बेबी कॉर्न और पालक पर फाइकोरेमेडिएटेड अल्गल बायोमास की खाद क्षमता का मूल्यांकन

EVALUATION OF MANURE POTENTIAL OF PHYCOREMEDIATED ALGAL BIOMASS ON BABY CORN AND SPINACH

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EVALUATION OF MANURE POTENTIAL OF PHYCOREMEDIATED ALGAL BIOMASS ON BABY CORN AND SPINACH

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*This is to certify that the thesis entitled "**Evaluation of Manure Potential of Phycoremediated Algal Biomass on Baby Corn and Spinach**" submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Environmental Sciences**, embodies the results of bonafide research work carried out by **MR. GULSHAN KUMAR SHARMA** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.*

It is further certified that any assistance and help availed during the course of the investigation as well as the source of information have been duly acknowledged by him.

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Dedicated To

MY PARENTS, FAMILY & Aarvi

FRIENDS

&

CHAIRPERSON

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Chapter 1 INTRODUCTION

Water is a source of life and energy, although millions of people worldwide are suffering from a shortage of fresh and clean drinking water. The rapid pace of industrialization, population expansion and unplanned urbanization have largely contributed to the severe water pollution. The total sewage wastewater generated in India is 61,754 million litre per day (MLD), out of which 22,963 MLD get treated (MoEF & CC, GOI, 2018).

Water pollution has become a great concern in the 21st century. Almost 70% of Earth surface is covered with water and it is continuously getting polluted by human activities. The release of untreated sewage wastewater rich in nutrients into aquatic systems is known to be one of the main causes of water pollution. The polluted water gets discharged to water bodies without any treatment (Sonune, 2004). Recycle and reuse and of fresh water is only a feasible approach to overcome the pressure of the growing population. Wastewater is the spent or used water containing dissolved or suspended solids such as pollutants and microbes, including nutrients etc. The nutrient-rich wastewater is discharged to water bodies' causes' eutrophication, which affects the aquatic ecosystem. Uncontrolled discharge of domestic and industrial wastewater contaminates both surface and underground water with erratic pollutants and high BOD and COD. With the limited availability and increasing pollution of the groundwater, it has become crucial to make the water reusable by removing the pollutants and therefore wastewater treatment has become both an ecological as well as an inevitable demand. The present wastewater treatment processes are expensive and energy-consuming. A large quantity of energy is required for pump operation, aeration, sludge disposal and other steps associated in the process. Due to the existing energy crisis, currently available conventional wastewater treatment methods do not promote a sustainable future. The chemical precipitation process is efficient in reducing the phosphorous content from wastewater, but the cost of reagent used in it is highly costly (De-Bashan and Bashan, 2004). The conventional treatment process removes organic pollutants in wastewater but an excess amount of sludge is generated in the activated sludge. Consequently, the wastes transferred from the liquid phase to a solid phase and the disposal of sludge is also highly energy-consuming. It was estimated that approximately one kWh of electricity is required for the removal of one kg of BOD in an activated sludge process (Oswald, 2003).

The alternative to the current treatment methods, microalgae-based remediation also called as phycoremediation are advised to remove the nutrients from wastewater (Mallick, 2002). Microalgae has a role in renewable energy production that will not compete with the agricultural resources and can remediate the wastewater as it requires a large amount of N and P for its growth. Phycoremediation is nothing but a type of phytoremediation, in which "the use of green algae to remove an excess nutrient load from wastewater". Hence the excess nutrient loads should be removed from the wastewater before its discharge into the nearby water bodies (Monfet and Unc, 2016) (Monfet and Unc, 2016). During wastewater treatment, wastewater undergoes mainly through primary, secondary and tertiary treatments. In the secondary treatment system at a place of activated sludge, algal-based technology can be used. Algae has an effective role in removing nitrate - nitrogen (NO_3 -N), ammonical - nitrogen (NH_4 -N), phosphorus (P), potassium (K), biological oxygen demand (BOD_5) and chemical oxygen demand (COD) (Khan *et al*., 2019). Wastewaters is also known as a low-cost media for growth of microalgae as it contains ample amount of nitrogen and phosphorus.

In addition to treating wastewater, the algae biomass obtained after phycoremediation can be effectively used as a manure to crops. The addition of algae manure into soil affects the chemical properties of the fertilized soil surface, as well as the biological activity of microflora, which in turn determines the biochemical status of soil fertility. Microalgal cultivation in wastewater has gained enormous popularity due to its efficiency for the production of biomass at reduced costs and lower environmental impacts as nutrients and freshwater are not a requisite. A feasibility analysis of dry biomass obtained by *C. minutisima* was conducted by Khan *et al.,* (2019) for a pond having an area of 1 ha (10,000 m^2). The dry biomass obtained after 25 days of inoculation was 0.44 g/L. So 3,91,111 kg of dry algal biomass can be obtained in a year. In their study the NPK content in dry algal biomass after phycoremediation was 5.87 % 1.15 % and 0.28 % respectively. Accordingly, from this dry weight, one could obtain N, P and K 22,958, 4,498 and 1,095 kg ha^{-1} y^{-1} respectively. Using this biomass in agriculture as biofertilizers, one can save the chemical fertilizers of about USD (United State Dollars) 55840 ha^{-1} y^{-1}. Algal biomass as a bio-fertilizer, contains both essential plant nutrients, carbohydrates, soluble proteins, and vitamins, which enhances the vegetative yield of the crop (Shaaban, 2001; Abd El-moniem and Abd-allah, 2008). Besides that application of algae biomass also improves the soil organic carbon which

has a favourable impact on the nutritional status of plants. Apart from that, algae biomass also has an important role in reducing soil erosion, by improving the bulk density of soil (Prasanna *et al*., 2008; Zhan and Sun 2012) and also act as a biocontrol agent for agricultural pests (Chaudhary *et al*., 2012; Prasanna *et al*., 2014). Recently microalgal cultivation in wastewater has gained immense popularity due to its effectiveness for the production of biomass at reduced costs and reduce environmental impacts as nutrients and freshwater are not needed. The microalgae assimilate available nitrogen and phosphorus from wastewater and convert it into a valuable biomass which can be used as manure to crops and, at the same time, reduces the use of chemical fertilizers and sewage disposal (Pittman *et al*., 2011). It has been reported that by 2020, the fertilizer demand would increase to about 41.6 million tons, inclusive of 23, 11.5 and 7.1 million tons of nitrogen (N), phosphorus (P) and potassium (K) respectively (Jaga and Patel 2012). The potential role of algae as a manure in agricultural systems has been already reported by (Venkataraman 1972; Kaushik 1998; Vaishampayan e*t al*., 2001; Prasanna *et al*., 2012). Wilkie and Mulbry (2002), have reported 4.9-7.1% N content and 1.5-2.1% phosphorus content in dry algae biomass while (Mulbry *et al*., 2007) reported 3.3-6.4% N in dry algal biomass of *Chlorella sp*.. Adey and Loveland (1998) reported 6-9% N and 1-2% P from harvested algal biomass. Khan *et al*., (2019) quoted that algae (*C. minutissima)* biomass had 5.87% nitrogen after phycoremediation (25 days) of sewage wastewater. The microalgae also have the capability to capture carbon at faster rate in comparison to terrestrial crops and can accumulate high percentage of carbohydrates, lipids or proteins in their biomass over a short period of time (Hernandez *et al*., 2014). The algal biomass contains up to 46% carbon, 10% nitrogen (N), 1% phosphates and 1 kg of dry algal biomass utilizes up to 1.7 kg CO_2 (Hu *et al*., 2008).

The present experiment was undertaken to study the phycoremediation potential of microalgae (*Chlorella minutissima)* for the treatment of sewage wastewater and to study impact of algae biomass of *C. minutissima* with treated wastewater on soil quality and crop productivity (spinach and baby corn). With this background the objectives of present study were:

1. To remediate the sewage wastewater using alga *Chlorella minutissima*
2. To produce manure from algal biomass using biogas slurry and gypsum
3. To evaluate the efficacy of algal manure on baby corn and spinach

To achieve these objectives the aim of the study were:

1. To quantify temporal dynamics (5 days interval) in physico-chemical quality of sewage wastewater after inoculation of algal culture.
2. To quantify manure production and nutrient content of algae biomass.
3. To assess impact of application of recommended dose of N in the form of algal biomass, manure obtained from mixture of (algal biomass + biogas slurry + gypsum) and urea on soil quality and crop productivity (spinach and baby corn).
4. To study nitrate leaching in spinach and baby corn grown soil column, irrigated with algal biomass suspension, biogas slurry, gypsum and urea.

Chapter 2 **REVIEW AND LITERATURE**

Water is a source of life but and millions of people worldwide are suffering from scarcity of clean drinking water. Rapid pace of industrialization, population expansion and unplanned urbanization have largely contributed to the severe water pollution. In India only 30% waste water get treated while rest discharge as such in water bodies without any treatment (MoEF&CC, GOI, 2018). The reason behind is the high cost and energy incurred in conventional wastewater treatment. As an alternative, phycoremediation integrated with conventional wastewater treatment is a sustainable approach. The wastewater grown microalgae are exceptional in providing the essential nutrients to the soil after phycoremediation. In addition to that left over wastewater can be used for irrigation.

In this chapter attempt has been made to review the previous work carried out on "Impact of algae biomass as manure on crops after phycoremediation of sewage wastewater" in India and at International level.

2.1 An overview of *Chlorella minutissima*

Chlorella minutissima is a unicellular green algae belonging to division Chlorophyta, living in both aquatic and terrestrial habitat. The microalgae is spherical in shape having size 2-10 µm diameter and devoid of flagella. It contains chlorophyll a and b. It is rich in amino acids and polyunsaturated fatty acids, particularly eicosapentaenoic acid (20:5co3), which makes it potentially useful in health foods and pharmaceuticals (Seto *et al*., 1984). The growth of *C. minutissima* does not require the presence of secondary carotenoids, thiamine and vitamin B12. It can grow in slightly acidic to saline water. It has temperature range for growth in between 15-40° temperatures and under irradiances from 30 to 550 µmol m^{-2} s^{-1}, in a light/dark cycle (Mojojidek 2008, Aleya *et al*., 2011).

2.2 Phycoremediation potential of microalgae *Chlorella minutissima* in sewage wastewater

Microalgae is a photosynthetic plant, so can absorb atmospheric carbon dioxide and remove excess nutrients from wastewater efficiently at marginal cost. It does not require arable land as it grow well on wastewater and complete its life cycle within 20-25 days. Hence, attracting the researcher as a potential alternative to conventional

wastewater treatment process. Phycoremediation is a process in which green algae are employed to remove an excess nutrient load from wastewater. The currently available secondary wastewater treatment process like - activated sludge, trickling filter, oxidation ponds for Nitrogen (N) and Phosphorus (P) removal are energy-consuming and too costly for wastewater treatment in developing countries (Wei *et al*., 2008). Microalgae are known to a major biotic component of wastewaters, which are involved both in eutrophication and bioremediation, as they can assimilate N and P from wastewater effluent (Boelee *et al*., 2011). Phycoremediation can be used in the secondary process at place of currently available conventional wastewater treatment. Phycoremediation efficiently removes TDS, nitrate, phosphate, ammonia, BOD, and COD from wastewater and leftover water after remediation can be used for irrigation purpose. In the current study algae, *Chlorella minutissima* was selected for remediation of sewage wastewater. *Chlorella* is known as one of the fastest-growing green microalgae. The algae-based remediation concept was initiated by Oswald (1963) for the tertiary treatment of municipal wastewater to many other applications in which microalgae and macroalgae are cultivated and utilized for specific bioremediation purposes. The use of various microalgae such as *Chlorella, Botryococcus, Chlamydomonas, Scenedesmus, Spirulina* and *Phormidium,* for treating domestic wastewater has been reported (Olguin, 2003; Chinnasamy *et al*., 2010; Kong *et al*., 2010). Research conducted by Chinnasamy *et al*., (2010) reported that a consortium of 15 algal isolates showed >96% nutrient removal in treated wastewater. (Martinez *et al*., 2000; Ruiz-Marin *et al*., 2010; Zhang *et al*., 2008) reported that *Chlorella* and *Scenedesmus* almost remove more than (80 %) ammonia, nitrate and total phosphorus from secondary treated wastewater. Dominic *et al*., (2009) has performed his study from Periyar of India on industrial polluted water and reported that *C. vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* reduce nitrate, phosphate and increase in dissolved oxygen content of industrial wastewater. (Munoz and Guieysse, 2006; Wilkie and Mulbry, 2002) has stated phycoremediation does not generate additional pollutants such as sludge, greenhouse gases etc. Tam and Wong (1989) used *C. pyrenoidosa* and *Scenedesmus sp*. for removal of excess nutrients from wastewater (settled and activated sludge filtrate). The higher growth of algae was recorded in settled sewage than in activated sewage. Although, both nitrogen and phosphorus content in wastewater was decreased significantly. The 75 % reduction of N and P was found within 7 days of growth and later removal of N and P was decreased gradually.

At the end of the experiment, more than 80 % N and P was reduced significantly. Tam and Wong (1996), in another study found that as nitrogen content was decreased in wastewater, the growth of algae *Chlorella vulgaris* was affected significantly. Therefore cultivation of *Chlorella* seems to be one of the feasible methods to reduce the amount of nitrogen and phosphorus entering the nearby coastal water, thus preventing the eutrophication. It is also clear that algal ponds with high inoculum size might be more suitable to be installed as a secondary rather than a tertiary treatment process. In a study conducted by (Riano *et al*., 2011) on phycoremediation potential of microalgae grown in wastewater. They analysed the total solids, total organic carbon, total nitrogen and total phosphorus at the different hydraulic retention times (HRTs). Influent and effluent samples were analysed for pH, chemical oxygen demand, soluble chemical oxygen demand, total phosphorous, soluble phosphorus, ammonia (NH_4^+ -N), nitrite (NO_2^- -N) and nitrate (NO_3^- -N). The result of the study shows that algae removes chemical oxygen demand, soluble chemical oxygen demand, total phosphorous, soluble phosphorus, ammonia (NH_4^+ -N), nitrite (NO_2^- -N) and nitrate (NO_3^- -N) from wastewater. Lam and Lee (2012) found *C. vulgaris* can grow in its best when we provide initial nitrate content of 26.67 mg/L, 24 h of continuous illumination and pH of 5. Nevertheless, a slow growth rate was observed when cultivating *C. vulgaris* under open environment; a reduction of nitrate (27 %) was recorded in comparison with controlled environment. V Sivasubramanian *et al*., (2010) studied the remediation of chrome-sludge from an electroplating industry using the microalga *Desmococcus olivaceus*. It has been found that the *D. olivaceus* was very efficient in treating the chrome-sludge of electroplating industry using an open pond. Su *et al*., (2012) observe that if NH_4-N and NO_3-N both are available microalgae prefer to utilize NH_4-N first. Tredici *et al*., (2010) have explained that microalgae have been identified as a superior feedstock for biodiesel and manure production, mainly due to their fast growth rate (100 times faster than terrestrial plant) and able to double their biomass in less than one day under favourable culture conditions. Biomass can be used as feedstock for biofuel production and also use as manure in a crop field. Reith *et al*., (2004) developed a process using microalgae to purify wastewater. They identified four beneficial by-products: fine chemicals (e.g. colourants and bioactive substances) biofuel, carbon-dioxide fixation, and water purification. They experimented with a “bubble column” photobioreactor system. This method removed ammonium, nitrate, and phosphate from wastewater at the level of less than 10 μL^{-1}. The resulting biomass can be used as feed

for aquaculture, agrochemicals and other bioactive substances, and energy sources such as biodiesel, hydrocarbons, methane, ethanol, and manure. Kothari *et al*., (2012) observe the potential of *C. pyrenoidosa* for treatment of dairy wastewater and biofuel extraction. The physical and chemical parameters of wastewater quality such as nitrate, phosphate, chloride, fluoride, hardness, etc. were studied. The level of nitrate and phosphate reduced by 60% and 87% in influent, 49% and 83% in the effluent, respectively. Rao *et al*., (2011) found that *C. vulgaris* exhibited appreciable nutrient scavenging properties under both laboratory and field conditions, although phycoremediation carried out in sunlight (field study) gave better results. Moreover, the growth of *Chlorella vulgaris* was faster under field conditions. Das *et al*., (2018) worked on *Chlorella* and *Scenedesmus* species and reported that both species were very effective in treating the low-strength municipal wastewater. Both these strains were able to recover a high amount of total nitrogen from the municipal wastewater; however, these strains were able to recover only a fraction of total phosphate, because of its higher concentration than cellular requirement in municipal wastewater. (Brar *et al*., 2019) reported that the microalgal species *C. pyrenoidosa*, *Anabaena ambigua* and *Scenedesmus abundans* have effectively reduced the BOD by 56%, COD by 77%, nitrate by 88%, and phosphate by 85% after a 25 days of the cultivation in the 3:1 dairy wastewater. Choudhary *et al*., (2016) have reported that algae removed COD and nitrate below permissible limit from rural sector wastewater by 87 % and 70 % respectively in the 12 days. Malla *et al*., (2015) stated that *C. minutissima* effectively removes about 90–98% TDS, 70–80% N, 60–70% P and 45–50% K from the wastewater within 12 days after inoculation. Similarly about (82%) reduction of TDS, nitrate, ammonia, and P from dairy effluent were also reported by Ummalyma and Sukumaran (2014) within 15 days of incubation. Hena *et al*., (2015) had reported the reduction of BOD up to 82% from dairy effluent by using consortium of microalga. Sharma *et al*., (2013) had reported that microalgae *C. minutissima* reduced TDS, BOD and ammonium-N by more than 90% and COD and Nitrate-N by >80% and total P > 70% in the raw waste water. Govindan (1984) in his study on a mixture of dairy and sewage wastewater reported 75 % to 95 % BOD and 72% to 91% COD reduction within 5 days of algae inoculation. The DO decreases initially up to 5 days and later continuously increases, it is because of high microbial activity and low algal growth in sewage wastewater during initial period. The same trend of DO was also observed by (Senger *et al*., 2011). The algal biomass harvested after phycoremediation can be used in pharmaceuticals

industry, biofertilizers, biodiesel, fish feed, bio-hydrogen, ethanol etc. (Chisti, 2007; Khan *et al*., 2009; Sturm and Lamer, 2011).

