selected as experimental plant. As it fulfills the need of food of people and used in different preparations like chapaties, daliya, bread, pizza, biscuits etc.

In the present study, the objective was to study effect of fly ash mixed with cyanobacteria on wheat plant. The method implemented in this study could be used

for fly ash inoculants routinely prepared in large quantities for agricultural use as Biofertilizers.

Addition of fly ash to the soil of poor buffering capacity increases soil pH due to presence of basic metal oxides and alters the availability of some nutrients. Fly ash can be used for reclaiming the problematic soil and enhance the biomolecules and chlorophyll pigment of wheat depending upon the nature of soil and fly ash. It affects physical, chemical and biological properties of soil and has impact on the available macro and micronutrients of plants.

Suratgarh Thermal Power Station (STPS) is one of coal based thermal power station of Rajasthan situated at Suratgarh, Sriganganagar and it fulfills the energy requirement of the region. It, however, has an adverse impact on environment i.e., atmospheric pollution by gaseous emission and solid waste pollution by ash dumping on land. Due to the large quantities of ash generated, there is an increasing scarcity of land for disposal of ash. The present ash disposal systems which are in use are causing serious disposal and ecological problems due to contamination of surface &groundwater and surrounding air causing serious problems to various biotic life forms. The inspiration for the presented study was in the light of above facts, i.e. to study the impacts of flyashmixed with 'Cyanobacteria' on the growth performance of 'Wheat Plant'.

Present study can be summarized as following-

- Collection of soil sample and collection of flyash.
- Physiochemical analysis of soil and flyash.

- Collection of cyanobacteria from ICAR (Indian Council of Agricultural Research), New Delhi and its*In-vitro*culture to scale up as per requirement of the work.
- The preparation of biofertilizerby using the different concentration of fly ash and cyanobacteria in soil.
- The setup of pot culture experiments of wheat using fly ash and cyanobacteria mixed as a biofertilizer.
- The Biochemical Analysis i.e. Carbohydrate, Starch, Protein, Phenol and Pigments (Chlorophyll a, Chlorophyll b & Total Chlorophyll) of wheat samples from pot culture experiments to find out optimum concentrations.

Soil samples were collected from Thakrawali village, Sriganganagar i.e. site of study. The fly ash was collected from Suratgarh Thermal Power Plant. Certified seeds of wheat plants (Raj-1482) were collected from Rajasthan State Seed Corporation Limited. Physicochemical analysis of soil and flyash was performed at CEG Test House and Research Center, Jaipur.

The testing of some heavy metals like cadmium, lead, zinc and nickel was performed at Soil Testing Laboratory, Department of Agriculture, Government of Rajasthan,Hanumangarh. This analysis suggested that flyash is a potential source of many macro and micro element to the plants and also including some heavy metals.

A set of 5 experiments was performed to observe the optimum levelsof fly ash mixed with cyanobacteriabiofertilizerand their effect on growth of wheat plant. Each set of pot culture experiment was performed in replica of five. So, two hundred and fifty-five pots were used to setup these experiments.

Table:1 Design of Pot Culture Experiments

S. No.	Experiments (combinations)	Number of Pots
1	Experiment I (Soil + Fly ash (0%- 20%)	10×5+5(control) =55
2	Experiment II (Soil + 12% Fly ash + *Anabaena* (10% - 100%)	10×5=50
3	Experiment III (Soil + 12% Fly ash + *Nostoc* (10% - 100%)	10×5=50
4	Experiment IV (Soil + 12%Fly ash + *Nostoc* 60% + *Anabaena* (10% - 100%)	10×5=50
5	Experiment V (Soil + 12%Fly ash + *Anabaena* 40% + *Nostoc* (10% -100%)	10×5=50

Air dried samples of soil and flyash were mixed accordingly i.e. 0%, 2%,4%, 6%,8%,10%,12%,14%,16%,18% and 20%fly ash by weight. Here normal soil with 0% flyash was control sample.

These different batches of soil were used for wheat production.For analyzing the best impact on growth performance and biochemistry, samples of wheat were analyzed biochemically– Carbohydrate contents by method ofDubois *et al*., (1956), Protein contents by method of Lowry*et al*., (1951), Phenol content by method of Malik and Singh, (1980), Starch contents by method of Anthrone reagent, (Henson and Moller, 1975) and Chlorophyll content by method of Arnon, (1949). Then reading were recorded and calculated.

It was observed that soil with 12% flyash produced maximum concentration of Biomolecules (Carbohydrate, Starch, Protein and Phenol) and Chlorophyllpigment (Chlorophyll a, b & Total Chlorophyll).

In the experiments, the initial control was set to be normal soil. Later, in the subsequent experiments control was changed to normal soil fixed with 12% fly ash (which produced maximum concentrations for the photosynthetic pigments and biomolecules). This changed control was then used with different concentrations of cyanobacteria i.e. *Anabaena&Nostoc* were added to this control (Table-1) to estimate the maximum concentration of photosynthetic pigments and biomolecules.

To analyze the impact of flyash mixed with cyanobacteria on biochemistry of wheat, results of various experiments (pot culture andbiochemical analysis) are presented in tables, images and graphs.

In order to test the significance of data, the statistical analysis of recorded data was performed. The method of standard deviation is used to find out the deviation between various parameters of present study. Interrelationship between various parameters, are presented by graphs. ANOVA one-way statistical tool was applied.

Physico-chemical analysis of flyash collected from Thermal Power Station showed that flyash is slightly alkaline in nature and a potential source of macro and micro nutrients. During the present work soil properties is influenced by fly ash addition in soil as improvement reported in wheat plants.

In biochemical analysis the value of chlorophyll and biochemical contents are also considered as an important factor during growth and differentiation of cells in

plants. Carbohydrate, Starch, Protein, Phenol, Chlorophyll a, Chlorophyll b and Total Chlorophyll contents, showed increase after using fly ash mixed with soil.Results are also indicating negative impact of higher concentration of fly ash on which further research can be done.

In light of the above facts, it is concluded that 12% fly ash mixed with cyanobacteria is optimum for wheat plant. So fly ash can be used mix with cyanobacteria as biofertilizer in the future for soil amendments and wheat production in different ways.

Our ultimate effort is to study the impact of fly ash mix with cyanobacteria on biochemical analysis of wheat. It will be suggested that thegenera can be used for the inoculation on wheat after open field investigation. It may enhance the nitrogen fixation which leads to improvement in soil quality. Cyanobacteria proved to be the best agent for flyash to support plant growth which may be due to the nitrogen fixed by cyanobacteria.

Results of the present study also revealed important finding that the use of flyash in experiment I showed comparatively lower range of biomolecules & pigments while in experiment II, III, IV, & V where cyanobacteria was mixed with flyash showed higher range of biomolecules & pigments composition. It shows that mixing of cyanobacteria with flyash is more beneficial than single use of flyash.

On the basis of overall study, it can be concluded that when the wheat plants are grown in soil containing 12% flyash mixed with 40% *Anabaena* and60% *Nostoc,*

which was used as biofertilizer, the concentration of biomolecules i.e. Carbohydrate, Starch, Protein, Phenol, Chlorophyll a, Chlorophyll b& Total Chlorophyll was optimum.

Finally the study revealed that the combination: 12% fly ash + 40%-50% *Anabaena* + 50%-60% *Nostoc* isbest as biofertilizerin soil for wheat plant.

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J. Phytol. Res. **30** (1 & 2) : 83-88, 2017 **ISSN70-5767**

EFFECT OF FLY ASH APPLICATION ON THE CHLOROPHYLL CONTENT OF WHEAT (*Triticum aestivum)*

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Thermal power plants use pulverized coal as a fuel source to generate power by obtaining fly ash as a by-product. Its generation in the country has increased from 40 Million ton (MT)/yr (1994) to about 235 MT/yr (2013). It is projected to be 325 MT/yr (2016-17), 500 MT/yr (2021-22) and 1000 MT/yr (2031-32). If it is not used, then it would demand large area of land for ash ponds and would pose a threat for air and water pollution. Fly ash utilization has increased from 1 MT/yr during 1994 to 130 MT/year during 2013, primarily as an outcome of concerted efforts under Fly Ash Mission-India. In the present study, it was shown that utilization of fly-ash as a carrier in bio-fertilizer formulations emerged as safe and effective alternatives. Use of fly-ash as a carrier in these formulations is an effective way of utilization of problematic fly-ash waste in a useful manner. Fly ash has similar physicochemical properties with soil. Fly ash addition to the soil in different doses improves photosynthetic pigments concentration beneficial for a wheat plant. We can conclude that though fly ash is a waste of concern but now has become a boon for sustainableagriculture

Keywords: DAT; Fly ash; Photosynthetic pigments; Sustainable agriculture; Wheat.