2.3 Manure potential of algae after phycoremediation of sewage wastewater

Wastewater is a rich source of N and P, so can be used as a cost-effective media for microalgae. The microalgae assimilate excess N, P and TDS from wastewater resulted in reduction of pollution load and solve the problem of sewage disposal. At the same time, algae biomass generated after phycoremediation can be used as a biofertilizer for agricultural crops and hence reduces the use of chemical fertilizers (Pittman *et al*., 2011). Biofertilizers includes both micro and macro-organism which maintain soil organic carbon and enhance the crop productivity. India is one of the largest producers and consumers of fertilizers in the world, but the increase in the fertilizer consumption in the country raised the severe environmental problem. It has been estimated that in India by 2020, the fertilizer demand would increase to about 41.6 million tons, which includes 23, 11.5, and 7.1 million tons of N, P and K respectively (Jaga and Patel 2012). The use of chemical fertilizers is also lead to the environmental pollution, due to the presence of heavy metals (Hg, Cd, As, Pb, etc.) and natural radionuclides (U238, Th232, etc.) (Savci 2012). The macronutrient NPK is foremost important for agriculture. Microalgal biomass contains a higher amount of nitrogen as compare to available organic manure, therefore microalgae can be used as biofertilizers in cropping systems. Unfortunately, limited research is done on the efficacy of algae as a manure, especially in agriculture. As stated earlier microalgal cultivation in wastewater has gained enormous popularity due to its efficiency for the production of biomass at reduced costs and lower environmental impacts as external nutrients, arable land and freshwater are not required. Microalga can capture nutrients at a faster rate in comparison to terrestrial crops and can accumulate a high percentage of carbohydrates, lipids or proteins in their biomass over a short period of time (Hernández *et al*., 2014). Different types of microbial biofertilizers are available such as carrier-based or liquid formulations or pellets (Motsara *et al*., 1995; Lata *et al*., 2002; Yan *et al*. 2013). It is very well documented that chemical fertilizer adversely affects soil, plant, and the environment. To reduce this effect in recent years, interest to organic farming has been increasing. The bio-fertilizer, organic manure, and bio-control agents have appeared to be as an important component of integrating nutrient supply system in arable land. Bio-fertilizers include the nitrogen-fixing, phosphate solubilizing and plant growth-

promoting microorganisms (Goel *et al*., 1999). The main biofertilizers are *Azospirillium, Azotobacter*, BGA, Azolla, P-solubilizing microorganisms, mycorrhizae and *Sinorhizobium* (Hegde *et al*., 1999). Algal biomass as a new bio-fertilizer contains an appreciable amount of macronutrients, micronutrients, natural enzymes carbohydrates, proteins, vitamins and growth regulators (El Fouly *et al*., 1992; Mahmoud, 2001; Shaaban, 2001 and Abd El-moniem and Abd-allah, 2008). Application of algal biomass to the soil improves soil characteristics which have a positive effect on the nutritional status of plants (Al-Gosaibi, 1994). Microalgae when added to soil can result in improving the pH and electrical conductivity of soil. In spite of that, algae manure also enhances the available NPK content in the soil (Renuka *et. al*., 2016). Microalgae can also be used in agriculture for soil reclamation purposes. Wastewater algae have a very favourable C: N ratio of 8.7. With the high nitrogen content, ideal C: N ratio, and easy mineralization in soil (Brady and Weil 2007), wastewater grown algae is a useful by-product of a wastewater treatment processes. Algae biomass contain high percentage of protein. Fowden (1952; Brady and Weil 2007) stated that out of total nitrogen in *Chlorella sp*., about 90% occurs as protein, so one of the easiest organic compound to decompose. Oysal *et al*., 2015 reported that phototrophic microalgae have better germination rate and plant height in maize and wheat crop compare to heterotrophic algae. Organic carbon and nutrients that are locked in the microalgae biomass can be released by the bacterial activity when it is applied to the soil and enhance the organic content of the soil. (Mulbry *et al*., 2005; Bird *et al*., 2012). In a study conducted by Mulbry *et al*., (2005) on seedlings of corn and cucumber in growth chambers and applied dried algal biomass produced on anaerobically digested dairy manure as a fertilizer. After twenty days, seedlings supplied with algae manure have higher tissue P and similar tissue N compare to conventionally fertilized plants. The size and shape of corn in algae treated soil was similar to soil treated with chemical fertilizer. In another conducted by (Mulbry *et al*., 2007) using dairy manure effluent in algal turf scrubber, successfully demonstrated the potential use of microalgae biomass as a slow release bio-fertilizer for growing plants. Microalgae biomass has the potential to substitute fish feed due to its easy culturing methods with rapid growth rates compared to terrestrial crops. Moreover, microalgae cells can be easily digested by fishes due to their very small size (Hemaiswarya *et al*., 2011; Apandi *et al*., 2018). The role of algae biomass as an effective bio fertilizer and as soil conditioner is already reported by various researcher (Faheed & Fattah 2008; Mata *et al*., 2010; Coppens *et*

al., 2015; Uysal *et al*., 2015; Abdel-Raouf *et al*., 2016; Renuka *et al*., 2016). Gougoulias *et al*., (2016) reported that a dried algae biomass applied in small amounts into a sandy loam soil amended with sheep manure ensured nitrification process and had positive effects on the availability of nutritional elements K, Cu, Zn, and Mn without any significant negative impact on soil chemical properties. In more recent work, algae biomass after the treatment of aquaculture effluents were used to grow tomatoes (Coppens *et al*., 2016). The tomato size of plants which are supplied with algae manure was of similar size and had nutrient concentrations similar to or higher than conventionally fertilized plants. However, fruit from algae-fertilized plants was higher in carotenoids and sugars. Austin (2018) reported that performance of algal biomass as manure after phycoremediation was similar to that of a conventional synthetic fertilizer and better than that of the commercial organic fertilizer. Microalgae have a unique property to fix atmospheric nitrogen in the soil and is therefore used for increasing the fertility of the soil. Mahmoud and Amara (2000) found that plants supplied with fresh and dry algae biomass have significantly higher growth compared with un-treated plant. Lozano *et al*., (1999) stated that the application algae on potato increased the ash, protein and carbohydrate content. A feasibility analysis of dry biomass obtained by *C. minutisima* was conducted by Khan *et al.*, (2019), for a pond having an area of 1 hactare (10,000 m^2). The dry biomass obtained after 25 days of inoculation was 0.44g/L. So 3,91,111 kg of dry algal biomass can be obtained in a year. In their study the nitrogen content in dry algal biomass after phycoremediation was 5.87 %. Accordingly, from this dry weight, one could obtain N, P and K 22,958, 4,498 and 1,095 kg ha^{-1} y^{-1} respectively. Using this biomass in agriculture as biofertilizers, one can save the chemical fertilizers of about USD (United State Dollars) 55,840 ha^{-1} y^{-1}

2.4 NPK content in algae biomass

Nitrogen is one of the most important macro-nutrient for the plant. The effectiveness of any organic manure depends on the percentage of nitrogen content in it. A few literatures available on the NPK content of green algae. Wilkie and Mulbry (2002), have reported 4.9-7.1% N content and 1.5-2.1% phosphorus content in dry algae biomass while (Mulbry *et al*., 2007) reported 3.3-6.4% N in dry algal biomass. Adey and Loveland (1998) reported 6-9% N and 1-2% P from harvested algal biomass. Khan *et al*., (2019) quoted that algae (*C. minutissima)* biomass had 5.87 % nitrogen after phycoremediation (25 days) of sewage wastewater.

2.5 Role of algae manure in nitrogen metabolism

Nitrogen (N) is a primary limiting factor for plant growth in agro-ecosystems. India is the second largest consumer of fertilizers in the world after China, consuming about 38.6 Mt per year (FAI, 2015-16). Algal manure is one of the potential organic manure, contains a high percentage of lipids and protein, so have a potential for biodiesel production. (Andrews's *et al*., 2013) conducted a field trial to assess the overall crop productivity of corn grown with algal meal fertilizer, feather meal, and urea. There was no significant difference in corn ear yield or ear N uptake among any of the algal meal treatment rates (101 kg N ha^{-1}, 146 kg N ha^{-1}, 190 kg N ha^{-1}) and the high rate of feather meal or urea (190 kg N ha^{-1}) application. The study indicated that algal meal will have a similar N mineralization rate, microbial respiration rate, and extracellular enzyme production, yield of sweet corn, and not have any negative impacts from the salt concentration. Mulbry *et al*., (2005) found that the algal biomass was similar in providing nitrogen and phosphorous when compared with Gardentone 4-6-6, a commercial slow release fertilizer. The study also showed that the biomass could be side-dressed on established crops without tilling because ammonia volatilization was not a concern. Approximately three percent of algal nitrogen was available immediately to plants. Mineralizable nitrogen increased an additional 26 to 30% after 21 days for a total of 29 to 33% plant available nitrogen (Mulbry *et al*., 2005). In a further study, Mulbry *et al*. (2007) used raw dairy manure, anaerobically digested dairy manure, and swine manure as feedstocks for algal growth. Algae were then evaluated to determine if the feedstock source caused differences in the effectiveness of algal biomass as a fertilizer. Initially, algal biomass from all sources had 5% mineralizable nitrogen and continued to increase until the study ended at 63 days. Corn grown with algal biomass or comparable levels of a commercial fertilizer was found to have similar shoot weight and nutrient content after 17 days in a growth chamber with potting soil (Mulbry *et al*., 2007). It was reported by (Swenson *et al*., 2009) that algae grown in wastewater was effectively works as fertilizer and perform equally to the organic, inorganic and commercially available fertilizers. (Andrews *et al*., 2013) reported that algae manure could also provide K, S, Ca, Mg in spite of providing nitrogen and phosphorus, and the full range of micronutrients needed for plant growth, so it could be a possible solution for organic producers struggling to find adequate organic nutrients to meet the ever growing demands on organic production. A major

drawback of algae based remediation of sewage wastewater is the presence of pathogenic bacteria, but microalgae growth is known to increase the pH of the media and suppress the growth of bacteria (Toha *et al.*, 1991). Sankar *et al.*, 2012 has reported that *C. vulgaris* biomass consists of nearly contains protein (40 %), oil (25 %), carbohydrate (20%) fiber (5%) and 10% minerals and vitamins. Despite its highly efficient photosynthesis which allows it to produce more oil and protein than most other plants. The lipid content in *Chlorella vulgaris* could be increased significantly between 50% and 70%.

2.6 Biodiesel potential of microalgae

Microalgae have many advantages over energy crops and therefore have a great possibility for energy production. Eukaryotic microalgae and cyanobacteria are contributing more than 40% of net primary productivity on Earth (McKenzie, 2011; NAS, 2012). Their high productivity and rapid growth rates led to an interest in their development as a renewable fuel source since before World War II (Shelef and Soeder, 1980). Microalgae are tolerant to harsh environmental stresses such as temperature and pH which enable them to reserve more food component in the form of energy (oil) (Chisti, 2007). One of the most valuable food components produced from microalga is lipid bodies, which turn into oil production that has the main application in the transport sector. Many countries including Germany, India, USA, China, and Japan are using the blending of biodiesel with traditional fuel in the transportation sector (Kumar *et al.*, 2004). However, the scarcity of existing fuels due to limited reserves is a heightened problem these days. Many available energy resources such as food and non-food crops as well as waste materials and microorganisms have been studied and executed practically with many success and failures. Every resource has its benefits and obstacles at a practical and industrial scale. But among all currently available energy resources microalgae seem like an incredible alternative in every respect due to its high product yield rate in the form of biofuels than other energy crops and microorganisms (He *et al*., 2016). Richa *et al*., (2012) analysed the dairy wastewater of cooperative milk union limited, Lucknow for physico-chemical parameters (colour, pH, turbidity, TDS, Cl-, F-, nitrate, phosphate, iron, ammonia). They treated the dairy wastewater with green algae and utilized the flush out algal biomass for biodiesel production Moreover, microalgae do not compete with the demand for food, water, and land for their cultivation. Additionally, microalgae are also involved in the carbon-neutral process of biofuel

production and emission which maintains the carbon cycle in the environment. Thus, shifting toward microalgae than other energy crops and microorganism can fulfil today's fossil fuel demand with providing a solution to existing problems. In spite of this, the combined advantages may also include the regional and rural development of the nation with the cultivation of microalgae on wasteland with the use of wastewater which would not affect natural resources. Consequently, the complete output of using microalgae may come in the form of energy supply and socioeconomic welfare of developing a country.

It is evident from the above discussion that the coupling of phycoremediation of sewage wastewater followed by biomass production as manure holds a great promise for the sustainable development and environmental waste management.

Chapter 3 **MATERIALS AND METHODS**

3.1 Experimental site

The study was carried out at the experimental farm of Centre for Environment Science and Climate Resilient Agriculture (CESCRA), ICAR-IARI, New Delhi, India (28° 35` N and 77°12`, 228.16 m above mean sea level). This region lies between humid subtropical and semi-arid climate (Köppen climate classification). The average annual temperatures is around 29°C. The month of May to June remains very hot, with extreme heat waves. In January month of winter season the sometimes minimum daily temperature goes below 5 °C.

The monsoon starts the month of June and lasts till mid-September, with mean annual rainfall of about 700 mm. The driest months (April-May) have average relative humidity of 20% to 30% whereas in the monsoon season (July-august), it is maximum 63% to 75% during morning hours.

3.2 Soil

The texture of soil in selected field is sandy loam with slightly alkaline to non-saline. The available organic carbon content is less than 1% (IARI soil fertility maps). The soils are alluvial in origin and affected by annual rain fall.

3.3 Site of sewage wastewater collection

The sewage wastewaters were collected from Indian Agricultural Research Institute (IARI) sewage treatment plant, New Delhi. The IARI campus is located in the middle of the NCT, Delhi. The sewage wastewater was having foul smell, high turbidity, black color, total dissolved solids and high biological oxygen demand (BOD).

3.4 Mass culture of microalgae *Chlorella minutissima*

The 10 mL pure culture of algae *Chlorella minutissima* was procured from National Centre for Blue Green Algae (NCBGA), IARI. The obtained culture of algae *C. minutissima* had multiplied for mass multiplication in culture room. The 10 mL culture was transferred in to 250 mL of culture media and kept in culture room for growth of *C. minutissima* in a controlled condition of temperature and light. Initially it took 30 days in preparation of 250 mL well grown culture. Later on this 300 mL prepared culture was used for preparation of 2 L of algae culture. Similarly 70 L of well grown culture of well grown culture was prepared for before transferring to sewage

wastewater. *C. minutissima* was grown on Bold's Basal Media (BBM) whose composition is given in Table 3.1.

Table 3.1 Composition of Bold's Basal Media

Chemicals	Concentration (g/ L)	Amount in culture medium (mL/ L)
$NaNO_3$	25	100
$MgSO_4.7H_2O$	7.50	10.0
NaCl	2.50	10.0
K_2HPO_4	7.50	10.0
KH_2PO_4	17.50	10.0
$CaCl_2.2H_2O$	2.50	10.0
Trace element a) $ZNSO_4.7H_2O$ b) $MnCl_2.4H_2O$ c) MoO_3 d) $CuSO_4.7H_2O$ e) $Cu(NO)_3.6H_2O$	8.82 1.44 0.71 1.57 0.94	1.0 - - - - -
H_3BO_3	11.40	1.0
EDTA-KOH Solution a) EDTA Na_2 b) KOH	50 31	1.0 - -
$FeSO_4.7H_2O$	4.98	-
Conc. H_2SO_4	1.0 mL/ l	1.0

3.5 Sampling of sewage wastewater

Near about 2000 L of sewage wastewater were transported from IARI sewage treatment plant raceway pond during the month of April. Subsequently sampling of sewage wastewater were done every month to enhance algae volume up to 20,000 L.

3.6 Inoculation of algae culture in sewage wastewater

Initially inoculated 700 mL centrifuged culture (*Chlorella minutissima)* in raceway pond (2000 L capacity) filled with sewage wastewater.

Plate 3.1 Raw sewage collected from IARI sewage treatment plant

3.7 Analysis of physico-chemical parameters

The sampling analyses were done in the laboratory of Division of Environmental Sciences, IARI New Delhi. The parameters like EC, pH, total dissolved solids (TDS), nitrate-nitrogen, ammonical-nitrogen, phosphorus, potassium, biological oxygen demand (BOD), chemical oxygen demand (COD), heavy metals (Fe, Cu, Pb Cr, As, Zn) were analyzed. The selected parameters were analyzed initially and after 25 days of *C. minutissima* inoculation. The difference between initial and final value (after 25 days of inoculation) gave the phycoremediation potential of algae (*C. minutissima*). Meanwhile, temporal dynamics (5 days interval) in physico-chemical quality of sewage wastewater after inoculation of algal culture was also analysed. The methods and techniques being employed for the physico- chemical analysis is given in Table 3.2. APHA (2000) standard methods for examination of water and wastewater were followed for wastewater analysis.

Table 3.2 The standard methods for estimation of physical and chemical parameters in sewage wastewater

S No.	Parameter	Units	Method
1	*Physical parameters*		
	Acidity/basicity		pH meter
	Electrical conductivity (EC_w)	dS/m	Portable water testing kit (WTW Vario Cond. Set 2X00-001A).
	Total dissolved salts(TDS)	mg/L	Portable water testing kit (WTW Vario Cond. Set 2X00-001A)
2	*Chemical Parameters*		
	Total ammonical nitrogen	mg/L	Colorimetric method
	Nitrate- nitrogen	mg/L	Colorimetric method
	Total phosphate phosphorus	mg/L	Colorimetric method
	Potassium	mg/L	Flame photometer
	Heavy metals	mg/L	Atomic absorption spectroscopy (AAS)
4	Biological oxygen demand (BOD)	mg/L	Winkler method
5	Chemical oxygen demand (COD)	mg/L	Chemical titrimetric method

Plate 3.2 Growth of algae *C. minutissima* in collected sewage wastewater in raceway ponds

3.8 Biochemical Oxygen Demand (BOD_5)

Biochemical oxygen demand of wastewater samples was determined by Winkler method. The 300 mL capacity of DO bottles were used for collecting wastewater sample. For fixing of oxygen present in sewage wastewater 1 mL each of $MnSO_4$ and alkali-iodide-azide reagent was added. 1 mL of concentrated sulphuric acid was added to the solution after settling of precipitates. Bottles were re-stoppered and mixed by inverting several times until dissolution is complete. 201 mL of this sample was taken in titration flask and titrated with 0.025M sodium thiosulphate using starch solution as indicator to an endpoint of pale straw colour. Finally initial D.O. of sample was calculated using following equation:

For titration of 200 mL sample, 1 mL 0.025 M $Na_2S_2O_3$ = 1 mg DO/ L

Another bottle of same sample was tightly closed and incubated for 5 days at 20 °C which was analysed after 5 days of incubation for DO of samples with same procedure as described above. A blank and standard were also analysed after 5 days.

3.9 Harvesting of *Chlorella minutissima*

It has been found that the microalgae grew up to 20 to 25 days. After 25 days the microalgae were harvested through muslin cloth. The fresh weight of algae biomass was measured. Later on fresh biomass of algae was air-dried followed by oven dry (60°C).

3.10 Estimation of nitrogen, phosphorus and potassium content in algae

Algae biomass was harvested after 25 days from sewage wastewater. The algae biomass was air dried and later oven dried. NPK content were analyzed from dry algae biomass as per standard protocol of plant analysis.

3.10.1 Estimation of nitrogen

For estimation of nitrogen content Kjelplus (micro kjeldhal apparatus) was used. It consists of 3 steps, viz. digestion, distillation, and titration. For digestion used concentrated H_2SO_4 than kept it for cold digestion for 24 hours. Transferred the distillate into kjeldahl distillation flask and kept a 250 mL conical flask containing 25 mL of 4 % boric acid with mixed indicator at the end of delivery tube. After completion of distillation, took out the conical flask containing the distillate and titrate the contents against 0.01 N standards H_2SO_4 till the bluish green colour turns pink.

Calculation

Weight of the plant sample taken = 0.1 g

Blank titre value = B mL of 0.01 N H

Sample titre value = S mL of 0.01 N H

Actual titre value = (S – B) mL

1000 mL of 1 N H_2SO_4 = 14 g N

(S – B) mL of 0.01 N H_2SO_4 = (S – B) 0.01x1/1000

= Present in 0.1 g plant sample

100 g plant sample contains = (S – B) 0.01x14 x 100 g of N

1000 x 0.1

(S – B) x 0.14 % N

3.10.2 Estimation of phosphorus (Watanabe, 1965)

Algae biomass was digested in a di-acid mixture 5:1 of HNO_3: $HClO_4$ or a tri-acid mixture of HNO_3: H_2SO_4: $HClO_4$ in the ratio of 9:4: 1. Took 1 g algae in 100 mL conical flask. Add 10 mL of acid mixture and swirl the content of the flask gently. Kept it for overnight for pre digestion. After cooling the flask, add 20 mL of distilled water.

Make up the volume with distilled water and filtered through Whatman No. 1 filter paper. Took 5 mL of plant digest into a 50 mL of volumetric flask. Add 10 mL of Barton's reagent shake well and make up the volume. Read the intensity of the colour at 420 nm in a spectrophotometer. Plot the absorbance value in the standard graph to obtain the concentration of phosphorus.

Calculations

Concentration of P in colored solution = **X** ppm

i.e., 1 mL of colored solution contains **X** µg P

50 mL of colored solution contains 50 x X µg P

Which is present in 5 mL of the algae digest

100 mL of algae digest consists of 50 x X x 100/5 = X x 1000 µg P

Which is obtained from1 g algae sample

100 g of plant sample consists of = X x 1000 x 100/1

= X x $105x10^{-6}$ g P

= X x 0.1 %

3.10.3 Estimation of potassium (Black, 1965)

Took out 5 mL of algae digest into a 25 mL volumetric flask and diluted to 25 mL with distilled water. Aspirated the standards and then the sample to the flame photo meter and calculated the K content by referring to the standard curve prepared.

Calculations

Concentration of K in the algae digest = *X* ppm

1 mL of the algae digests =*X* µg of K

100 mL of the algae digest = 100 x *X* µg of K

1 g of algae sample =100 x *X* µg of K.

100 g of algae sample = 100/1 x 100 x X µg of K.

= *X* x *104* x *10-6 g K* = *X* x *0.01%*

3.11 Production of manure from algal biomass using biogas slurry and gypsum

Algal biomass harvested from raceway ponds. Initially the fresh and oven dry weight of algae *C. minutissima* was recorded. Algal biomass was mixed with biogas slurry and gypsum in the ratio of 100:20:1 and 100:10:0.5 on weight basis. After mixing, mixture was kept for 15 days for aerobic digestion to produce manure. The pH of experimental field was alkaline in nature, therefore gypsum was added to reduce the alkalinity of the soil. The mixture was aerobically digested for 15 days followed by estimating the nitrogen and phosphorous content by standard protocol as shown in section 3.10.1 and 3.10.2.