Introduction

Wheat is the second most important food crop of the country after rice both in areaand production. The total area under the crop is about 29.8 million hectares in the country. India stands second in the production of wheat in the world contributing over 13 percent of the total area and 12 percent of the total production of wheat in the world. Wheat is a species of *Poaceae* Family and it has caryopsis fruit. In India, it is a winter crop grown in Rabi season with a temperature between 10-l5°C and rainfall between5-15cm.Wheat cropping season is from October-November to March-April in Rajasthan. There are many species of wheat which together make up the genus *Triticum* the most widely grown is common wheat (*T. aestivum*). Fly ash which is a by-product of Thermal power plants also plays an important role and combination of fly ash mixed with soil. Fly ash has similar physicochemical properties with soil. It can mix homogeneously and can improve agronomicproperties of soil. The $_1$ high concentration of micronutrient and macronutrient presents in fly ash increases the yield of many crops in the agricultural

field. The physicochemical properties and biological properties of soil were improved by fly ash at proper amendment lead to improving the productivity. Application of fly ash in soil improved the physicochemical properties of soil viz., bulk density, porosity and water holding capacity[2]. Fly ash could be successfully utilized to increase the yield of maize crop in terms of growth parameters like plant height, root height, dry matter percentage and chlorophyll and carotene content[3]. Therefore, the present study was carried out to evaluate the beneficial dose of fly ash that will help to increases crop productivity without any loss.

Material and Methods

A field experiment was conducted during the Rabi season of 2016-17 in the pots in Sri Ganganagar District to study the efficacy of fly ash as fertilizers on phytopigments of wheat (*Triticumaestivum*).

The fly ash used in this study collected from the Suratgarh Thermal power plant (TPP) Sriganganagar, Rajasthan, India. The soil was collected from the test field form 30 cm from organic places before sowing and after harvest, air dried, sieved (<10 mm) and analyzed for physicochemical properties. The observations on thecropwere recorded at pre-harvest 30, 60, 90 days after transplantation (DAT) and at maturity in January 2017 on phytopigments parameters. Chlorophylls are the essential and important components of photosynthesis. They occur in chloroplasts as green pigments in all photosynthetic plant tissues. Biochemical assay Chlorophyll content of plant leaves was estimated by Arnon's method using 80% acetone for preparing leafextract[4].Result and Discussion

The impact of different concentration of fly ash in soil on Wheat plant chlorophyll content was analyzed and the results are presented in Table 1. Total chlorophyll and carotenoid contents also decreased significantly with increasing concentrations of FA as compared to that of the control at 50 days. Maximum Chlorophyll-a showed in 12% fly ash with soil (Chlorophyll-a0.239

μg/gm) (Table 1, Fig.1), maximum Chlorophyll-b showed in 8% fly ash with soil (Chlorophyll-b 0.045 μg/gm) (Table 1, Fig.2), maximum Chlorophyll-total showed in 12% fly ash with soil(Chlorophyll-total 0.430 μg/gm) (Table 1, Fig.3).

Table 1. Effect of different concentration Fly ash incorporation in soil on Chlorophyll status of the wheat crop (2016-17

Treatment	Chlorophyll A	Chlorophyll B	Chlorophyll Total
Control (Soil)	0.119	0.021	0.233
Fly ash (2%)	0.125	0.021	0.230
Fly ash (4%)	0.204	0.028	0.298
Fly ash (6%)	0.211	0.041	0.321
Fly ash (8%)	0.135	**0.045**	0.393
Fly ash (10%)	0.192	0.028	0.388
Fly ash (12%)	**0.239**	0.042	**0.430**
Fly ash (14%)	0.219	0.039	0.389
Fly ash (16%)	0.187	0.031	0.293
Fly ash (18%)	0.137	0.028	0.243
Fly ash (20%)	0.163	0.026	0.282

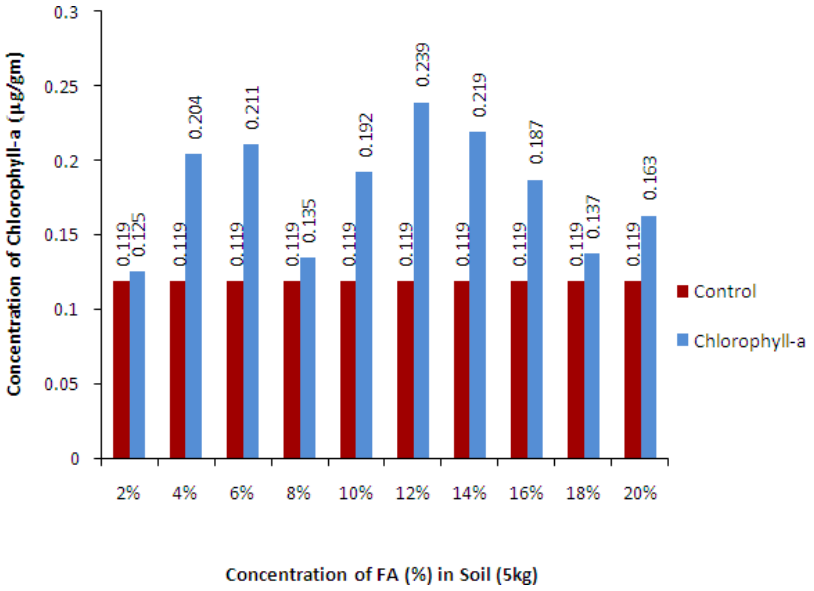

Fig.1 Standard Graph of Chlorophyll-a estimation

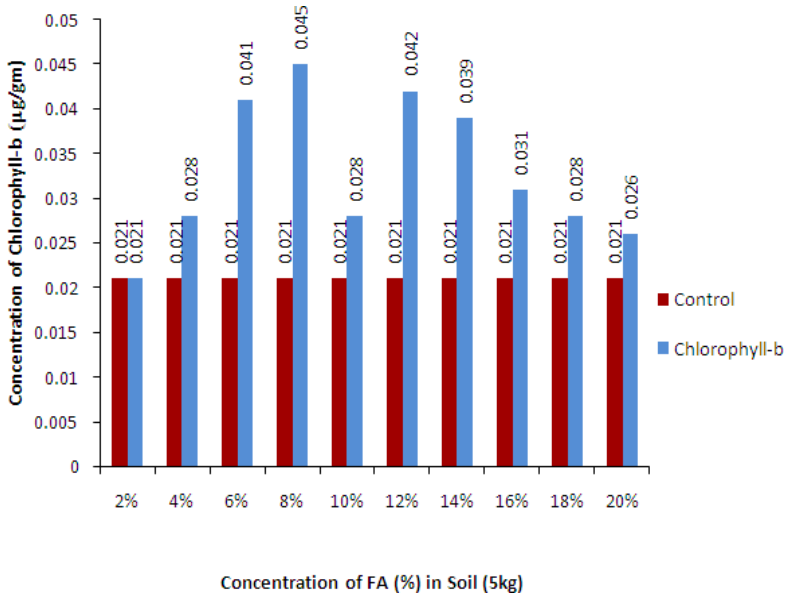

Fig. 2 Standard Graph of Chlorophyll-b estimation

Though the beneficial use of fly ash has been recognized in various areas like in concrete, brick making, soil stabilization treatment and other applications. Flyash having considerable both the soil amending and nutrient - enriching properties as macro and micronutrients is helpful in improving crop growth and yield[5]. The low dose rate of fly ash increased chlorophyll contents significantly[6]. The chlorophyll alkalinity caused soluble salts on the leaf surface[7]. Thus present study Wheat plant chlorophyll content was analyzed and the results are presented like as Maximum

quantity of Chlorophyll-a (0.239µg/gm) was recorded by mixing 12% fly ash in soil, maximum quantity of Chlorophyll-b (0.045µg/gm) was recorded by mixing 8% fly ash in soil, maximum quantity of Chlorophyll-total (0.043µg/gm) was recorded by mixing 12% fly ash in soil, (Table 1, Fig. 1 to 3).