3.12 Sowing of baby corn and spinach

The field was prepared for growing baby corn and spinach. Experiments were setup with eight different treatments having three replications for each. In this way total field size for experiment was 48 m^2 with 1m x 1m for each treatment. The 0.5 m^2 space is left between rows for channel. Hence 24 m^2 plot size is allotted for spinach and baby corn. Spinach and baby corn were sown on 20th December 2018 and 2nd February 2019 respectively.

3.13 Field application of algae manure, urea, algae manure with biogas slurry and gypsum

The *Chlorella*: biogas slurry: gypsum in (100:20:1) and (100:10:0.5) (dry w/w/w) were applied in field. The algae manure on the basis of recommended dose of N, algae manure with urea in 75:25, 50:50 and 25:75 and recommended dose of NPK was applied to spinach and baby corn. The algae manure was applied to field with treated water on the basis of recommended dose of nitrogen. The example of calculation for algae culture needed as manure per plot is given below (treatment T_3, baby corn). The calculation is based on nitrogen while P and K is supplied additionally.

Calculation

Recommended dose of baby corn150:60:60 NPK kg/ha

Algae dry biomass-0.44g/L (Table 4.2) 1

Nitrogen content in algae dry biomass-6% (Table 4.3) 2

Treatment T_3 of baby corn (algae manure on the basis of recommended dose of nitrogen)

For $1m^2$ field of baby corn-15 g nitrogen is required

It was observed that 6 g nitrogen is present- 100g algae biomass ….(1)

So for 15 g Nitrogen- 100*15/6=250g dry algae biomass required

0.44 g dry algae biomass obtained from- 1 L ….(2)

Hence 250 g algae will be supplied by- 250/0.44=570 L algae with treated wastewater.

It mean that in treatment T_3 (algae biomass on the basis of nitrogen), 570 L of algae present in treated wastewater was required, which is equivalent to recommended dose of nitrogen for $1m^2$ field size in baby corn. The algae culture needed for various treatments of spinach and baby corn is given in Table 3.3 and 3.4 respectively. The details of treatment selected for application of *Chlorella*: biogas slurry: gypsum in (100:20:1) and (100:10:0.5) (dry w/w/w), algae manure on the basis of recommended dose of N, algae manure with urea in 75:25, 50:50 and 25:75 and recommended dose of NPK was applied to spinach and baby corn as follows.

Objectives

1. To remediate the sewage wastewater using alga *Chlorella minutissima*
2. To produce manure from algal biomass using biogas slurry and gypsum
3. To evaluate the efficacy of algal manure on baby corn and spinach

Treatments

T_0 Control condition with no nutrient application

T_1 Aerobic digested *Chlorella* manure 1 (algal biomass+ Biogas slurry+ Gypsum in 100:20:1 (w/w/w) ratio)

T_2 Aerobic digested *Chlorella* manure 2 (algal biomass+ Biogas slurry+ Gypsum in 100:10:0.5 (w/w/w) ratio)

T_3 Algal biomass on the basis of nitrogen

T_4 75 % dose of N by algal biomass + 25 % by urea

T_5 50 % dose of N by algal biomass + 50 % by urea

T_6 25 % dose of N by algal biomass + 75 % by urea

T_7 Recommended dose by chemical fertilizer

Table 3.3 Algae manure with treated wastewater, biogas slurry and gypsum needed for spinach per plot

Treatments	Details	Algae culture needed/per plot ($1m^2$)
T1	Aerobic digested *Chlorella* manure 1 (algal biomass+ biogas slurry+ gypsum 100:20:1 w/w/w)	230 L algae + 20 L Biogas slurry + 1 g gypsum
T2	Aerobic digested C*hlorella* manure 2 (algal biomass+ Biogas slurry+ gypsum: 100:10:0.5 w/w/w)	230 L algae + 10 L biogas slurry + 0.5 g gypsum
T3	Algal biomass on the basis of nitrogen	190 L
T4	75 % dose of N by algal biomass + 25 % by urea	142 L
T5	50 % dose of N by algal biomass + 50 % by urea	95 L
T6	25 % dose of N by algal biomass + 75 % by urea	48 L

Table 3.4 Algae manure with treated wastewater, biogas slurry and gypsum needed for baby corn per plot

Treatments	Details	Algae culture needed
T1	Aerobic digested *Chlorella* manure 1 (algal biomass: Biogas slurry: Gypsum:: 100:20:1 w/w/w)	230 L algae + 20 L Biogas slurry + 1 g gypsum
T2	Aerobic digested *Chlorella* manure 2 (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w	230 L algae + 10 L biogas slurry + 0.5 g gypsum
T3	Algal biomass on the basis of nitrogen	570 L
T4	75 % dose of N by algal biomass + 25 % by urea	426 L
T5	50 % dose of N by algal biomass + 50 % by urea	285 L
T6	25 % dose of N by algal biomass + 75 % by urea	142 L

3.14 Size of field

The experiment was conducted in 1m x 1m plot size with following eight treatments. The experiment was laid out in RBD design with three replication. The recommended dose of fertilizer for spinach and baby corn was 50:50:50 NPK kg/ha and 150:60:60 NPK kg/ha respectively.

3.15 Nitrate leaching

The setups were installed for determining leaching of nitrate in treatments with different depths. For that hollow pipes of PVC (column) having 100 cm length and 30 cm diameter were kept in triplicate. Equal numbers of seeds of spinach were sown in all columns. The top 10 cm of column left for irrigation. At depth of 15 cm, 30 cm, 45 cm, 60 cm, 75 cm and 90 cm a plastic syringe of 15 mL capacity were installed for collecting leachate. The collected leachates were filter though filter paper whatman No. 1 and analyzed for nitrate content though spectrophotometer at 220 nm and 275 nm wavelength.

Plate 3.3 Nitrate leaching set up and collection of leachate.

3.16 Nitrate + nitrite and Ammonium content in soil after harvesting of crop

The soils were collected at three different depth 0-15 cm, 15-30 cm and 30-45 cm from column through auger after harvesting of spinach. The collected soil was extracted through 2N KCl solution and analyzed through scalar N-flow analyser for nitrate + nitrite and ammonium content.

3.17 Determination of physical and chemical properties of soil

The chemical parameters pH, EC, organic carbon, available nitrogen, and available phosphorus were analyzed in the soil initially and after harvesting of the both crops. The standard protocol for estimation of above selected parameters is given below.

3.17.1 Soil texture

Mechanical composition of field soil *i.e.*, proportion of sand, silt and clay size particles was determined by hydrometer method (Bouyoucos, 1962). The texture of the soil was determined according to textural triangle proposed by USDA (Brady and Weil, 2002).

3.17.2 Soil pH and electrical conductivity (EC)

The pH of soil was determined in 1:2.5 (soil: water) suspension using portable water testing kit (WTW Vario Cond. Set 2X00-001A). The electrical conductivity (EC) was also determined in the supernatant liquid of the same extracts with the help of portable water testing kit (WTW Vario Cond. Set 2X00-001A) at 25˚C

3.17.3 Organic carbon

For determination of oxidizable organic carbon, soil samples grounded and passed through 0.2 mm sieve. Organic carbon content in soil was determined by wet oxidation method using $K_2Cr_2O_7$ as outlined by Walkley and Black (1934).

3.17.4 Total available nitrogen in soil

Available nitrogen in soil was determined by alkaline potassium permanganate method as described by Subbiah and Asija (1956). The procedures involve two steps a) distilling the soil with alkaline potassium permanganate solution and, b) absorb the ammonia liberated in boric acid which is then titrated with standard sulphuric acid.

3.17.5 Total available phosphorus in soil

Available phosphorus forms (Olsen P) were extracted with 0.5 mol/L $NaHCO_3$ and measured calorimetrically according to the method described by Olsen and Watanabe (1965).

3.18 Determination of soil enzymatic activity

Soil enzymes dehydrogenase, urease and nitrate reductase were analyzed in all treatments after 30 days of sowing in baby corn and spinach grown soil.

3.18.1 Dehydrogenase (EC 1.1.1)

Dehydrogenase activity in soil was estimated by standard method described by Tabatbai, (1994). In a test tube one gram soil was taken and added 1 mL of 3 % TTC (2,3,5- Triphenyl tetrazolium chloride solution) to saturate the soil. Added 1 g glucose in each tube. Incubate tubes at 28◦ C for 24 h. Added 10 mL of methanol after incubation. Allowed to stand for 6 h in open condition after shaking. After 6 h withdrew clear pink colour supernatant and took reading at 485 nm in spectrophotometer. Expressed the result in µg TPF $h^{-1}g^{-1}$ of soil.

3.18.2 Dissimilatory nitrate reductase (EC: 1.7.99.4)

Nitrate reductase in soil was estimated by standard method described by Tabatbai, (1994). The nitrite-N, extracted with a 2.5M potassium chloride solution, is treated with a diazotizing agent (sulfanilamide) in hydrochloric acid solution to convert nitrite to a diazonium salt, and subsequently treated with a coupling reagent (N-1 napthyl-ethylenediamine) to convert the diazonium salt to an azo compound. The intensity of reddish purple colour of the resultant compound is measured at 540 nm.

3.18.3 Urease (EC 3.5.1.5)

Urease activity in soil was estimated by standard method described by Tabatbai, (1994). Took 5 g soil sample in 125 mL polypropylene bottle. Added 5mL urea solution. Incubated at 37˚C for 5 hour. After incubation added 50 mL 2*M* KCL-PMA (Phenyl mercuric acetate) solution. Shaken for 1 h. The suspension was filtered the after 1 hour by Whatman no. 42. Took 1-2 mL of extract in 50 mL volumetric flask. Volume was make up to 10 mL with 2*M*- KCL-PMA after that added 30 mL colouring agent and put in water bath. After 30 mL of water bath, remove flask and cooled immediately (ice) 15 min. Volume was make up to 50 mL with water. Measures absorbance at 527 nm in spectrophotometer.

3.19 Agronomical yield parameters in spinach and baby corn

The agronomical yield parameters selected for spinach and baby corn is given in table. In spinach total four cuttings were taken during the experiment from 1 m^2 plot (Table 3.4).

Table 3.5 Growth and yield parameters of spinach and baby corn

Economical yield parameter	Spinach	Baby corn
	Shoot length, shoot biomass, recoverable root length, root biomass	Baby corn yield with husk, baby corn yield without husk, baby corn husk yield and cob length

3.20 Field Layout Plan- Each plot 1m x 1m and 0.5 x 0.5 m left between each row for channel

T_1R_1	T_0R_2	T_7R_3
T_0R_1	T_6R_2	T_5R_3
T_3R_1	T_7R_2	T_0R_3
T_2R_1	T_3R_2	T_4R_3
T_4R_1	T_5R_2	T_2R_3
T_5R_1	T_2R_2	T_3R_3
T_7R_1	T_5R_2	T_6R_3
T_6R1	T_1R_2	T_1R_3

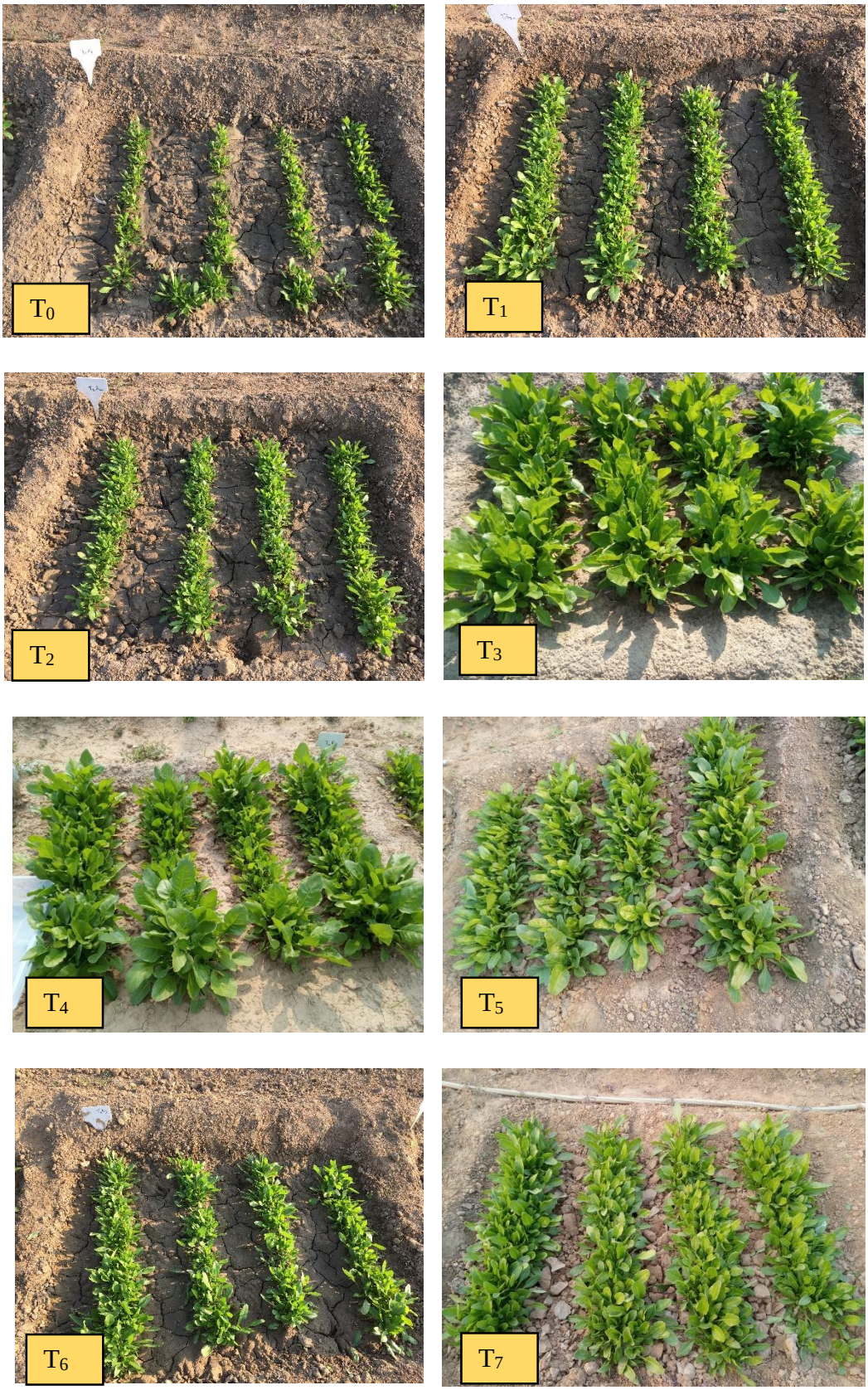


Plate 3.4 Effect of treatments on the spinach

Plate 3.5 Effect of treatments on baby corn

3.21 Statistical analysis

The triplicate sets of data were analysed in accordance with the experimental design (Randomized Block Design). The measurement of growth parameters were subjected to one-way analysis of variance (ANOVA) to test difference among means corresponding to algae and chemical fertilizer. Least significant difference (LSD, P=0.05) was used to determine whether means differed significantly. Duncan's Multiple Range test (DMRT) is a post hoc test to measure specific differences between pairs of means. To evaluate the potential relationship between the soil enzymatic parameters and nutrient content the Pearson correlation coefficient were made through (SPSS 21).

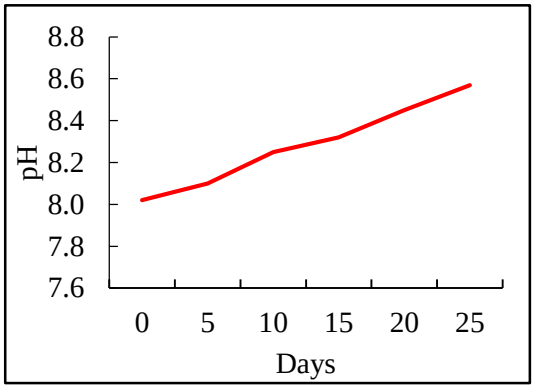
Chapter 4 **RESULTS**

The present experiment was undertaken to study the phycoremediation potential of microalgae (C*hlorella minutissima)* for the treatment of sewage wastewater and to study impact of algae biomass of C. *minutissima* with treated wastewater on soil quality and crop productivity (spinach and baby corn). The aim of study was 1) to quantify temporal dynamics (5 days interval) in physico-chemical quality of sewage wastewater after inoculation of algal culture, 2) to quantify manure production and nutrient content of algae biomass, 3) to assess impact of application of recommended dose of N in the form of algal biomass, manure obtained from mixture of (algal biomass+ biogas slurry+ gypsum) and urea on soil quality and crop productivity (spinach and baby corn) and 4) to study nitrate leaching in spinach and baby corn grown soil column, irrigated with algal biomass suspension, biogas slurry, gypsum and urea. The results of the experiment has been described in details in following sub sections.

4.1 Phycoremediation potential of *Chlorella minutissima* for sewage wastewater

4.1.1 Temporal dynamics in sewage wastewater quality

C*hlorella minutissima* was found very effective in the removal of electrical conductivity **(**EC), total dissolved solids, phosphorous (P), potassium (K), ammonium, nitrate, biological oxygen demand and chemical oxygen demand of sewage wastewater. There was significant decrease in the electrical conductivity, TDS, P, K, NH_4^+, NO_3^-, BOD and COD content while increase in pH (insignificant) and DO (significant) of sewage wastewater after the inoculation of algae (Table 4.1). The EC, TDS, P, K, NH_4^+ and NO_3^- value continuously decreased during 25 days of inoculation however, rate of decrease was higher during 0-15 days of inoculation (Fig. 4.1). It means *Chlorella minutissima* can effectively reduce the EC, TDS, P, K, NH_4^+, NO_3^-, BOD and COD within 15 days of inoculation. EC, TDS, P, K, NH_4^+, NO_3^- continuously decreased after algal inoculation however BOD, COD and DO followed different trend. BOD and COD initially increased up to 5 days of algal inoculation followed by continuous decrease while DO initially decreased up to five days of algal inoculation followed by continuous increase.


pH
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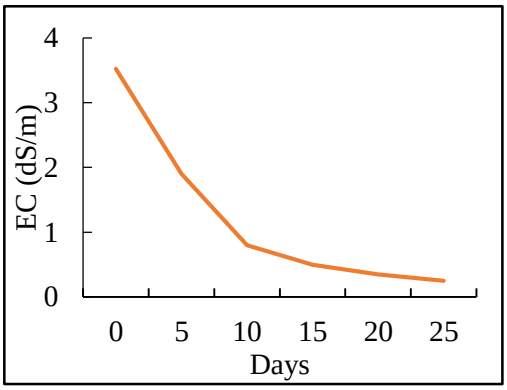
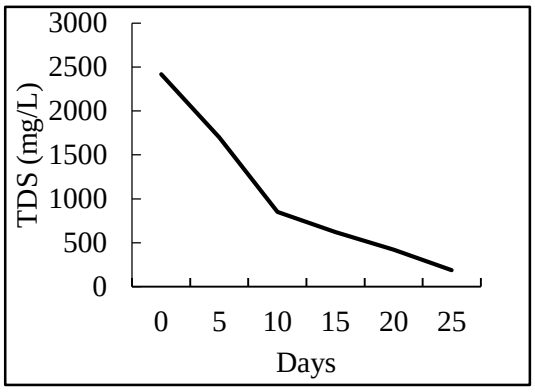
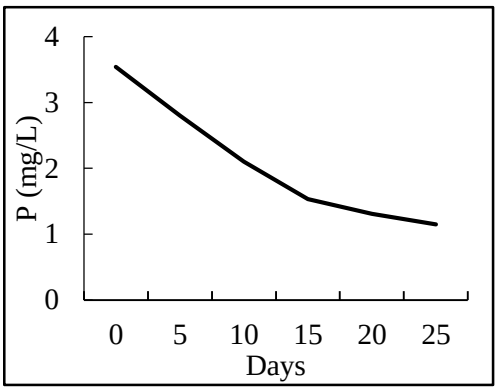
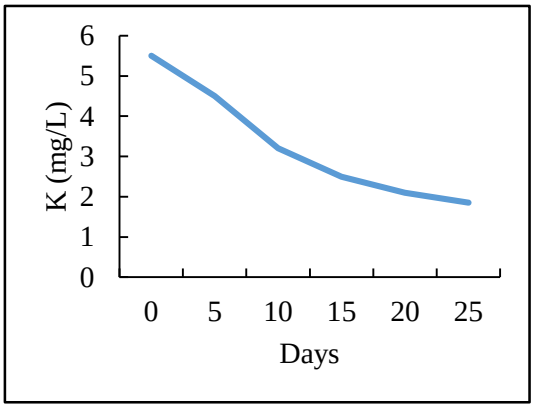
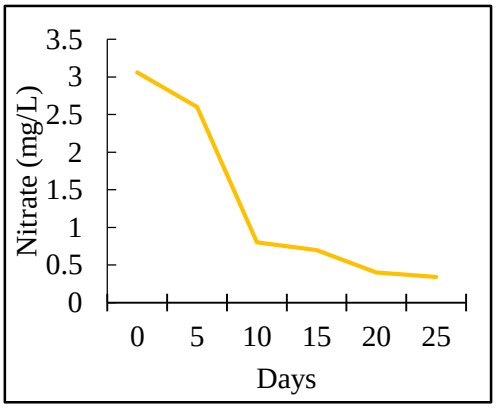
8.6
8.4
8.2
8.0
7.8
7.6
0
5
10
15
20
25
Days