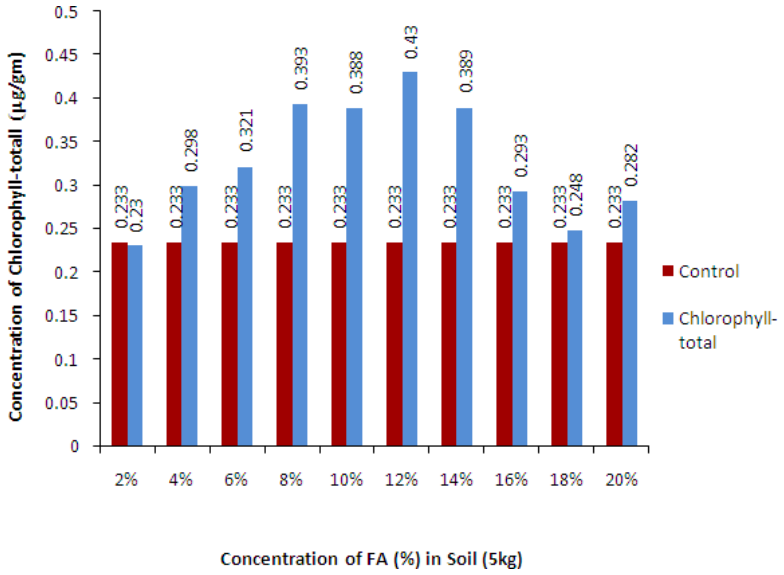

Fig. 3 Standard Graph of Chlorophyll-total estimation

Similar observations were made for cotton and wheat grain yield with 20% fly ash which increased N, P and K nutrients and increased the growth and yield[8]. Dry biomass yield of ryegrass, tomato, and growth of spinach significantly increased with fly ash application of acid soils[9]. The addition of fly ash in sandy soils as a replacement of P and K fertilizer increased the dry matter production of clover[10]. The plant height of barley and sorghum crops increased in concentration of available mineral nutrients in amended soils[11]. In our study, 12% fly ash levels proved to be optimally useful for the plant growth. Leaf area and leaf pigment content of the treated plants also increased. The observed responses of the plants are also supported by other workers, like Bharti et al., on green gram; Pathan et al., on *Cynodon dactylon* (L.) Pers., cv Wintergreen; Parveen et al.,on *Mentha citrata*; Hisamuddin and Singh, on *Pisum sativum*. Their findings indicated that the concentration of fly ash for better plant growth varied from plant to plant.

Sunflower plant (*Helianthus annuus* L.) plants treated with fly ash exhibited improved growth[12]. Relative growth rate (RGR) and net assimilation rate increased by over 20% at a low fly ash application rate. Leaf area and leaf pigment content of the treated plants also increased. Similarly, *Beta vulgaris* grown in fly ash – amended soil revealed that application of low amount (2%) of fly ash favored plant growth and improved yields[13]. It is also observed that tomato plant grown in fly ash mixture showed luxuriant growth with bigger and greener leaves. Plant growth, yield, carotenoids, and chlorophylls were enhanced in 40- 80 % fly ash amended soils.

At 100% fly ash, yield was considerably reduced[14].

Many researchers added to fly ash in the soil to evaluate the long-term consequences of fly ash on soil environment[15,16]and crop productivity[17]. Fly ash incorporation in the sandy loam soil (up to 40%) modified the soil environment, mainly moisture retention, release and transmission behavior, pH, EC and organic carbon. The texture of the soil-ash admixture remained sandy loam up to 10% ash application, beyond this level the texture turned to loamy soil. Numerous studies report the impact of fly ash addition on the yields of different crops with either depressions or enhancements in yield [18,19,20,21,22,23]. Maize and soybean receiving fly ash through aerial spray with different doses increased leaf area and metabolic rate, as well as photosynthetic pigments and drymatter compared with their respective controls[24].

The above study proves that the effect of the quantity of fly ash occurs on the qualities of photosynthetic pigments.

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Journal of Agroecology and Natural Resource Management

p-ISSN: 2394-0786, e-ISSN: 2394-0794, Volume 6, Issue 1; January-March, 2019, pp. 9-12

The Impact of Fly ash Application on the Carbohydrate Content of Wheat (*Triticum aestivum*)

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Abstract—*Fly ash is a waste material predominantly generated in the production of electricity. Thermal power plants use pulverized coal as a fuel source to generate power by obtaining fly ash as a by- product. Its generation in the country has increased from 40 Million ton (MT)/yr (1994) to about 235 MT/yr (2013). It is projected to be 325 MT/yr (2016-17), 500 MT/yr (2021-22) and 1000 MT/yr (2031-32). If it is not used then it would pose a threat for air and water pollution. In India landfill covers a vast area thus depleting the land for agricultural use (Ahmaruzzaman, 2010). In the present study, we tried to assess the feasibilities of possible effective and safe utilization of fly ash as a soil amendment in north Rajasthan wheat field and its impact on wheat plants, especially at Biochemical carbohydrate properties. Fly ash addition to the soil in different doses improves carbohydrate concentration beneficial for a wheat plant. Experimental examination shows the best result in wheat physiological response on 12% fly ash from vegetative part ofwheat.*