EC (dS/m)
4
3
2
1
0
0
5
10
15
20
25
Days


TDS (mg/L)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Days


P (mg/L)
4
3
2
1
0
0
5
10
15
20
25
Days


K (mg/L)
6
5
4
3
2
1
0
0
5
10
15
20
25
Days


Nitrate (mg/L)
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Days

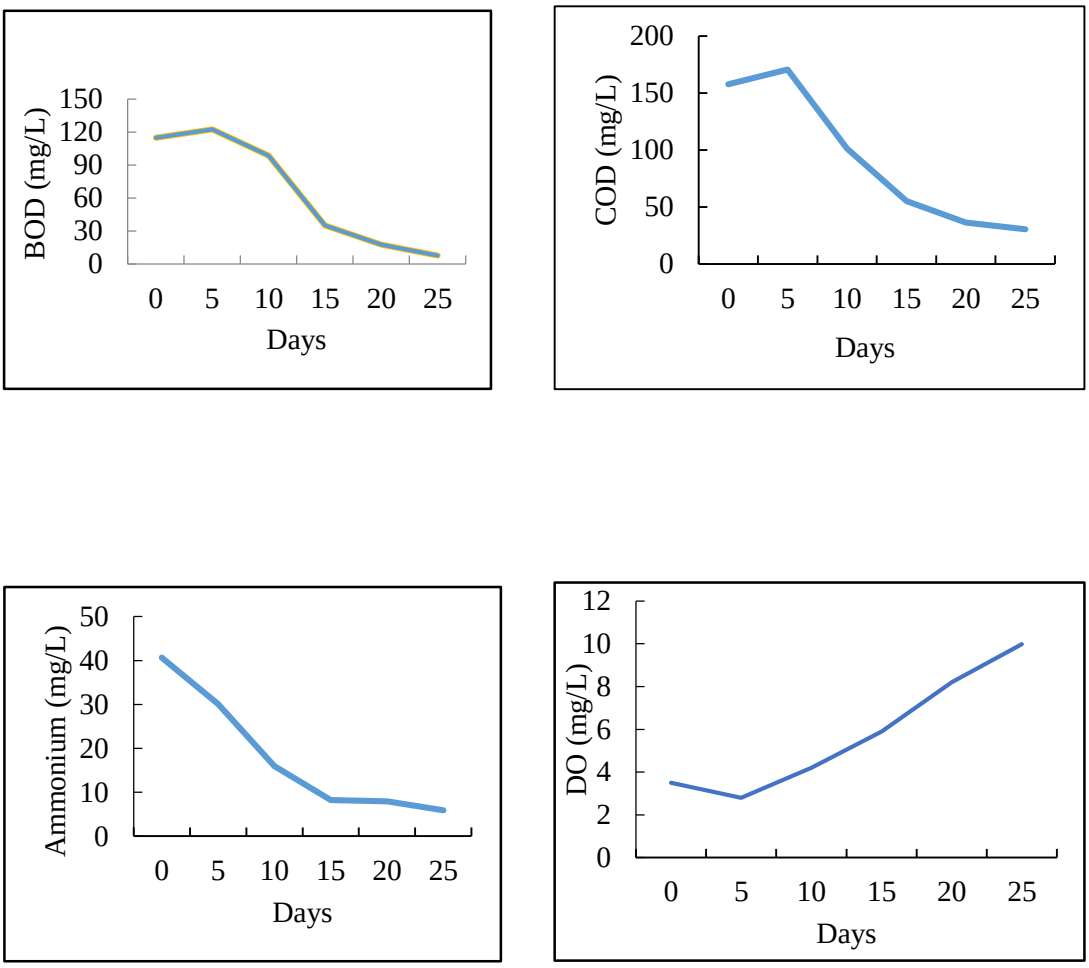


Fig 4.1. Temporal variation in quality of sewage wastewater after inoculation of *Chlorella minutissima* for pH, EC (ds/m), TDS (mg/L), phosphorous (mg/L), potassium (mg/L), nitrate (mg/L), BOD (mg/L), COD (mg/L), ammonium (mg/L) and DO (mg/L) at 5 day interval for 25 days

4.1.2 Changes in electrical conductivity (EC) and total dissolved solids (TDS)

Electrical conductivity and TDS are the most important parameters for determining quality of water for irrigation. Total dissolved solids (TDS) is total amount of any metals, cations, anions, minerals and salt dissolved in water. Irrigation with high EC and TDS water lead to accumulation of salt and higher soil water osmotic potential that ultimately reduces water availability to the crop and their yield. According to FAO, irrigation water quality parameters, the electrical conductivity and TDS of irrigation water should be in the range of 0.7-3 dS/m and 450- 2000 mg/L respectively to avoid secondary soil salinization. The initial EC and TDS of sewage wastewater in this study was 3.52±0.07 dS/m and 2416±202 mg/L (Table 4.1). After 25 days of phycoremediation this value significantly decreased to 0.25±0.03 dS/m and 136±12.58 mg/L respectively. The decrease in EC and TDS was about 93% and 94.3% respectively (Fig. 4.2) The EC and TDS level in sewage wastewater after phycoremediation were within prescribed maximum limit for the irrigation.

4.1.3 Changes in Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Dissolved Oxygen (DO)

BOD and COD are indicator of organic pollutant load in the wastewater. Wastewater with high BOD and COD reduces dissolve oxygen availability to the aquatic organism. Therefore, BOD and COD load of wastewater should be reduced before discharging it in to the fresh water bodies. A standard biological oxygen demand test conducted over a five-day period (BOD_5) is typically used to estimate the decomposable organic compounds in a waterbody. In present study, C. *minutissima* was found very effective in reducing BOD and COD while increasing DO content of the sewage wastewater. The BOD and COD were reduced from 114±7.08 mg/L and 157±0.68 to 7.75±1.12 mg/L and 30.58±1.39 mg/L respectively after 25 days of algal inoculation (Table 4.1). While DO increased from 3.50±1.04 mg/L to 8.10±0.4 mg/L. The BOD and COD were reduced by 93.2 % and 80.5 % respectively (Fig 4.2). While DO increased by more than twice compare to initial. The decrease in BOD and COD might be due to an increase in DO through photosynthesis of algae that might have led to aerobic decomposition of organic matter in the wastewater.

4.1.4 Changes in NPK content

Nitrogen, phosphorus and potassium are primary plant nutrients. Nitrogen is absorbed by plants and algae in the form of nitrate and ammonia, while phosphorus is absorbed in the form of phosphate and potassium in the form of K^+. Therefore, inoculation of algae in the sewage wastewater significantly reduced amount of the nitrate, ammonia, phosphate and potassium. The initial content of nitrate, ammonium, phosphate and potassium in sewage wastewater were 3.06±0.01 mg/L, 5.6+0.18 mg/L, 3.54±0.05 mg/L and 5.5±0.09 mg/L respectively (Table 4.1.). They get reduced to 0.34±0.05 mg/L, 2.9±0.15 mg/L, 1.15±0.01 mg/L, and 1.85±0.03 mg/L respectively after 25 days of phycoremediation by *C. minutissima*. The percentage reduction was 89 %, 48.2 %, 67.4 % and 66.3 % respectively for nitrate, ammonium, phosphate and potassium (Fig.4.2).

Table 4.1- The physical and chemical characteristic of wastewater before and after harvest (25 days) of *Chlorella minutissima*

Parameters	Initial value	Final value	P value (t test)	Water quality for irrigation*
	Average ± Stand. dev			
pH	8.02±0.05	8.57±0.27	0.06	6.0 – 8.5
EC (dS/m)	3.52±0.07	0.25±0.03	0.00	0.7 – 3.0 dS/m
TDS (mg/L)	2416±202	136±12.58	0.00	450 – 2000 mg/L
P (mg/L)	3.54±0.05	1.15±0.01	0.00	0-2 mg/L
K (mg/L)	5.5±0.09	1.85±0.03	0.00	0-2 mg/L
NH_4^+ (mg/L)	5.6±0.18	2.9±0.15	0.00	0-5 mg/ L
NO_3^- (mg/L)	3.06±0.01	0.34±0.05	0.00	5 – 30 mg/ L
DO (mg/L)	3.5±1.04	8.1±0.40	0.00	0-7 mg/ L
BOD_5 (mg/L)	114±7.08	7.75±1.12	0.00	100 mg/ L
COD (mg/L)	157±0.68	30.58±1.39	0.000	NA

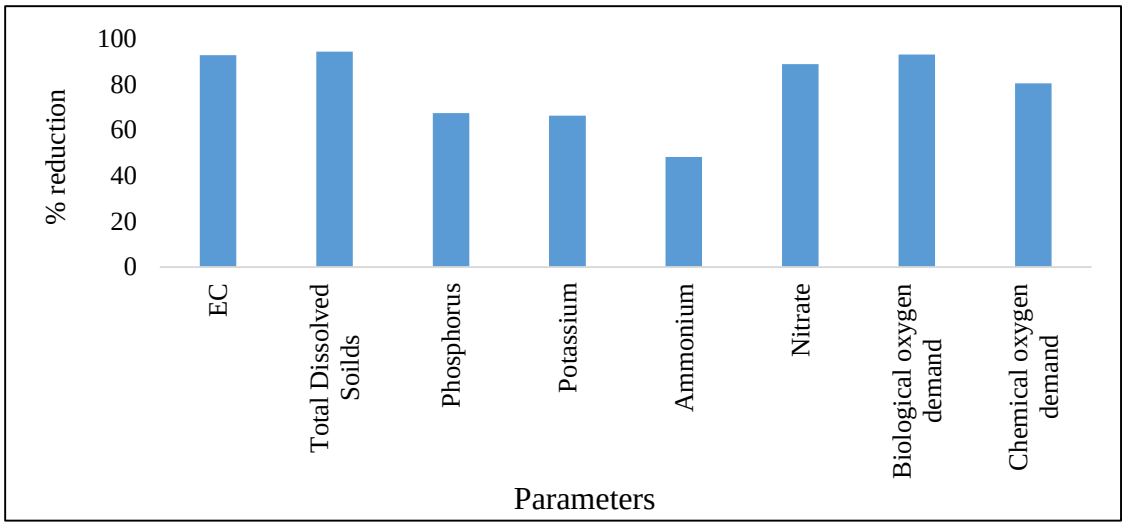


Fig. 4.2 Phycoremediation potential of *Chlorella minutissima* (removal of pollutants) in collected sewage wastewater (% reduction)

4.2 Algal biomass production and its NPK content

4.2.1 Algal biomass production

The algal biomass produced per unit of wastewater after inoculation was determined by filtering algal treated wastewater after 25 days of phycoremediation by muslin cloth. The obtained fresh and dry biomass was 1.26±0.07 g/L and 0.44±0.04 g/L of wastewater respectively (Table 4.2).

Table 4.2 Fresh and dry weight of algae after 25 days of innoculation in wastewater

Parameters	Concentration with Std. dev
Fresh weight (g/L)	1.26±0.07
Dry weight (g/L)	0.44±0.04

4.2.2 NPK content in algal biomass

The dried algal biomass was used for the determination of NPK content by standard methods. The NPK content in dry algae biomass was 6.0±0.17%, 1.0±0.10% and 0.48±0.04 % respectively as shown in Table 4.3.

Table 4.3 NPK content in dry algae biomass

Parameter	Concentration with Std. dev
Nitrogen (%)	6.0±0.17
Phosphorus (%)	1.0±0.10
Potassium (%)	0.48±0.04

4.2.3 NPK content of manure produce from a mixture of algae, biogas slurry and gypsum

Algal biomass was mixed with biogas slurry and gypsum in the ratio of 100:20:1 and 100:10:0.5 on (w/w/w) basis. After mixing, mixtures was kept for 15 days for aerobic digestion to produce manure. The pH of experimental field was alkaline in nature, therefore gypsum was added to reduce the alkalinity of the soil. After 15 days of digestion of mixture, the nitrogen and phosphorus content in obtained manure was determined by standard protocol as described in material and methods section. The obtained manure contained 3.9% and 3.5% nitrogen in 100:20:1 and 100:10:0.5 mixtures respectively (Table 4.4). While phosphorus content was 0.58% and 0.55% in 100:20:1 and 100:10:0.5 mixtures respectively. The nitrogen and phosphorus content in biogas slurry were 1.2 % and 0.7 % respectively while in algal biomass it were 6.0 % and 1.0% respectively. Therefore, resultant mixtures should have 5.15 % N and 0.94 % P in 100:20:1 mixtures while 5.54 % N and 0.97 % P in 100:10:0.5 mixtures (Table 4.4). It indicated loss of N and P after addition of biogas slurry and gypsum. However the percentage decrease in phosphorus content was insignificant and lower than nitrogen content. The reason for reduction in nitrogen might be due to volatilization loss of ammonia. Therefore, result indicated that application of sole algal biomass as manure will supply more N and P than mixture of biomass algae, biogas slurry and gypsum.

Table 4.4 Nitrogen and phosphorus content in aerobic digested manure obtain from mixture of algal biomass, biogas slurry and gypsum

Mixture	Theoretical N and P content [Algae (6.0% N and 1.0 P) + Biogas slurry (1.2% N and 0.7% P)]		Observed N and P content in the manure		Loss	
	N_t (%)	P_t (%)	No (%)	P_o (%)	N (%)	P (%)
Algae + biogas slurry + gypsum (100:20:1)a	5.2	0.94	3.9	0.58	1.3	0.36
Algae + biogas slurry + gypsum (100:10:0.5)b	5.54	0.97	3.5	0.55	2.04	0.42

P value of Nta x Noa = 0.00, P-value of Pta x Poa = 0.06;
P value of Ntb x Nob = 0.00, P-value of Ptb x Pob = 0.065;

4.3 Impact of algal biomass application on soil and productivity of spinach and baby corn

To study the impact of application of biomass algae as manure on the plant growth and soil quality, a field study was carried out in 1 m x 1m plot size. The experiment was laid out in RBD design with three replication. The selected crops were spinach and baby corn while source of N were i) algal biomass, ii) manures obtained from 15 days digestion of algal biomass + biogas slurry + gypsum mixture in 100:20:1 and 100:10:0.5 ratio and iii) urea. The biomass algae, manure and urea was applied at the rate of recommended dose of nitrogen for the selected crops in the study area i.e. 150 kg N/ha for baby corn and 50 kg N/ha for spinach. The total eight treatment combinations was selected: T_0 (control, zero N application), T_1 (algal biomass+ Biogas slurry+ Gypsum+ 100:20:1 w/w/w), T_2 (algal biomass+ Biogas slurry+ Gypsum+ 100:10:0.5 w/w/w), T_3 (100% N dose by Algal biomass), T_4 (75 % dose by algal biomass + 25 % by urea), T_5 (50 % Dose by algal biomass + 50 % by urea), T_6 (75 % Dose by algal biomass + 25 % by urea and T_7 (recommended dose of NPK by chemical fertilizer)**.** The detailed description of treatments has been outlined in material and method section. The obtained results has been described below

4.3.1 Impact on growth and yield of spinach

To study the impact, the leaf biomass, leaf length, root biomass and recoverable root length of spinach in each treatments were recorded.

4.3.2 Biomass yield of spinach

Leaves are economic product of spinach therefore, total four cuttings of leave were obtained from each plot during the experiment. The total leaf biomass yield was found significantly different among the treatments while there was insignificant difference in the leaf length. The maximum leaf length/plant varied from 11.09 cm to 12.88 cm among all the treatments (Table 4.5). Highest fresh leaf biomass (7.15 kg/m^2) was recorded in T_3 (100% N dose by algal biomass) followed by (7.12 kg/m^2) in T_5 (50 % N dose by algal biomass + 50 % by chemical fertilizer), while lowest (5.02 kg/m^2) in T_0 (control, zero N application) (Table 4.5). Leaf biomass yield in T_3 was significantly higher (42.43 %) than T_0 (control) while higher, but insignificantly different from other treatments. Since, farmers supply recommended dose of NPK

through chemical fertilizer, therefore we compared leaf biomass yield of each treatments with T_7 which has been depicted in Fig. 4.3. As compared to T_7, the leaf biomass yield was higher in T_3 (5.6 %) and T_5 (5.1 %) while lower in other treatments T_0, T_1, T_2, T_4 and T_6 (25.8 %, 22%, 22.7%, 0.7 % and 1.9 % respectively). It was also observed that leaf length of T_3 (100% N dose by Algal biomass) was 3.5 % higher than T_7 (recommended dose of NPK by chemical fertilizer) while in other treatments found to be lower comparatively. Thus result indicated that, supply of recommended dose of N through algal biomass may lead to higher or equivalent leaves yield of spinach as compared to leaves produced by supply of recommended dose of fertilizer.

Table 4.5 Effect of treatments on the yield of fresh leaf biomass and leaf length of spinach

Treatments	Biomass (kg/m^2)	Leaf length/plant (cm)
T0	4.55^a	11.09^a
T1	5.28^{ab}	11.31^a
T2	5.23^{ab}	11.30^a
T3	7.15^b	12.88^a
T4	6.72^b	11.81^a
T5	7.12^b	12.31^a
T6	6.64^b	12.18^a
T7	6.77^b	12.45^a
LSD (P=0.05)	1.01	3.32

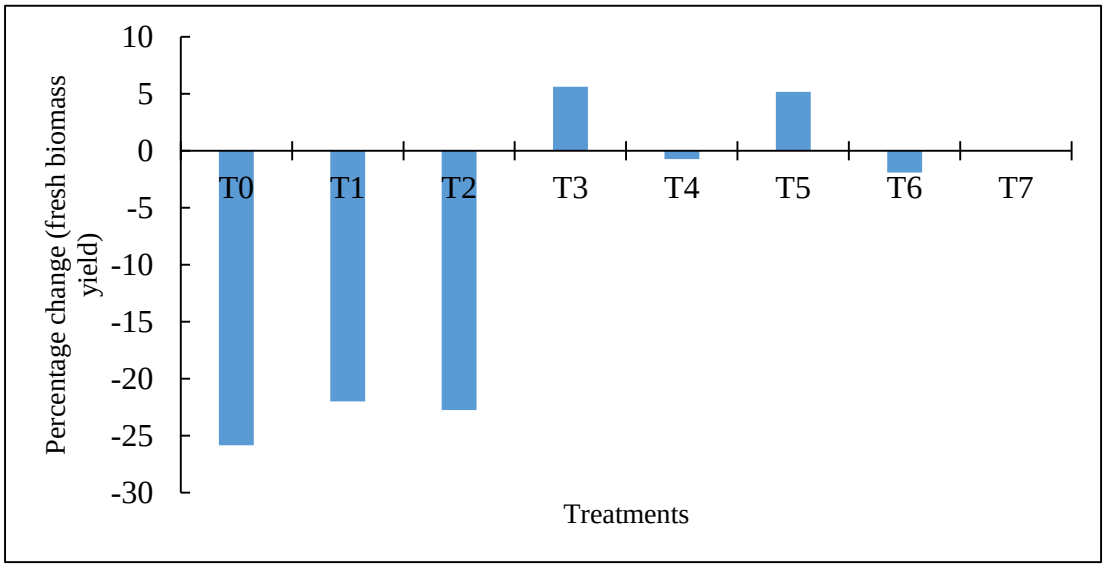
T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

4.3.3 Recoverable root length/plant (cm) and root biomass of spinach

The maximum recoverable root length/plant (17.04 cm) was obtained in treatment T_7 (recommended dose of NPK by chemical fertilizer) followed by 16.54 cm in T_2, while least (14.35 cm) was obtained in T_1 (algal biomass+ biogas slurry+ gypsum: 100:20:1) (Table 4.6). However, differences was statistically insignificant. The increase/decrease of root length with respect to T_7 has shown in Fig. 4.4.

As compared to T_7, the recoverable root length in treatment T_0, T_1, T_2 T_3, T_4, T_5 and T_6 was (13.9 %, 15.8 %, 2.9 % 3.1 %, 10.7 %, 9.7 % and 6.5 % respectively) lower.

The fresh weight of root biomass/plant is shown in the Table 4.5. The maximum root biomass/plant (18.32 g) was obtained in T_7 followed by (18.17 g), in T_3 (100 % N dose by chemical fertilizer) while the lowest (10.33 g) was obtained in T_1. It was observed that, statistically T_3 was significantly higher than treatment T_0 by 74 % but on par with treatment T_7. The increase/decrease of root biomass with respect to T_7 was revealed in Fig 4.4. The root biomass/plant in treatment T_0, T_1, T_2 T_3, T_4, T_5 and T_6 was 43.2 %, 43.6 %, 26.2 %, 0.8 %, 32.7 %, 1.5 % and 19.4 % respectively lower than T_7.



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.3. Percentage change in spinach leaf biomass yield with respect to T_7 (recommended dose of chemical fertilizer)

4.3.3 Recoverable root length/plant (cm) and root biomass of spinach

The maximum recoverable root length/plant (17.04 cm) was obtained in treatment T_7 (recommended dose of NPK by chemical fertilizer) followed by 16.54 cm in T_2, while least (14.35 cm) was obtained in T_1 (algal biomass+ biogas slurry+ gypsum: 100:20:1) (Table 4.6). However, differences was statistically insignificant. The increase/decrease of root length with respect to T_7 has shown in Fig. 4.4.

As compared to T_7, the recoverable root length in treatment T_0, T_1, T_2 T_3, T_4, T_5 and T_6 was (13.9 %, 15.8 %, 2.9 % 3.1 %, 10.7 %, 9.7 % and 6.5 % respectively) lower.

The fresh weight of root biomass/plant is shown in the Table 4.5. The maximum root biomass/plant (18.32 g) was obtained in T_7 followed by (18.17 g), in T_3 (100 % N dose by chemical fertilizer) while the lowest (10.33 g) was obtained in T_1. It was observed that, statistically T_3 was significantly higher than treatment T_0 by 74 % but on par with treatment T_7. The increase/decrease of root biomass with respect to T_7 was revealed in Fig 4.4. The root biomass/plant in treatment T_0, T_1, T_2 T_3, T_4, T_5 and T_6 was 43.2 %, 43.6 %, 26.2 %, 0.8 %, 32.7 %, 1.5 % and 19.4 % respectively lower than T_7.

Table 4.6 Impact of treatments on recoverable root length/plant and root biomass/plant of spinach

Treatments	Recoverable root length/plant (cm)	Root Biomass/plant(g)
T0	14.67^{a}	10.40^{a}
T1	14.35^{a}	10.33^{a}
T2	16.54^{a}	13.51^{ab}
T3	16.50^{a}	18.17^{b}
T4	15.21^{a}	12.32^{ab}
T5	15.38^{a}	18.04^{b}
T6	15.92^{a}	14.75^{ab}
T7	17.04^{a}	18.32^{b}
LSD (P=0.05)	2.42	5.80

T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

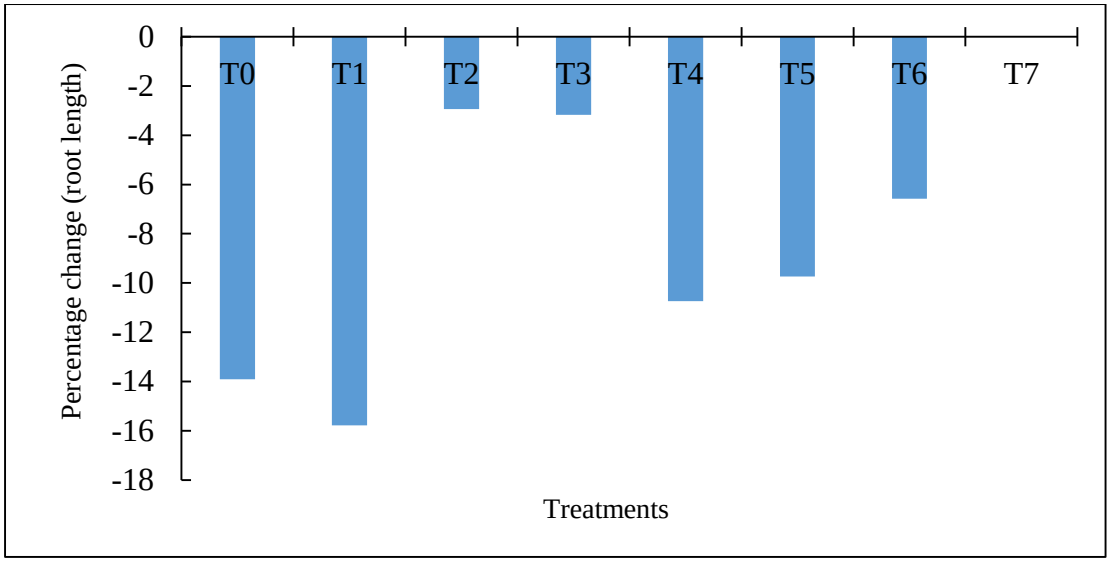
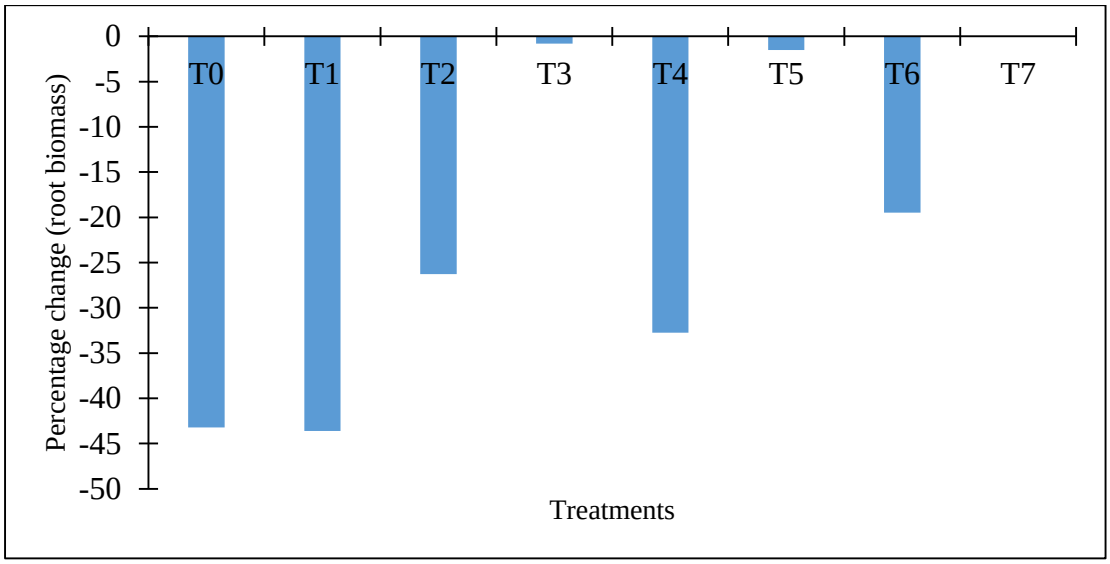


Fig. 4.4 Percentage change in spinach root length with respect to T_7 (recommended dose of chemical fertilizer)



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.5 Percentage change in spinach root biomass with respect to T_7 (recommended dose of chemical fertilizer**)**

4.4 Impact on growth, yield and quality of Baby corn

To study the impact, the baby corn yield with husk, baby corn yield without husk (cob), husk yield and cob length were recorded in each treatment

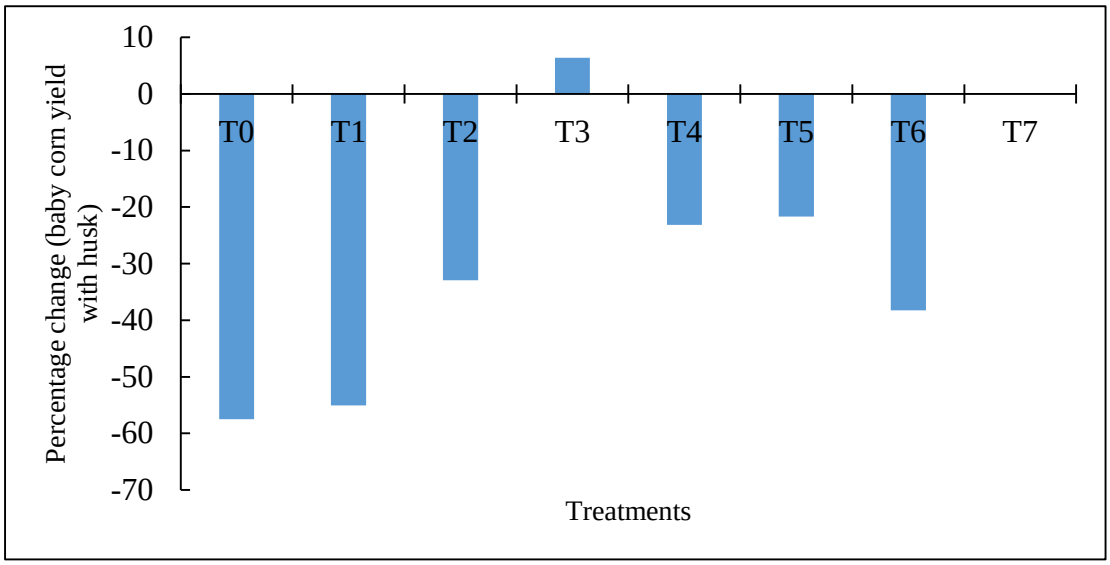
4.4.1 Baby corn yield with husk

It was recorded that highest (2.83 kg) baby corn yield with husk was obtained in T_3 (100 % N dose by algal biomass) followed (2.6 kg) by T_7 (recommended dose of NPK by chemical fertilizer), while lowest (1.13 kg) obtained in T_0 (100 % N dose by algal biomass) (Table 4.7). Statistically among the treatments baby corn yield with husk of T_3 is significantly higher than T_0 by 1.5 times while non-significant with T_7. Since, farmers supply recommended dose of NPK through chemical fertilizer, therefore we compared baby corn yield with husk of each treatments with T_7 which has been depicted in Fig. 4.6. The baby corn yield with husk in T_3 was 6.3 % higher than T_7 while in other treatments found to be lower comparatively.

Table 4.7 Effect of treatments on baby corn yield with husk, without husk, husk yield and cob length

Treatments	Baby corn yield			
	With husk (kg)	Without husk (kg)	Husk yield (kg)	Cob length (cm)
T0	1.13^{a}	0.24^{a}	0.88^{a}	6.06^{a}
T1	1.19^{a}	0.26^{a}	0.93^{a}	6.24^{ab}
T2	1.78^{b}	0.24^{a}	1.54^{c}	6.29^{b}
T3	2.83^{d}	0.66^{d}	2.16^{d}	7.48^{e}
T4	2.04^{c}	0.38^{b}	1.66^{c}	6.53^{c}
T5	2.08^{c}	0.53^{b}	1.55^{c}	6.65^{c}
T6	1.64^{b}	0.27^{a}	1.37^{b}	6.92^{d}
T7	2.66^{d}	0.54^{b}	2.12^{d}	7.38^{e}
LSD (P=0.05)	0.15	0.04	0.14	0.18

T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

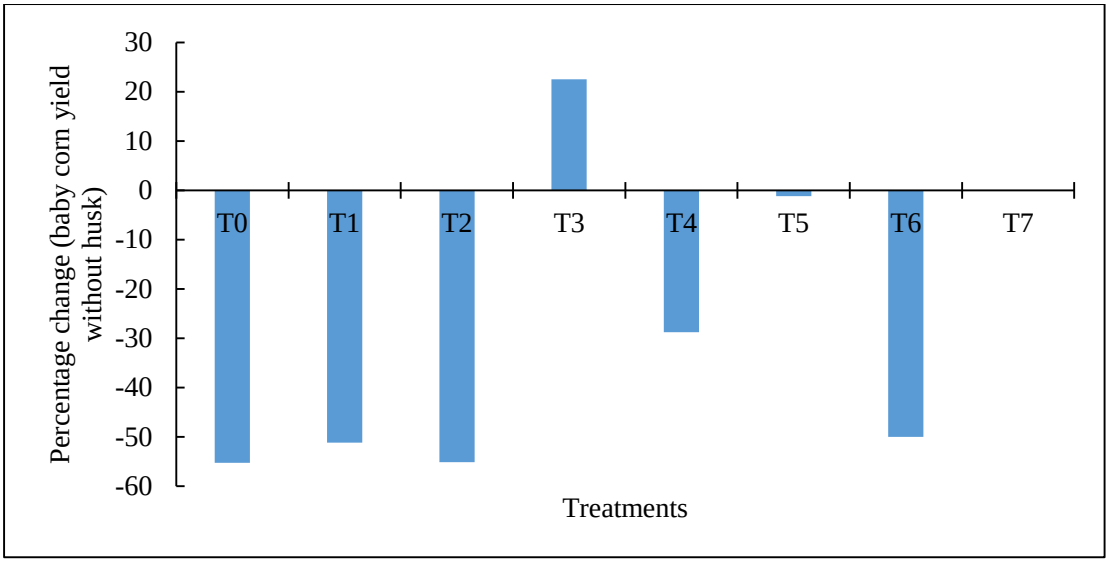


T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig 4.6. Percentage change in baby corn yield with husk with respect to T_7 (recommended dose of chemical fertilizer)

4.4.2 Baby corn yield without husk (cob)

The cob is the economic product of baby corn therefore, it was harvested from each plot during the experiment. The mean value baby corn yield without husk is given in Table 4.7. It was observed that highest (0.66 kg) baby corn yield without husk was obtained in T_3 (100 % N dose by algal biomass) followed (0.54 kg) by T_7 (recommended dose of NPK by chemical fertilizer) while lowest (0.24 kg) were obtained in T_0 (control, zero N application). Statistically among the treatments baby corn yield without husk of treatment T_3 is significantly higher than T_7 and T_0 by 22.6% and more than 1.5 times respectively. The increase/decrease of baby corn yield without husk with respect to T_7 is depicted in Fig 4.7. The cob yield of T_3 was 22.5 % higher than T_7, while others found to have lower yield comparatively. Thus result indicated that, supply of recommended dose of N through algal biomass may lead to higher cob yield as compared to cob produced by supply of recommended dose of fertilizer.

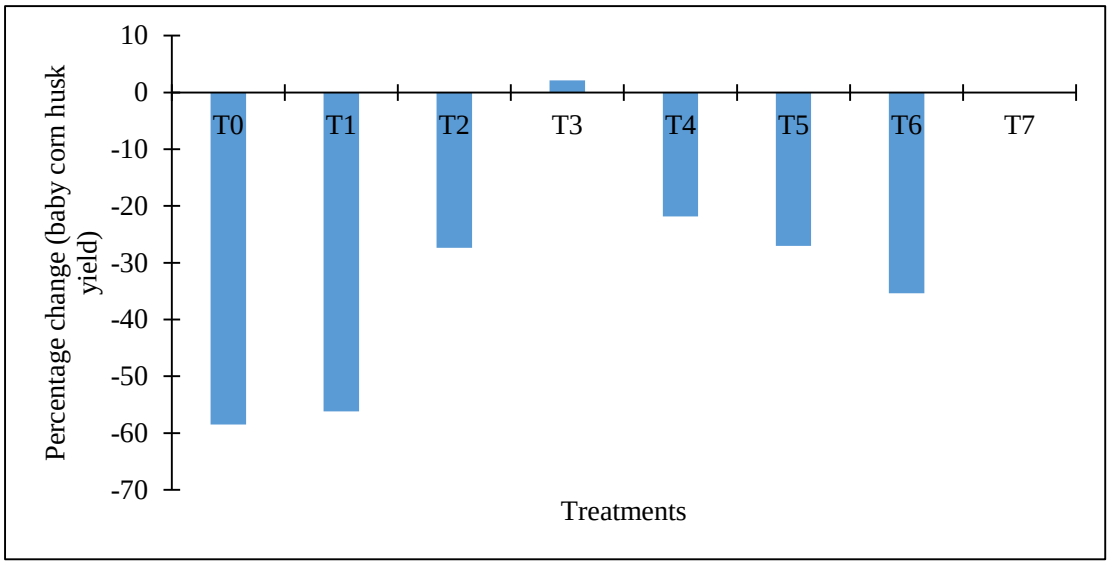


T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig 4.7. Percentage change in baby corn yield without husk with respect to T_7 (recommended dose of chemical fertilizer)

4.4.3 Baby corn husk yield

Baby corn husk also an important product obtained from baby corn. It is mainly used in silage preparation and feed for cattle. It was observed that highest (2.16 kg) baby corn husk yield was obtained in T_3 (100 % N dose by algal biomass) followed (2.12 kg) by T_7 (recommended dose of NPK by chemical fertilizer), while lowest (0.88 kg) were obtained in T_0 (control, zero N application). Statistically among the treatments baby corn husk yield of T_3 is significantly higher than other treatments, while non-significant with T_7. The increase/decrease of baby corn husk yield with respect to T_7 is given in Fig. 4.8. As compared to T_7, the baby corn husk yield of T_3 was 2.09 % higher while lower in other treatments T_0, T_1, T_2, T_4, T_5 and T_6 (58.4 %, 56.2 %, 27.3 %, 21.8 %, 27 % and 35.3 %) respectively. Thus result shown that, supply of recommended dose of N through algal biomass may lead to higher or equivalent husk yield of baby corn as compared to husk produced by supply of recommended dose of fertilizer.

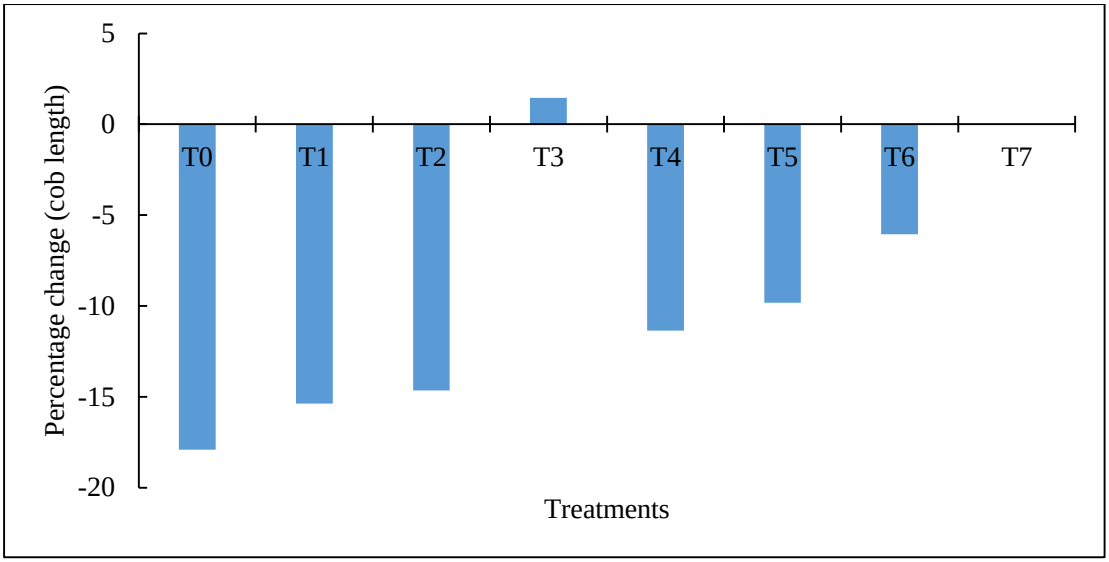


T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig 4.8. Percentage change in baby corn husk yield with respect to T_7 (recommended dose of chemical fertilizer)

4.4.4 Cob length

Cob size is a very important parameter in baby corn. The cob with highest size fetch maximum market price. It was observed that highest (7.48 cm) baby corn cob length was obtained in T_3 followed by T_7 (7.38 cm), while lowest (6.06 cm) were obtained in T_0 (Table 4.7). Statistically among the treatments baby corn cob length of T_3 is significantly higher than T_0 by 23.4 % while non-significant with T_7. The increase/ decrease of baby corn cob length with respect to T_7 is shown in Fig 4.9. The baby corn cob length of T_0, T_1, T_2, T_4, T_5 and T_6 was 18 %, 15.3 %, 14.7 %, 11.3 %, 98 % and 6 % respectively lower than T_7, while higher obtained in T_3 (1.4 %).