Keywords: *Fly ash (FA); carbohydrate; Sustainable agriculture; Soil; Wheat.*

1.INTRODUCTION

Fly ash consists mainly of amorphous glass and a few crystalline phases. The crystalline phases of fly ash consist gypsum ($CaSO_4$ $2H_2O$), aluminosilicate glass, mullite ($3Al_2O_3$ $2SiO_2$), quartz (SiO_2), magnetite (Fe_3O_4), anhydrite ($CaSO_4$), ettringite ($3CaO$ Al_2O_3 $3CaSO_4$ $32H_2O$), opaline SiO_2, hematite (Fe_2O_3), lime (CaO), chlorite, feldspars and spinel ($_{FeAlO4}$) depending on the mineralogy of the feed coal [10, 17]. The degree of soil pH change on FA application is dependent on the factors like the difference between the pH of FA and soil, the buffering capacity of the soil, and the FA capacity as determined by the amount of CaO, MgO, and Al_2SiO_5 present[26]. FA improves the physical properties of soil and nutrient status of soil [20]. FA has been used for the correction of sulfur and boron deficiency in acid soils [1]. Elemental composition of FA (both nutrient and toxic elements) varies due to types and sources of used coal [3]. Its use inagriculture was initially due to its liming potential and the presence of essential nutrients, which promoted plant growth and also alleviated the nutrient deficiency in soils [19]. Although, the lower levels of FA in the soil caused enhancements of both growth and yield, however, the adverse effects at higher levels were observed for crops [16]. The effect of FA on soil fertility largely depends upon the properties of original coal and soil. FA, which can be acidic or alkaline depending on the source, can be used to buffer the soil pH [7]. An extensive variation in the BD of FAs (0.81–1.16Mg/m3) [2, 22), was observed. A marked decrease in the BD of a variety of agricultural soils (1.25–1.65 mg/m3) after FA addition [18], and improvement in soil property, workability, WHC, and permeability of different soil types after the decrease in their BD on FA improvement are well recognized. Soil moisture is a key variable of the climate system which has impacts on water, energy, and biogeochemical cycles. FA helps to preserve soil moisture [23]. Lime in FA readily reacts with acidic components in soil and releases nutrients such as S, B, and Mo in the form and amount beneficial to crop plants. The low dose rate of fly ash increased chlorophyll contents significantly [4]. The chlorophyll alkalinity caused soluble salts on the leaf surface [6]. Nowadays in India, the use of fly ash in agriculture has become of much concern. Therefore, an attempt was made to summarize the information available on the effect of fly ash on soil properties and cropgrowth.

Wheat is the staple food of millions of people, being one of the three globally produced Cereals (Maize and Barley being the other two). Although rice is the second largest produced cereal in the world, its production is localized to Western and Eastern Asia. Wheat is a species of Poaceae Family and it has caryopsis fruit. In India, it is a winter crop grown in Rabi season with a temperature between 10-l5°C and rainfall between5-15cm. Wheat cropping season is from October- November to March-April in Rajasthan. There are many species of wheat which together make up the genus *Triticum*

the most widely grown is common wheat (*T.aestivum*). Fly ash which is a by-product of Thermal power plants also plays an important role and combination of fly ash mixed with soil. Fly ash has similar physicochemical properties with soil. Fly ash can mix homogeneously and improve agronomic properties of soil. Therefore, the present study was carried out to evaluate the beneficial dose of fly ash that will help increases crop productivity without any loss.

1. MATERIAL AND METHODS

A field experiment was conducted during the Rabi season of 2017-18 in the pots in Sri Ganganagar District to study the efficacy of fly ash as fertilizers on carbohydrate of wheat (*Triticum aestivum*). The fly ash used in this study collected from the Suratgarh Thermal power plant (TPP) Sriganganagar, Rajasthan, India. The soil was collected from the test field form 30 cm from organic places before sowing and after harvest, air dried, sieved (<10 mm) and analyzed for physicochemical properties. The observations on the crop were recorded at pre-harvest 30, 60, 90 days after transplantation (DAT) and at maturity in January 2018 on Biochemical carbohydrate. Carbohydrate is an essential and important component of plants. The biochemical assay carbohydrate content of plant leaves was estimated by the Anthronemethod.