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig 4.9. Percentage change in baby corn cob length with respect to T_7 (recommended dose of chemical fertilizer)

4.5 Impact of algal biomass application on soil quality

Before sowing and after the harvest of spinach and baby corn, soil was sampled from 0-15 cm from each treatment plots. The sampled soil was used to determine physico-chemical (pH, EC, Organic C, Available N and P) and enzymatic activity (dehydrogenase, urease and nitrate reductase activities). The obtained result has been described below:

4.5.1 Physico-chemical properties

4.5.1 pH and EC

Soil pH and EC were insignificantly different while soil organic-C, available phosphorus and available N content were significantly different among treatments in both the crops. The pH of soils was found higher than initial (8.26) value in all the treatments in both the crops. It varied from 8.41 to 8.68 in the spinach grown soil while 8.4 to 8.6 in baby corn grown soil. However, increase was statistically insignificant (Table 4.8). Among the treatments, the soil pH was lowest in treatment T_3 in the soil of both the crops. The soil EC also showed insignificant difference and was below the safe limit in all the treatments. It varied from 0.22 dS/m to 0.5 ds/m in the spinach grown soil while 0.24 dS/m to 0.52 ds/m in baby corn grown soil.

Table 4.8 pH and EC after harvest of spinach and baby corn

Treatments	Spinach		Baby corn	
	pH	EC (dS/m)	pH	EC (dS/m)
Initial	8.26	0.43	8.26	0.43
T0	8.56^{a}	0.22^{a}	8.40^{a}	0.24^{a}
T1	8.61^{a}	0.43^{ab}	8.50^{ab}	0.45^{ab}
T2	8.49^{a}	0.50^{ab}	8.60^{ab}	0.52^{b}
T3	8.41^{a}	0.44^{ab}	8.41^{ab}	0.46^{ab}
T4	8.51^{a}	0.46^{ab}	8.45^{ab}	0.48^{ab}
T5	8.59^{a}	0.31^{ab}	8.60^{ab}	0.33^{ab}
T6	8.68^{a}	0.26^{ab}	8.46^{a}	0.28^{ab}
T7	8.56^{a}	0.44^{ab}	8.58^{a}	0.46^{ab}
LSD (P=0.05)	NS	0.23	0.26	0.23

T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

4.5.2 Soil organic carbon

All the treatments showed higher organic carbon content as compared to initial soil organic carbon in the soil of both the crops. However, difference was statistically insignificant. The initial organic carbon of soil before showing was 0.25 % (Table 4.9). After harvest of the crop, it varied from 0.23 % to 0.33 % in the soil of spinach and 0.24 % to 0.32 % in the soil of baby corn. The maximum Soil organic carbon (SOC) content was found in the T_3 (100 % N dose by algal biomass) of the both crops. It was 3.12 % higher in the soil of spinach and 6.6 % higher in the soil of baby corn as compared to the soil cultivated with recommended dose of chemical fertilizer i.e. T_7 (recommended dose of NPK by chemical fertilizer). The increase/decrease in organic carbon (%) with respect to treatment T_7 has been shown in Fig. 4.10 and Fig 4.11. The result indicated that application of algae biomass led to higher build-up of SOC as compared to NPK applied by chemical fertilizer.

4.5.3 Available soil nitrogen

All the treatments showed higher available nitrogen content as compared to initial available nitrogen content in the soil of both the crops. After harvest of the crop, it varied from 240-361 kg/ha in the soil of spinach and 236-415 kg/ha in the soil of baby corn (Table 4.9). The maximum available nitrogen content in spinach and

baby corn was found in T_3 (361 kg/ha) and T_5 (415 kg/ha). Statistically in the soil of both the crops, available nitrogen content in treatment (T_3) supplied with only algae and treatment (T_7) supplied with recommended dose of NPK by chemical fertilizer was non-significant. However, T_3 was statistically significantly higher than T_0 by 50.41 % in the soil of spinach and 74.67 % in the soil of baby corn. The available nitrogen content of T_3 in the soil of spinach was 4.22 % higher than treatment T_7 while in other treatments found to be lower comparatively (Fig. 4.12) In baby corn grown soil T_0, T_1, T_2, T_4, T_5 and T_6 was 47 %, 22.2 %, 9.2 %, 5.5 %, 5.4 % and 8.4 % respectively lower than T_7 (Fig. 4.13).

Table 4.9 Organic carbon (%), available nitrogen and phosphorus (kg/ha) content in the spinach and baby corn grown soil with different treatments

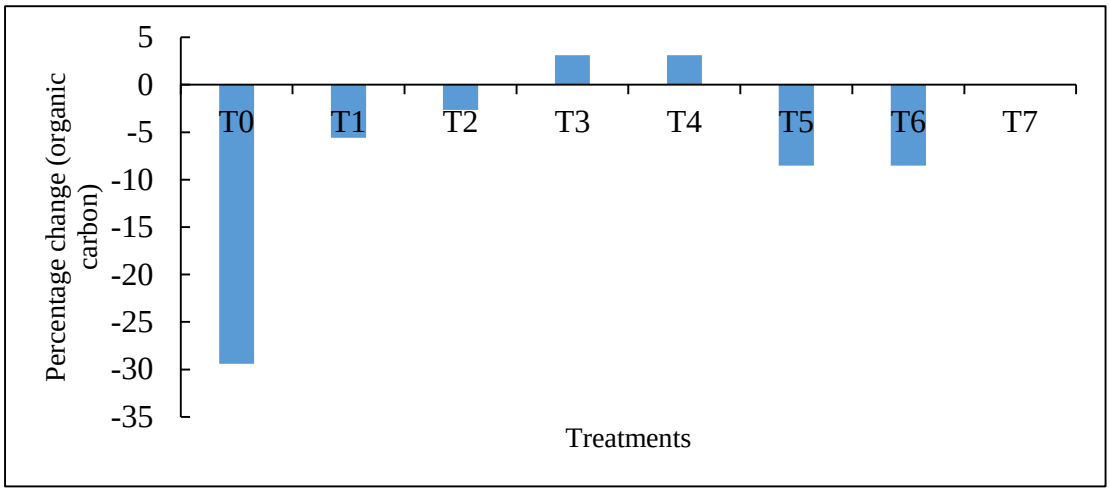
Treatments	Spinach			Baby corn		
	Organic carbon	Available Nitrogen	Available Phosphorus	Organic carbon	Available Nitrogen	Available Phosphorus
Initial	0.25	228	41.15	0.25^{a}	228	41.15
T0	0.23^{a}	240^{a}	47.46^{b}	0.24^{a}	236^{a}	43.22^{a}
T1	0.32^{ab}	311^{bc}	44.95^{a}	0.29^{ab}	323^{b}	43.66^{ab}
T2	0.31^{ab}	295^{b}	46.08^{ab}	0.29^{ab}	377^{c}	44.26^{ab}
T3	0.33^{b}	361^{e}	47.63^{b}	0.32^{b}	413^{e}	45.38^{b}
T4	0.33^{b}	330^{cd}	47.46^{b}	0.27^{ab}	392^{cd}	44.78^{ab}
T5	0.26^{ab}	331^{cd}	46.42^{ab}	0.30^{ab}	392^{d}	44.43^{ab}
T6	0.31^{ab}	343^{de}	45.56^{ab}	0.30^{ab}	380^{c}	43.57^{ab}
T7	0.32^{b}	346^{de}	47.63^{b}	0.30^{ab}	415^{e}	45.12^{ab}
LSD (P=0.05)	0.17	23.44	1.91	0.17	27.50	1.75

T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

4.5.4 Available phosphorus (kg/ha)

All the treatments showed higher available phosphorous content as compared to initial available phosphorous content in the soil of both the crops. After harvest of the crop, it varied from 44.95-47.63 kg/ha in the soil of spinach and 43.22-45.38 kg/ha in the soil of baby corn (Table 4.9). The maximum available phosphorous content was found in T_3 in spinach (47.63 kg/ha) and baby corn (45.38 kg/ha) soil. Statistically in the soil of both the crops, available phosphorous content in treatment (T_3) supplied

with only algae and treatment (T_7) supplied with recommended dose of NPK by chemical fertilizer was non-significant. However, T_3 was statistically significantly higher than T_1 (5.9 %) in the soil of spinach and T_0 (4.9 %) in the soil of baby corn. The available phosphorous content in T_0, T_1, T_2, T_4, T_5 and T_6 was 0.3 %, 5.6 %, 3.2 %, 0.3 %, 2.5 % and 4.3 % respectively lower than T_7 in the soil of spinach (Fig. 4.14). On the other hand in baby corn soil available phosphorous content in T_0, T_1, T_2, T_4, T_5 and T_6 was 4.2 %, 3.2 %, 1.9 %, 0.8 %, 1.5 % and 3.4 % respectively lower than T_7 (Fig. 4.15).



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.10. Percentage change in organic carbon content with respect to T_7 (recommended dose of chemical fertilizer) in the soil of spinach

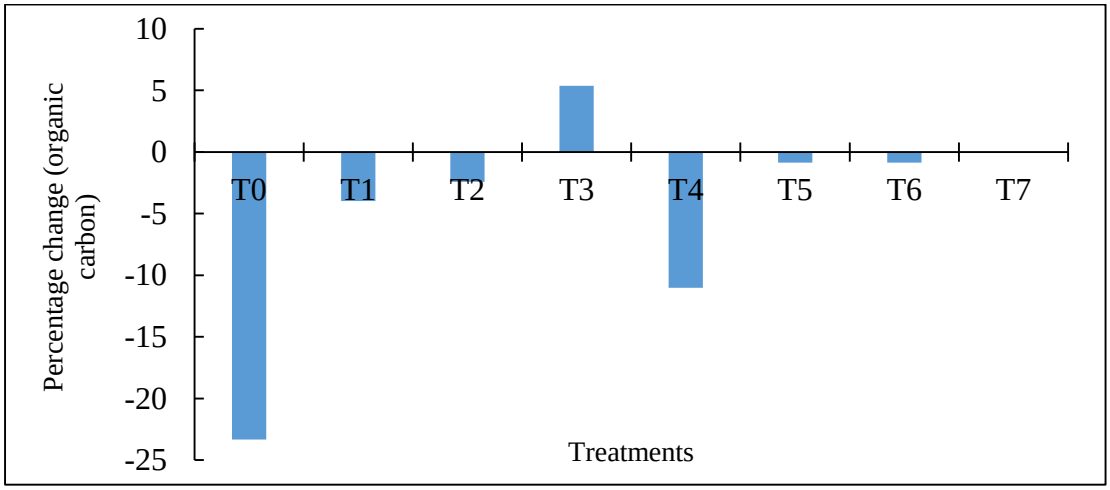
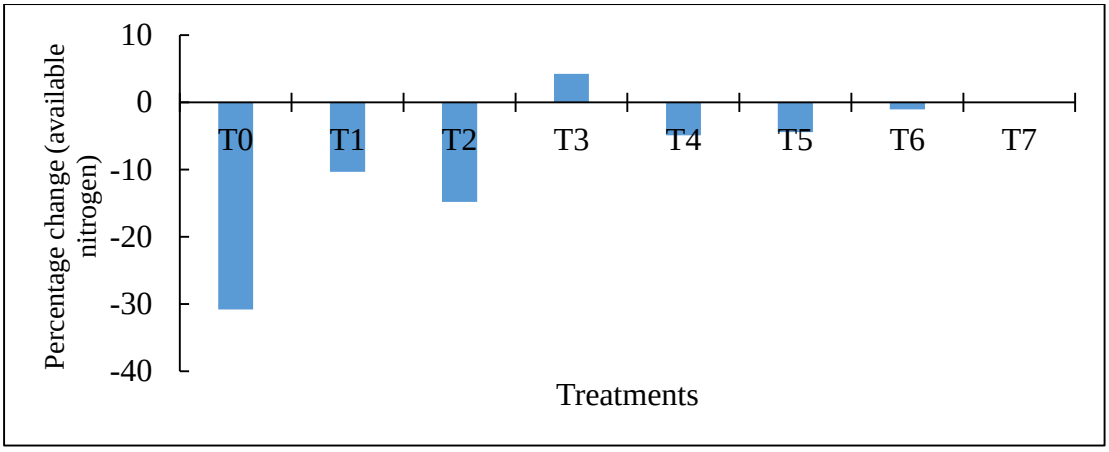


Fig 4.11. Percentage change in organic carbon content with respect to T_7 (recommended dose of chemical fertilizer) in the soil of baby corn



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.12 Percentage change in available nitrogen content in the soil of spinach with respect to T_7 (recommended dose of chemical fertilizer)

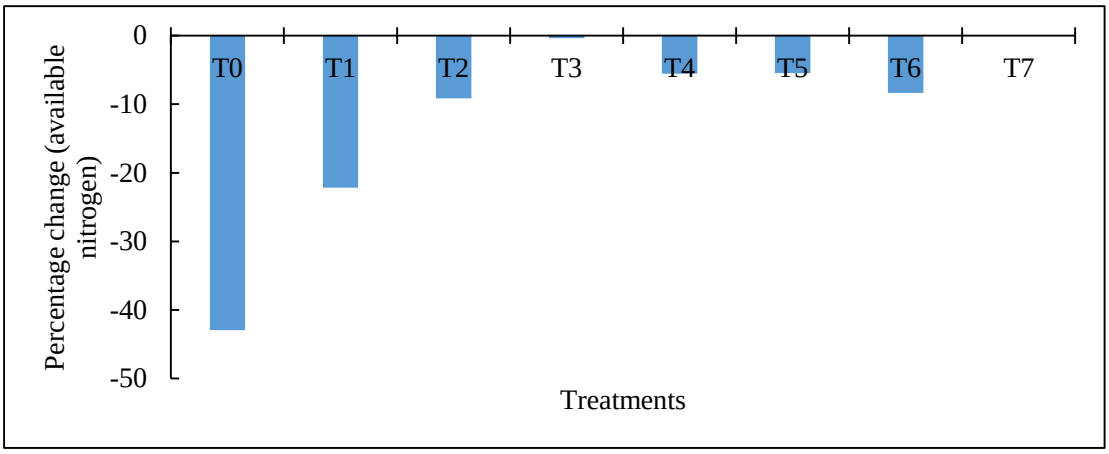


Fig. 4.13 Percentage change in available nitrogen content in the soil of baby corn with respect to T_7 (recommended dose of chemical fertilizer)

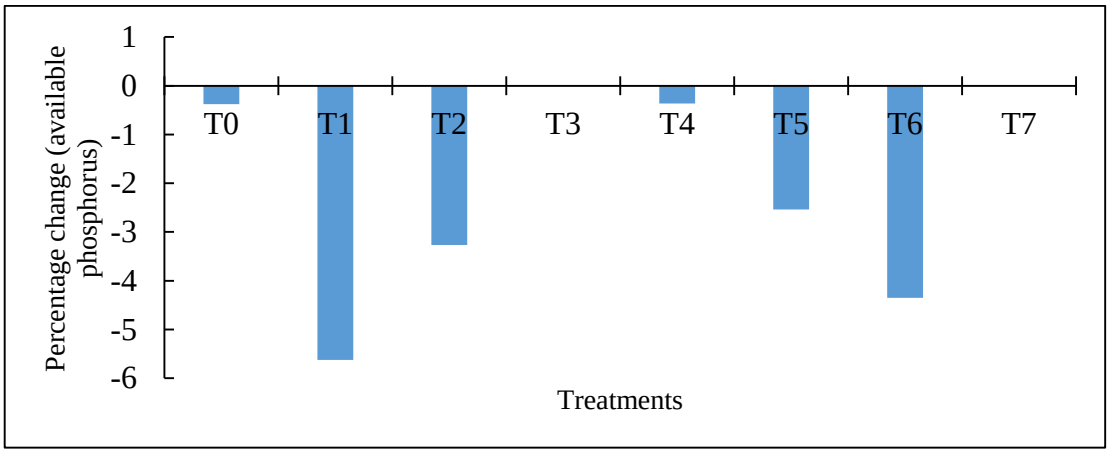
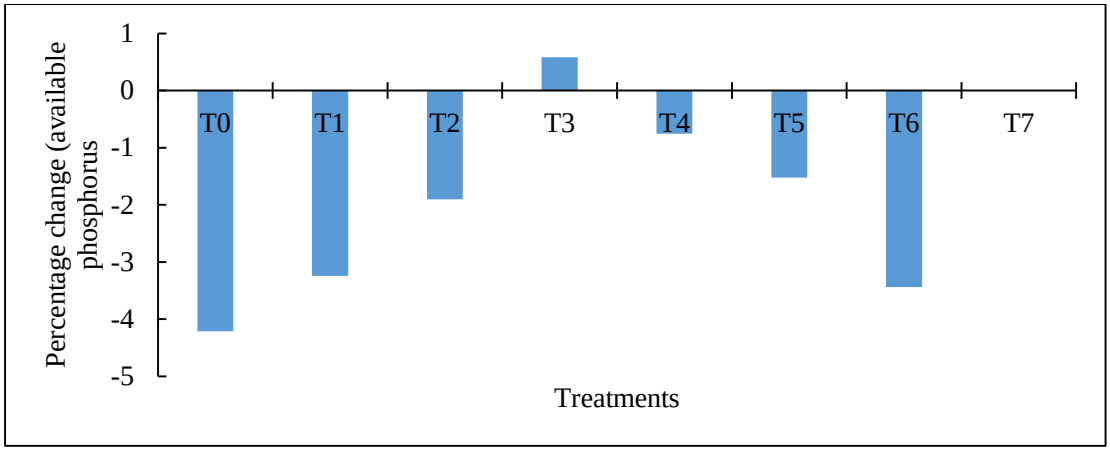


Fig. 4.14. Percentage change in available phosphorus content in soil of spinach with respect to T_7 (recommended dose of chemical fertilizer)



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.15. Percentage change in available phosphorus content in soil the soil of baby corn with respect to T_7 (recommended dose of chemical fertilizer)

4.6 Nitrate leaching

The nitrate content in leachate was determined through a column study in the field. The hollow pipes of PVC (column) of 100 cm length and 12 inches diameter were kept in triplicate. The top part (10 cm) of the column was left for irrigation. At a depth of 15 cm, 30 cm, 45 cm, 60 cm, 75 cm, and 90 cm a plastic syringe of 15 mL capacity was installed for collecting leachate. The cumulative nitrate content in leachate was found to be maximum (24.63 mg/L) in treatment T_7 (recommended dose of NPK by chemical fertilizer) while lowest (15.81 mg/L) in T_0 (control, zero N application) followed by (17.19 mg/L) T_3 (recommended dose of NPK by chemical fertilizer).

4.6.1 Nitrate leaching (0-15 cm) depth

The mean value of nitrate content in water extracted from 0-15 cm depth of experimental field is presented in Table 4.10. The maximum leaching of nitrate was taken place from T_7 (8.57 mg/L) followed by T_6 (7.89 mg/L), while least was recorded from T_0 (5.03 mg/L). Statistically among the treatment nitrate content in leachate in T_3 was significantly lower than T_7 by 29.9% while higher than control by 19.4 %. The increase/decrease of nitrate in leachate with respect to treatment T_7 has shown in Fig. 4.16. The nitrate content in treatment T_1, T_2, and T_3 was 27.1%, 26.7%, and 29.8% respectively lower than treatment T_7.

4.6.2 Nitrate leaching (15-30 cm) depth

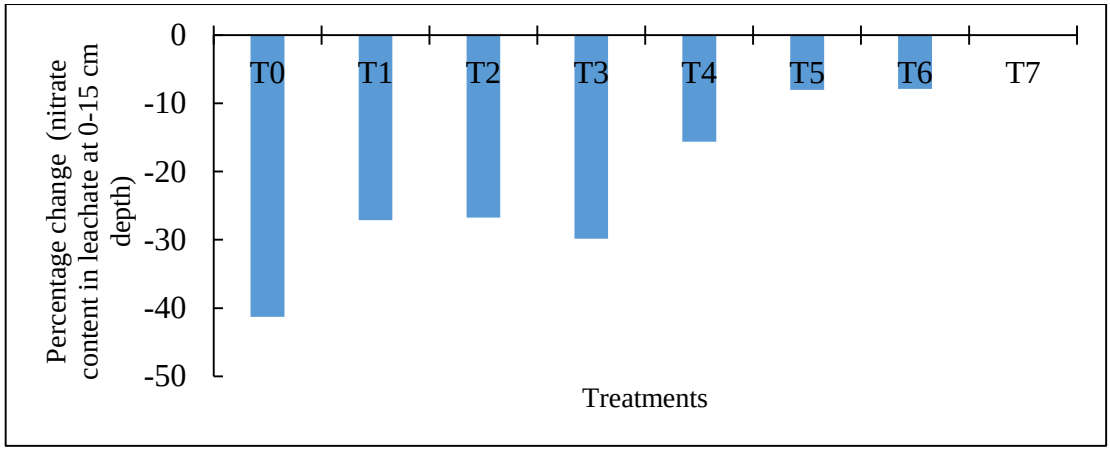
The mean value of nitrate content in water extracted from 15-30 cm depth of experimental field is presented in Table 4.10. The maximum leaching of nitrate was recorded in treatment T_6 (7.98 mg/L) followed by T_7 (7.97 mg/L), while least was observed from T_0 (5.80 mg/L). Statistically among the treatment nitrate content in leachate in T_3 was significantly lower than T_7 by 25.6% while significantly higher than T_0 by only 2.24%. The increase/decrease of nitrate leaching from 15-30 cm during experiment with respect to T_7 is shown in Fig. 4.17. The nitrate content in treatment T_1, T_2, and T_3 was 15.12%, 17.85% and 25.63% respectively lower than treatment T_7.