Principal

Carbohydrates are dehydrated with concentrated H_2SO_4 to form "Furfural", which condenses with anthrone to form a green color complex which can be measured by using colorimetrically at 620nm (or) by using a red filter. Anthrone reacts with dextrins, monosaccharides, disaccharides, polysaccharides, starch, gums, and glycosides. But they yields of color where is to form carbohydrate tocarbohydrate.

Regents

Anthrone reagent: Dissolved 200mg of anthrone reagent in 100ml of concentrated H_2SO_4.

Standard Glucose solution: a) Stock standard: Weigh 100mg of Glucose and transfer it carefully into a 100ml with Distilled water.(100mg of Glucose in 100ml of Distilled water). **b) Working standard:** Dilute 10ml of stock standard solution in 100ml with distilled water in a volumetric flask.

Procedure

To take 0.2 to 1ml of working standard solution of five different test tube and added water to bring the volume to 1ml in each test tube added 4ml of anthrone reagent and mixed the contents as well and covered the test tube with bath for 10 min then cool the test tube to the room temperature and measured the optical density in a photoelectric colorimeter at 620nm (or) by using a red filter. Simultaneously prepared a blank with 1ml of distilled water and 4ml of anthrone reagent. Constructed a calibration curve on a graph paper, by plotting the glucose concentration (10 to 100mg) on x-axis and absorbance at 620nm on the y-axis.Computed the concentration of the sugar in the sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor has to be taken into account.

Plant Material Preparation and Extraction

The fresh plant was collected and washed with running tap water followed by double distilled water. It was subjected to extraction by phosphate buffer.10gm of each leaf; stem and rhizome were macerated with 50ml of phosphate buffer using mortar and pestle and filtered using what man filter paper by centrifuging at 4000 rpm for 20 minutes by discarding the palate. The above steps were performed for each leaf, stem and rhizome samples separately until a clear extract was obtained. The extract was stored in a refrigerator for further use.

3. RESULT AND DISCUSSION

The impact of different concentration of fly ash in soil on Wheat plant carbohydrate content was analyzed and the results are presented in Table 1. Total carbohydrate content also decreased significantly with increasing concentrations of FA as compared to that of the control at 50 days. Maximum carbohydrate showed in 12% fly ash with soil (carbohydrate 0.800 µg/ml) (Table 1,Fig.1).

Table 1: Effect of different concentration Fly ash incorporation in soil on another Biochemical status of the wheat crop (2017-18)

Treatment	Carbohydrate (µg/ml)
Control (Soil)	0.371
Fly ash (2%)	0.592
Fly ash (4%)	0.691
Fly ash (6%)	0.649
Fly ash (8%)	0.75
Fly ash (10%)	0.755
Fly ash (12%)	**0.800**
Fly ash (14%)	0.732
Fly ash (16%)	0.423
Fly ash (18%)	0.413
Fly ash (20%)	0.354