4.6.3 Nitrate leaching (30-45 cm) depth

The mean value of nitrate content in water extracted from 30-45 cm depth of experimental field is presented in Table 4.10. The maximum leaching of nitrate was found in treatment T_7 (8.16 mg/L) followed by T_6 (7.31 mg/L), while least was observed in T_0 (4.98 mg/L). Statistically among the treatment, nitrate content in leachate from T_3 was significantly lower than T_7 by 35.5%, while significantly higher than control by 5.4%. The increase/decrease of nitrate leaching from 30-45 cm during an experiment with respect to T_7 has shown in Fig. 4.18. The nitrate content in treatment T_3 was 35.6 % lower than treatment T_7.

Table 4.10 Nitrate leaching at different depth in spinach with different treatments

Treatments	Nitrate in leaching (mg/L)			Cumulative nitrate content
	(0-15 cm)	(15-30 cm)	(30-45 cm)	
T0	5.03^a	5.80^a	4.98^a	15.81
T1	6.25^b	6.76^b	5.17^a	18.18
T2	6.28^b	6.55^b	5.25^a	18.08
T3	6.01^b	5.93^a	5.25^a	17.19
T4	7.23^c	6.41^b	6.19^b	19.83
T5	7.88^d	6.40^b	6.22^b	20.50
T6	7.89^d	7.98^c	7.31^c	23.18
T7	8.57^e	7.97^c	8.16^d	24.69
LSD (P=0.05)	0.36	0.35	0.31	



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.16. Percentage change in nitrate leaching at 0-15 cm depth with respect to T_7 (recommended dose of chemical fertilizer)

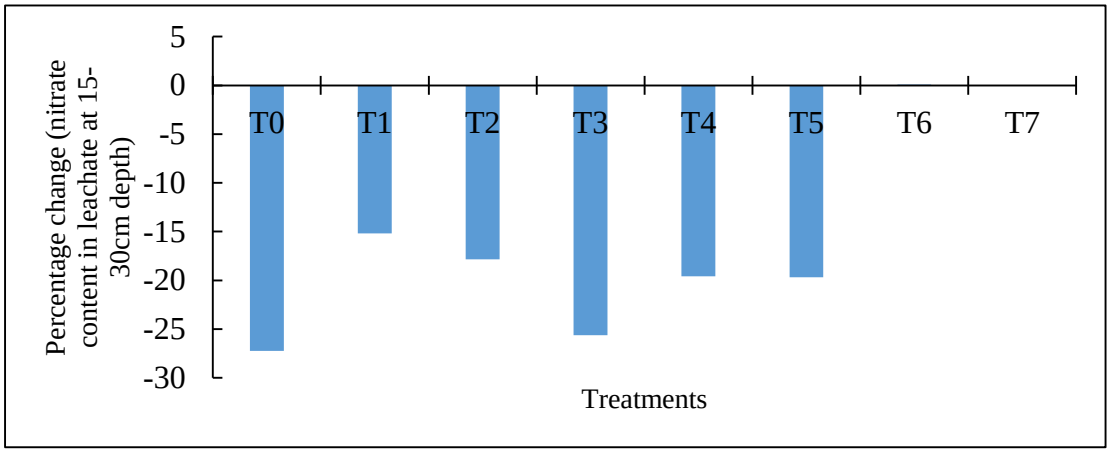


Fig. 4.17. Percentage change in nitrate leaching at 15-30 cm depth with respect to T_7 (recommended dose of chemical fertilizer)

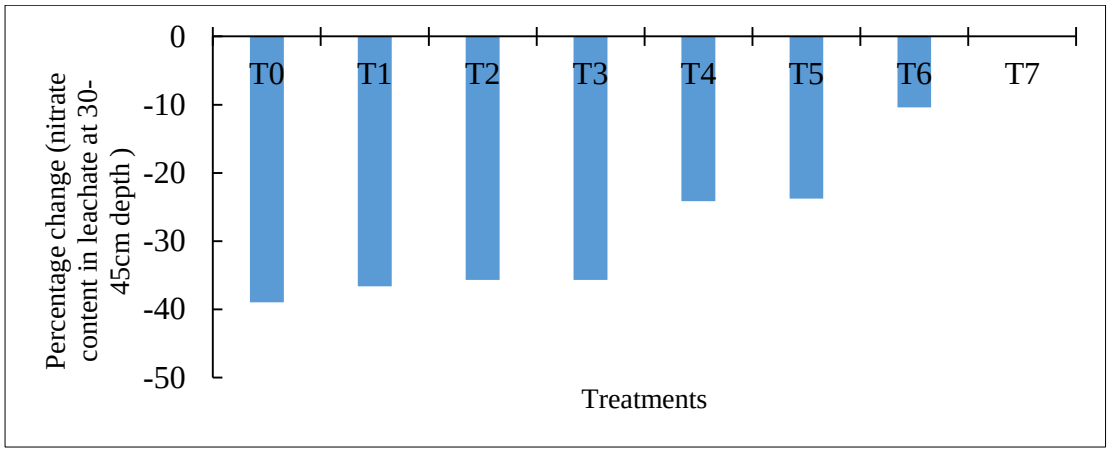


Fig. 4.18. Percentage change in nitrate leaching at 30-45 cm depth with respect to T_7 (recommended dose of chemical fertilizer)

4.7 Biological parameters

The soil enzymes like dehydrogenase, urease and nitrate reductase activity were analysed in soil after 30 days of sowing in both the crops (Table 4.7). Enzymatic activity provides the information about the soil health.

Table 4.11 Impact of treatments on enzymatic activity in the spinach and baby corn grown soil

Treatments	Spinach			Baby corn		
	DHA*	Urease	NR**	DHA*	Urease	NR**
T_0	0.15^{a}	1.04^{a}	0.06^{a}	0.16^{a}	1.01^{a}	0.02^{a}
T_1	0.20^{b}	1.14^{ab}	0.08^{ab}	0.21^{ab}	1.10^{ab}	0.04^{ab}
T_2	0.28^{cd}	1.18^{ab}	0.10^{b}	0.28^{c}	1.20^{bc}	0.06^{abc}
T_3	0.30^{e}	1.39^{c}	0.11^{b}	0.34^{c}	1.38^{d}	0.13^{d}
T_4	0.28^{cd}	1.29^{bc}	0.10^{ab}	$0.28b^{c}$	1.26^{cd}	0.09^{bcd}
T_5	0.27^{cd}	1.33^{c}	0.10^{ab}	0.30^{bc}	1.30^{cd}	0.10^{cd}
T_6	0.23^{bc}	1.06^{a}	0.08^{ab}	0.23^{ab}	1.06^{ab}	0.06^{abc}
T_7	0.21^{b}	1.35^{c}	0.08^{ab}	0.31^{bc}	1.33^{cd}	0.07^{abc}
LSD (P=0.05)	1.12	0.15	0.06	0.08	0.15	0.06

*DHA-Dehydrogenase, (ugTPF$g^{-1}h^{-1}$) ** NR-Nitrate reductase (mg NO_2-N produced g^{-1} soil 24 h^{-1}), Urease (ug urea g^{-1} soil h^{-1})

4.7.1 Dehydrogenase (EC 1.1.1)

The determination of dehydrogenase (DHA) in the soil samples provides an information about biological characteristic of the soil. Among all the enzymes in the soil, dehydrogenases are widely used as an indicator of overall soil microbial activity. The mean value of dehydrogenase activity in soil of spinach and baby corn is given in Table 4.11. The maximum DHA activity was found in the T_3 (100 % N dose by algal biomass) of the both crops. Statistically DHA activity in T_3 was significantly higher than T_7 (recommended dose of NPK by chemical fertilizer) by 42.85 %, while in the soil of baby corn DHA activity in T_3 was non-significant with T_7. The spinach grown soil maximum (0.30 µgTPFg^{-1} of soil h^{-1}) dehydrogenase activity was recorded in T_3 followed by (0.28 µgTPFg^{-1} of soil h^{-1}) T_2 (algal biomass+ biogas slurry+ gypsum+ 100:10:0.5 w/w/w) and T_4 (75 % dose by algal biomass + 25 %) by urea while least (0.15 µgTPFg^{-1} of soil h^{-1}) were obtained in T_0. The increase/decrease of

dehydrogenase activity with respect to T_7 in spinach and baby corn grown soil has given in Fig. 4.19, Fig. 4.20. The dehydrogenase activity in T_3, T_2, T_4, and T_5 was found to be 42.9 %, 33.3 %, 33.3 and 28.6 % higher respectively compare to T_7. On the other hand treatment T_0 and T_1 was 28.57 % and 4.76 % lower than T_7. In bay corn T_3 is 11.06 % higher than T_7, while T_0, T_1 and T_6 was 48.3 %, 33.3 % and 25.5 % respectively lower than T_7. The results shows that application of algae biomass as manure improves the microbial activity in soil equally or more than that of NPK applied by chemical fertilizer.

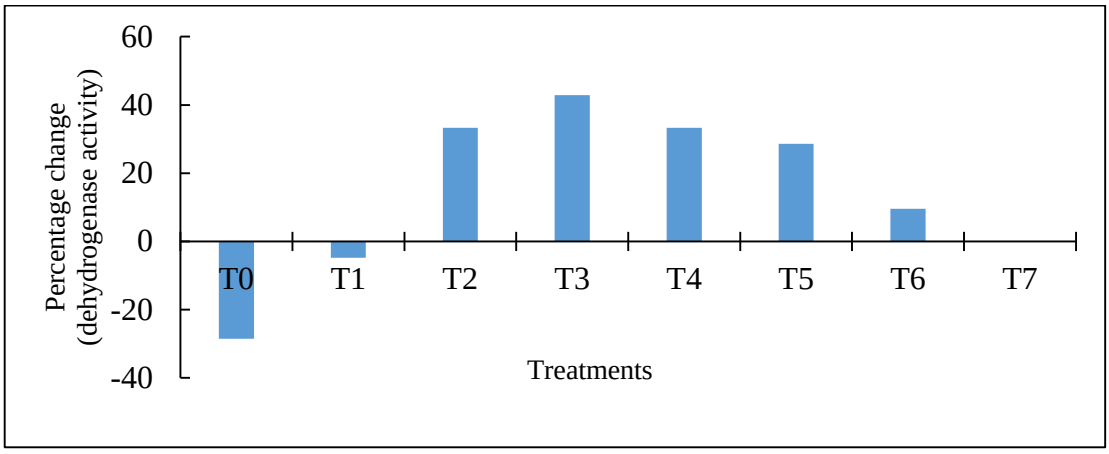
4.7.2 Urease (EC 3.5.1.5)

Urease is one of the important soil enzymes, plays a role in hydrolysis of urea to ammonia and carbamic acid followed by the chemical hydrolysis of carbamic acid to ammonia and carbon dioxide. The urease activity was analysed after 30 days of sowing. The mean value of urease activity in soil of spinach and baby corn is given in Table 4.11. Statistically among the treatments T_3 (100 % N dose by algal biomass) was significantly higher than T_0 (control, zero N application) by 33.65 %, while non-significant with T_7 (recommended dose of NPK by chemical fertilizer) in the soil of spinach. On the other hand T_3 is significantly higher than T_0 by 36.6 % while non-significant with T_7 in baby corn grown soil. The increase/decrease of urease activity with respect to T_7 in spinach and baby corn grown soil has given in Fig. 4.21, Fig. 4.22. As compared to T_7, urease activity was higher in T_3 (2.9 %) while lower in other treatments T_0, T_1, T_2, T_4 T_5 and T_6 (23 %, 15.6 %, 12.6 %, 4.4 % and 1.8 % and 21.4 % respectively) in spinach gown soil. On the other hand in baby corn grown soil urease activity was higher in T_3 (4 %) while lower in other treatments T_0, T_1, T_2, T_4 T_5 and T_6 (24 %, 17.7 %, 10 %, 5.2 %, 2.3 % and 20.3 % respectively)

4.7.3 Nitrate reductase (EC: 1.7.99.4)

Nitrate reductase is an important enzyme involved in denitrification, which reduces the NO_3^- to NO_2^-. The nitrate reductase activity was measured after 30 days of sowing in soil of both the crop. The mean value of urease activity in soil of spinach and baby corn is given in Table 4.11. Statistically among the treatments T_3 (100 % N dose by algal biomass) was significantly higher than T_0 (control, zero N application) by about 83 %, while non-significant with T_7 (recommended dose of NPK by

chemical fertilizer) in the soil of spinach. But in baby corn grown soil T_3 was statistically significantly higher from both T_0 and T_7 by 5 times and 85 % respectively. The increase/decrease of nitrate reductase activity with respect to T_7 in spinach and baby corn grown soil has depicted in Fig. 23 and Fig. 24. The treatment T_3 was 37.5 % higher than T_7 while T_2, T_4 and T_5 was 25 % higher than T_7 in spinach grown soil. In baby corn grown soil the treatment T_3, T_4 and T_5 was found to be 85.7 %, 28.6 % and 43.9 % respectively higher than T_7.



T0-Control condition with no nutrient application, T1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T3-Algal biomass on the basis of nitrogen, T4-75 % dose by algal biomass + 25 % by urea, T5-50 % Dose by algal biomass + 50 % by urea, T6-25 % Dose by algal biomass + 75 % by urea, T7-Recommended dose by chemical fertilizer

Fig. 4.19 Percentage change in dehydrogenase activity in soil of spinach with respect to T_7 (recommended dose of chemical fertilizer)

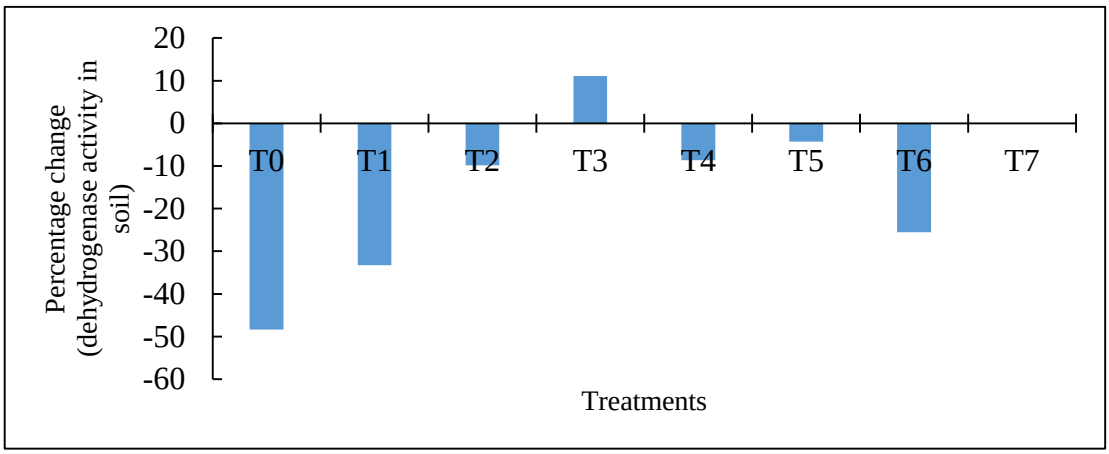


Fig. 4.20. Percentage change in dehydrogenase activity in soil of baby corn with respect to T_7 (recommended dose of chemical fertilizer)

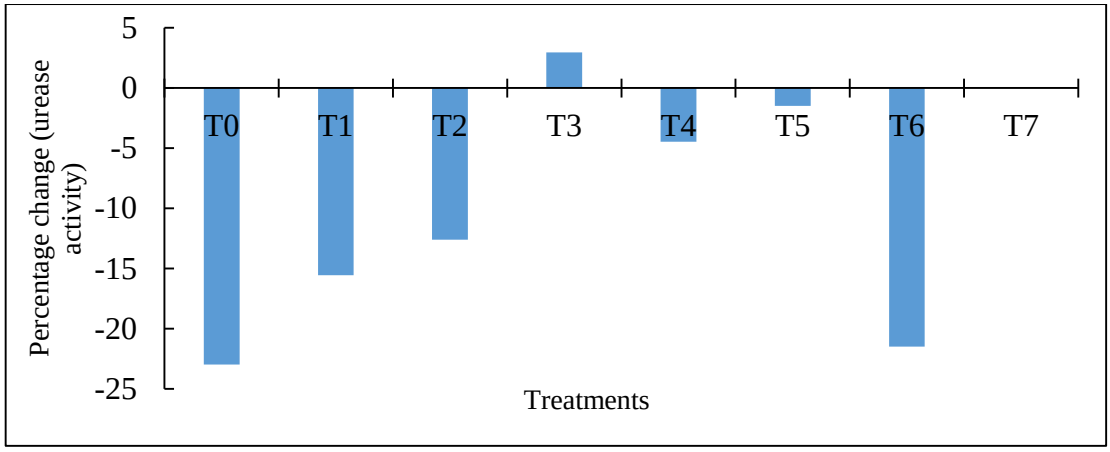
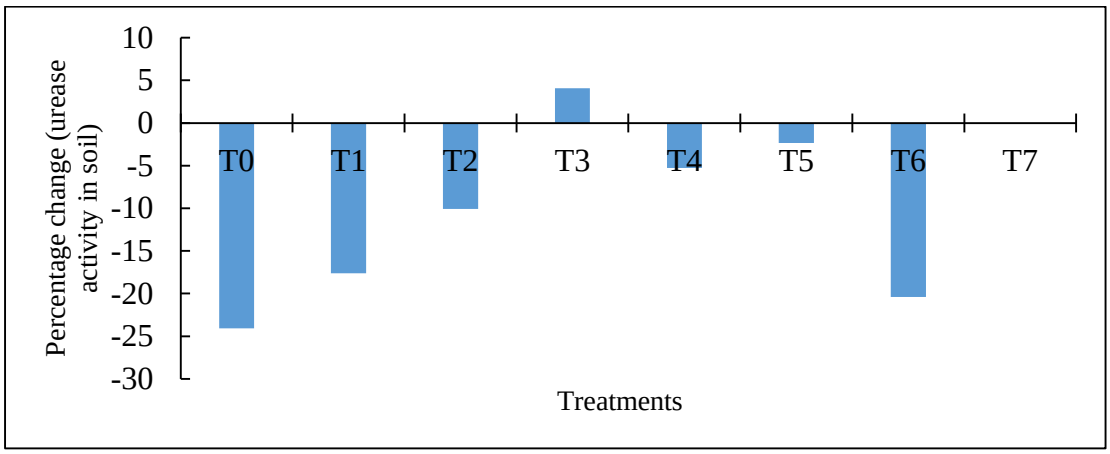


Fig. 4.21 Percentage change in urease activity in the spinach grown soil with respect to T_7 (recommended dose of chemical fertilizer)



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5¬-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilize

Fig. 4.22 Percentage change in urease activity in the baby corn soil with respect to T_7 (recommended dose of chemical fertilizer)