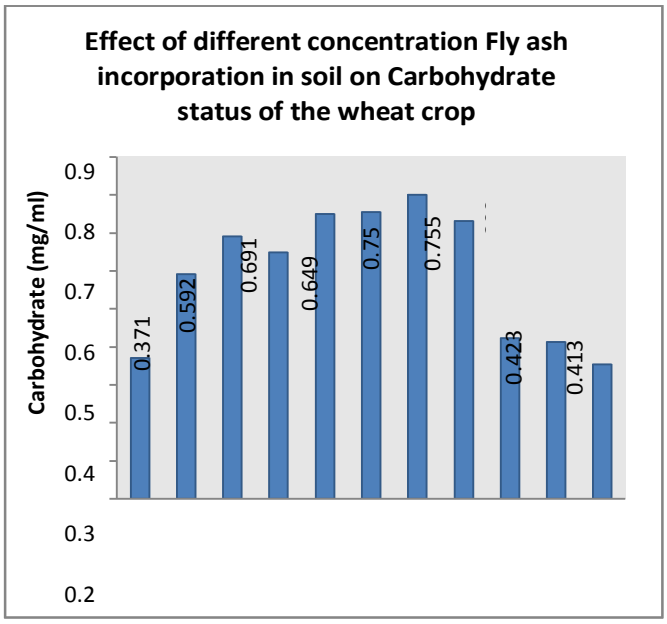

Fig. 1: Standard Graph of Carbohydrate estimation

Similar observations were made for cotton and wheat grain yield with 20% fly ash which increased N, P and K nutrients and increased the growth and yield [24]. Dry biomass yield of ryegrass, tomato, and growth of spinach significantly increased with fly ash application of acid soils [13]. Maize and soybean receiving fly ash through aerial spray with different doses increased leaf area and metabolic rate, as well as photosynthetic pigments and dry matter compared with their respective controls [15]. An enhancement in protein content due to fly ash application in soybean, wheat, gram, sorghum, and maize [8, 9]. A significant increase in the plant root biomass and nutrient content upon FA addition to soil [25] and higher nutrient concentrations (K, Ca, Mg, S, Zn, and B) in Brassica grown on soils treated with FA-amended compost as compared to control values were inferred. A significant increase in the nutrient uptake of oilseed crops and improvement in the fertility status of soil after the FA application were noticed. FA application improved the Si content of rice plants [11]. Fly ash has also been viewed as a source of plant nutrients such as calcium (Ca), boron (B), sulfur (S), and molybdenum (Mo)[21]. In our study, 12% of fly ash levels proved to be optimally useful for plant growth. Through a lot of research has been done on the use of fly ash in meliorating poor physical conditions and· nutritional deficiency of different soils for various crops. There was uncertainty as to the rates of coal fly ash needed for optimum physiological processes and growth. Addition of 10 t ha-1 fly ash increased growth rates and concentrations of chlorophylls a and b, but reduced carotenoid concentrations in barley (*Hordeum vulgare*) and ryegrass (*Secale cereale*) canola (*Brassica napus*), radish (*Raphanus sativus*), field peas (*Pisum sativum*), and Lucerne (*Medicago sativa)* [27]. Transpiration in barley was increased due to flyash application [27]. Investigation studies of fly ash with fertilizers and organic manures may give a better understanding of its use of sustainable cropproduction.

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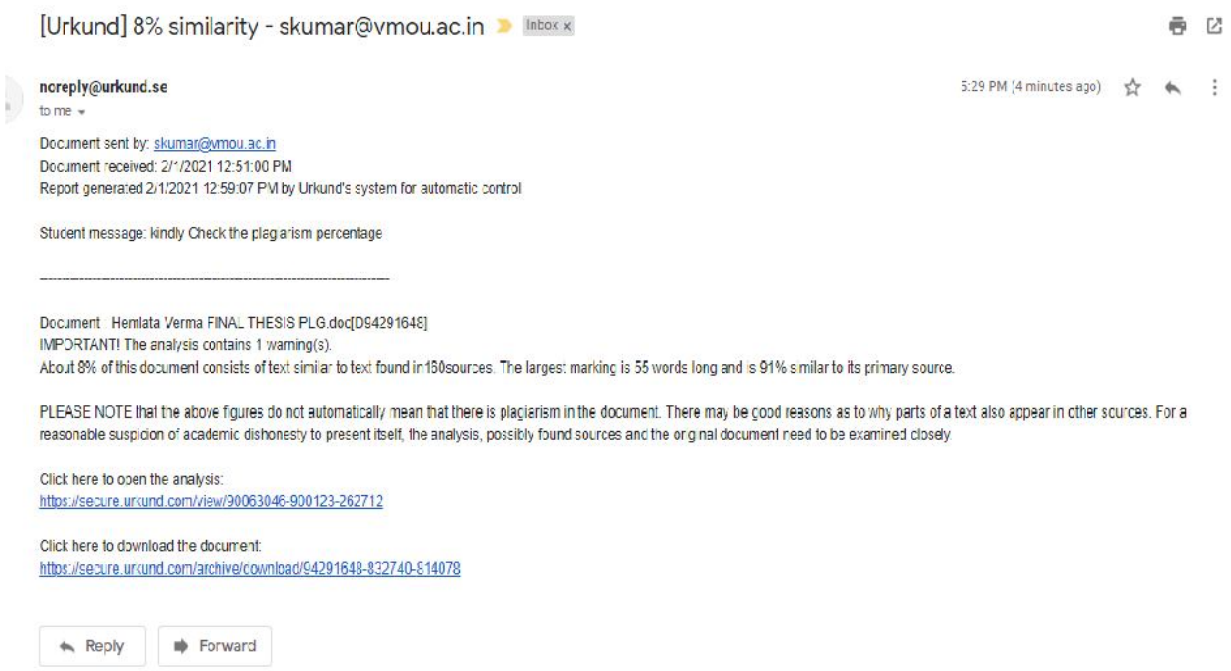

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