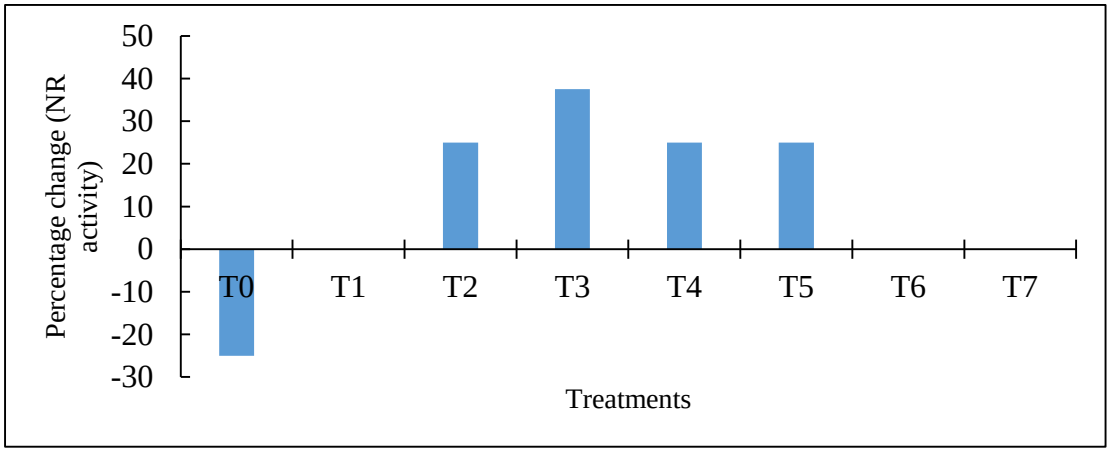


Fig. 4.23 Percentage change in nitrate reductase activity in spinach grown soil with respect to T_7 (recommended dose of chemical fertilizer)

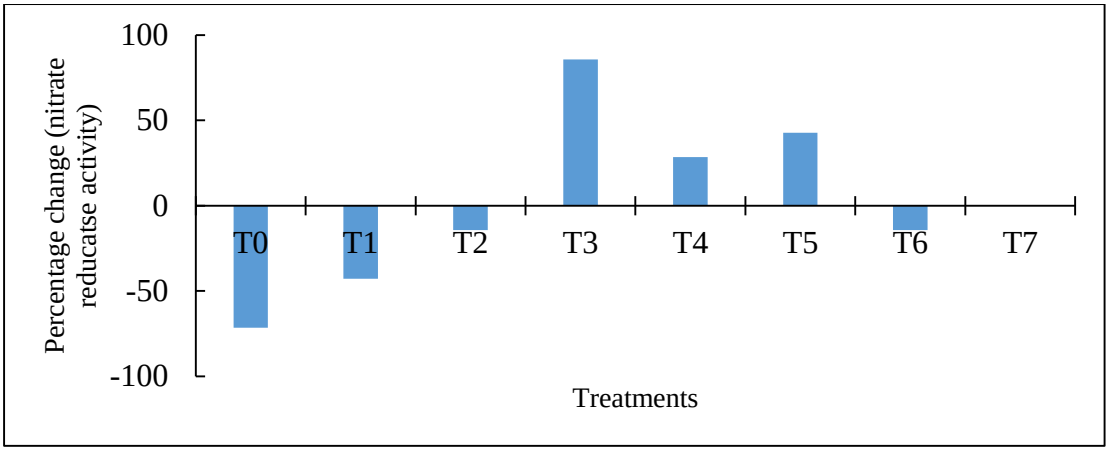


Fig. 4.24 Percentage change in nitrate reductase activity in baby corn grown soil with respect to T_7 (recommended dose of chemical fertilizer)

4.8 Nitrate + nitrite concentration after harvest of spinach

The soil samples were taken from depth of 0-15 cm, 15-30 cm and 30-45 cm by auger after harvest of spinach. The left-over nitrate + nitrite content was analysed in collected soil sample. The cumulative nitrate + nitrite content was found to be maximum in treatment T_7 (24.40 mg/kg) while lowest in T_0 (9.47 mg/kg) followed by treatment T_3 (10.27 mg/kg) (Table 4.12).

4.8.1 Nitrate + nitrite at 0-15 cm depth after harvest of crop

The mean value of nitrate content in soil extracted from 0-15 cm depth of experimental field is presented in the Table 4.12. The maximum concentration (8.53 mg/kg) of nitrate + nitrite was found in treatment T_7 (recommended dose of NPK by chemical fertilizer) followed by T_6 (8.20 mg/kg) (25 % Dose by algal biomass + 75 % by urea), while least (3.53 mg/kg) was observed in T_0 (control, zero N application). Statistically, among the treatment, the concentration of nitrate + nitrite in T_3 was significantly lower than T_7 by 51.5%, while higher than T_0 by 17%. The increase/decrease of nitrate + nitrite through soil column from 0-15 cm during an experiment with respect to T_7 has shown in the Fig. 4.25. It was recorded that the nitrate + nitrite concentration in treatment T_1, T_2, T_3, T_4, and T_5 was 58.6%, 57.7%, 50.7%, 51.5%, and 46.8% lower than T_7.

4.8.2 Nitrate + nitrite at 15-30 cm depth after harvest of crop

The mean value of nitrate + nitrite content in soil collected from depth 15-30 cm has shown in the Table 4.12. The maximum (8.33 mg/kg) content of nitrite + nitrate was found in T_7 (recommended dose of NPK by chemical fertilizer) followed

by (8.20 mg/kg) T_6 (25 % Dose by algal biomass + 75 % by urea) while least (2.87 mg/kg) was observed in T_3 (100 % N dose by algal biomass). Statistically, among the treatment, the concentration of nitrate + nitrite in T_3 was significantly lower than T_7 and T_3 by 65.5% and 23.4% respectively. The increase/decrease of nitrate + nitrite from depth 15-30 cm during an experiment with respect to T_7 has shown in Fig. 4.26. It was found that the nitrate + nitrite content in treatment T_0, T_1, T_2, T_3, T_4, T_5 were 54.3%, 45.4%, 43.9% 65.5%, 53.1 %, and 47.9% respectively lower than T_7.

4.8.3 Nitrate + nitrite at 30-45 cm after harvest of spinach

The mean value of nitrate + nitrite content in soil collected from depth 30-45 cm is shown in the Table 4.12. The maximum concentration of nitrite + nitrate was found in treatment T_6 (9.03 mg/kg) followed by T_7 (7.53 mg/kg), while least was observed in T_0 (2.13 mg/kg). Statistically, among the treatment, the concentration of nitrite + nitrate in T_3 was significantly lower than T_7 (56.5%), while significantly higher than T_0 by 53.5%. The increase/decrease of nitrate + nitrite from depth 30-45 cm during the experiment with respect to T_7 has shown in Fig. 4.27. It was observed that the nitrate + nitrite concentration in treatment T_1, T_2, T_3, T_4, and T_5 was 71.7 %, 71.2 %, 39.4 %, 56.6 %, 32.9 %, and 32.7 % respectively lower than treatment T_7, while T_6 was found to be 19 % higher than T_7.

Table 4.12 Nitrate + nitrite content in soil with depth after harvest of spinach

Treatments	Nitrate + nitrite(mg/kg)			Cumulative nitrate content
	(0-15 cm)	(15-30 cm)	(30-45 cm)	
T0	3.53^{b}	3.80^{b}	2.13^{a}	9.47
T1	3.60^{d}	4.55^{d}	2.17^{a}	10.31
T2	4.20^{d}	4.67^{d}	4.56^{c}	13.43
T3	4.13^{a}	2.87^{a}	3.27^{b}	10.27
T4	4.53^{bc}	3.90^{bc}	5.05^{d}	13.48
T5	6.80^{cd}	4.33^{cd}	5.07^{d}	16.20
T6	8.20^{e}	8.20^{e}	9.03^{f}	25.43
T7	8.53^{e}	8.33^{e}	7.53^{e}	24.40
LSD (P=0.05)	0.39	0.47	0.43	

T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

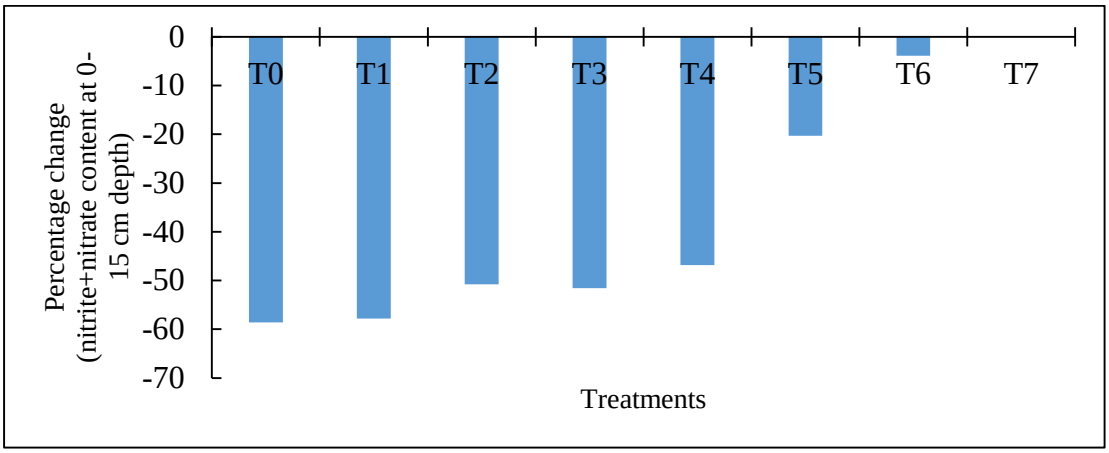
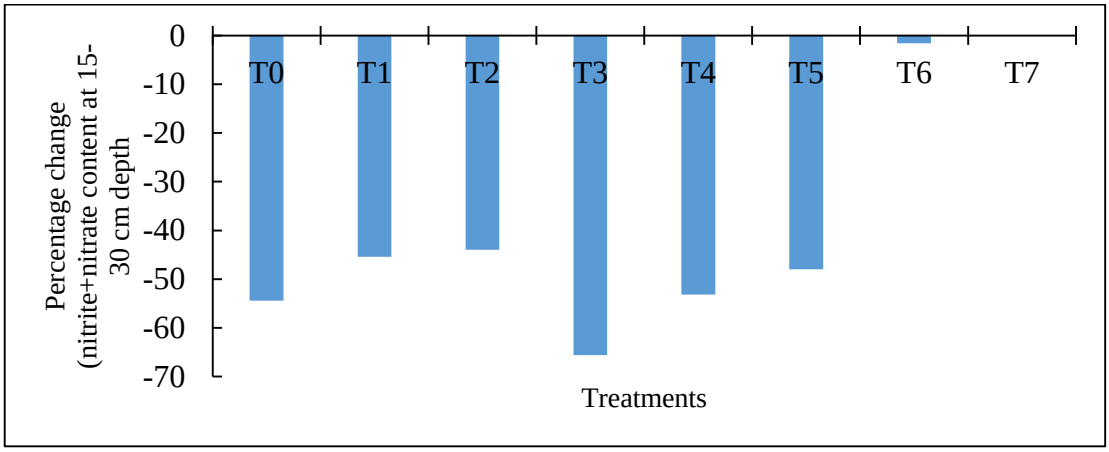


Fig. 4.25 Percentage change in nitrate+nitrite concentration in soil after harvest at 0-15 cm depth with respect to T_7 (recommended dose of chemical fertilizer)



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.26 Percentage change in nitrate + nitrite concentration in soil after harvest with respect to T_7 (recommended dose of chemical fertilizer) at 15-30 cm depth

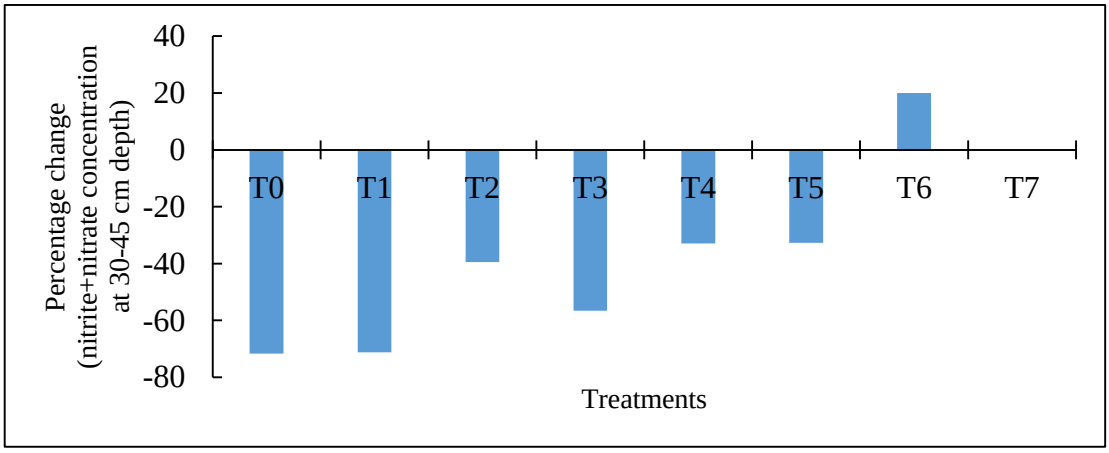


Fig. 4.27 Percentage change in nitrite + nitrate concentration in soil after harvest with respect to T_7 (recommended dose of chemical fertilizer) at 30-45 cm depth

4.9 Ammonium concentration after harvest of crop

Similar to nitrate + nitrite, ammonium content was also analysed after harvest of spinach in soil from depth of 0-15 cm, 15-30 cm and 30-45 cm by auger. The left-over ammonium content was analysed in collected soil sample. The cumulative ammonium content was found to be maximum in treatment T_7 (136.43 mg/kg) while lowest in T_0 (106.43 mg/kg) followed by treatment T_2 (119.87 mg/kg).

4.9.1 Ammonium content at depth (0-15 cm) after harvest of spinach

The mean value of ammonium content in soil collected from depth 0-15 cm is shown in the Table 4.12. The maximum content of ammonium was found in T_7 (46.37 mg/kg) followed by T_1 and T_2 (41.4 mg/kg), while least was observed in T3 (38.6 mg/kg). Statistically, among the treatment, ammonium content in T_3 was significantly higher than T_7 and T_0 by 16.8% and 4.7% respectively. The increase/decrease of ammonium from 0-15 cm during the experiment with respect to T_7 is shown in Fig. 4.28. It was observed that the ammonium content in treatment T_1, T_2, T_3, T_4 and T_5, and T_6 were 14.58 %, 12.25 %, 12.34 %, 19.17 %, 14.40 %, 18.60 and 16.21% respectively lower than treatment T_7.

4.9.2 Ammonium content at depth (15-30 cm)

The mean value of ammonium content in soil collected from 15-30 cm depth is presented in Table 4.13. The maximum content of ammonium was found in treatment T_1 (44.33 mg/kg) followed by T_5 (42.63 mg/kg), while least was observed in T_0 (31.40 mg/kg). Statistically among the treatment, ammonium content in T_3 was significantly higher than T_0 by 22.30 % while on insignificant with T_7. The increase/decrease of ammonium from 15-30 cm during experiment with respect to T_7 is shown in Fig. 4.29. It was observed that the ammonium content in treatment T_1 and T_3 was 14.5 % and 9.3 % respectively higher than treatment T_7 while T_0 was 18.86 % lower than treatment T_7.

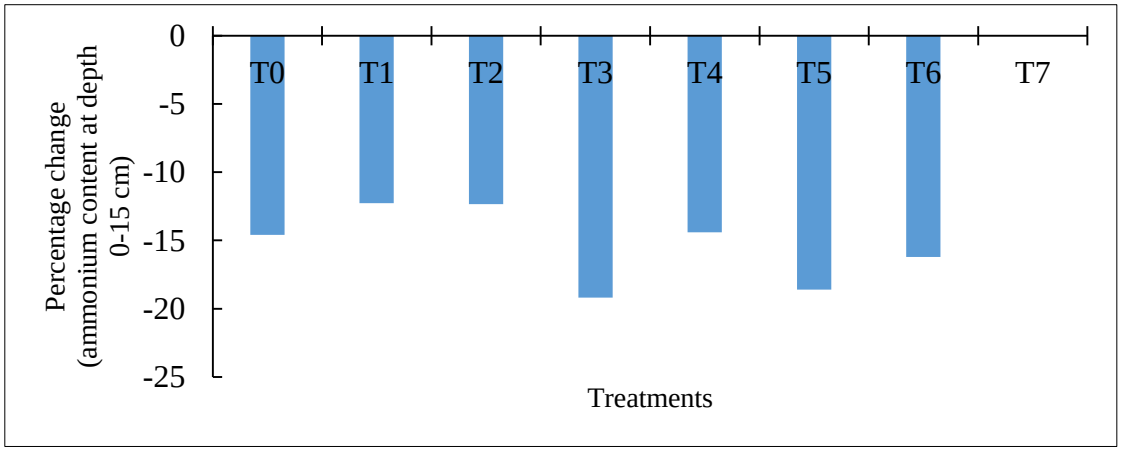
4.9.3 Ammonium content at depth (30-45 cm)

The mean value of ammonium content in soil collected from 30-45 cm depth layer is shown in Table 4.13. The maximum content of ammonium was found in treatment T_7 (51.37 mg/kg) followed by T_1 (44.90 %), while least was observed in T_0

(34.57 mg/kg). Statistically among the treatment, ammonium content in T_3 was significantly lower than T_7 by 25.7 % while significantly higher than T_0 by 10.3 %. The increase/decrease of ammonium from 15-30 cm during experiment with respect to T_7 is shown in Fig. 4.30. It was observed that the ammonium content in treatment T_0, T_1, T_2, T_3, T_4, T_5 and T_6 were 32.72 %, 12.59 %, 25.77 %, 20.58 %, 21.03 % 24.73 % and 19.6 % respectively higher than treatment T_7.

Table 4.13 Ammonium content in soil with depth after harvest of spinach

Treatments	Ammonium (mg/kg)			Cumulative ammonium content (mg/kg)
	0-15 cm	15-30 cm	30-45 cm	
T0	40.47^{cd}	31.40^{a}	34.57^{a}	106.43
T1	41.40^{d}	44.33^{c}	44.90^{d}	130.63
T2	41.37^{d}	40.37^{bc}	38.13^{b}	119.87
T3	38.60^{a}	42.30^{bc}	40.80^{bc}	121.70
T4	40.53^{cd}	39.67^{bc}	40.57^{bc}	120.77
T5	38.83^{ab}	42.63^{bc}	38.67^{bc}	120.13
T6	39.80^{bc}	41.90^{bc}	41.30^{c}	123.00
T7	46.37^{e}	38.70^{b}	51.37^{e}	136.43
LSD (P=0.05)	1.13	4.45	2.48	



T_0-Control condition with no nutrient application, T_1 -Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:20:1 w/w/w), T_2-Aerobic digested chlorella manure (algal biomass: Biogas slurry: Gypsum: 100:10:0.5 w/w/w), T_3-Algal biomass on the basis of nitrogen, T_4-75 % dose by algal biomass + 25 % by urea, T_5-50 % Dose by algal biomass + 50 % by urea, T_6-25 % Dose by algal biomass + 75 % by urea, T_7-Recommended dose by chemical fertilizer

Fig. 4.28 Percentage change in ammonium concentration in soil after harvest with respect to T_7 (recommended dose of chemical fertilizer) at 0-15 cm depth

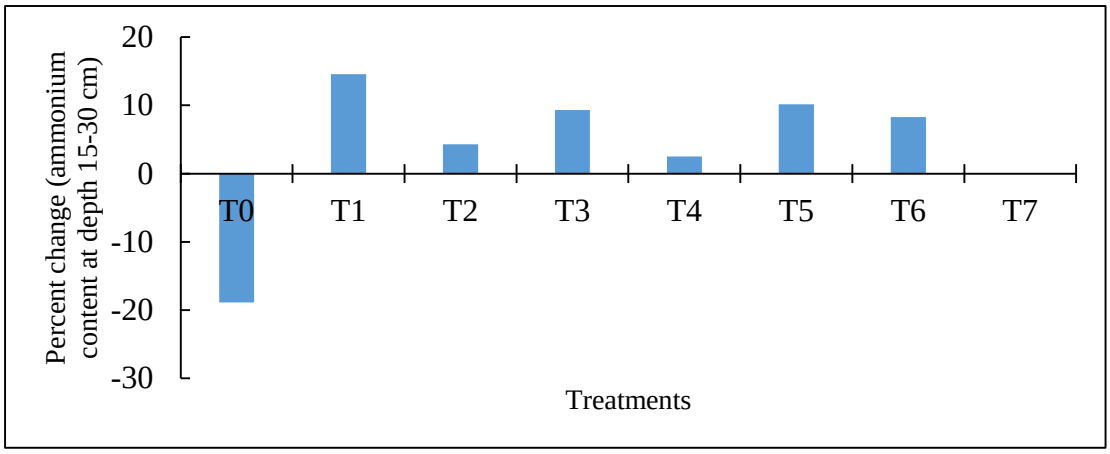


Fig. 4.29 Percentage change in ammonium concentration in soil after harvest with respect to T_7 (recommended dose of chemical fertilizer) at 15-30 cm depth

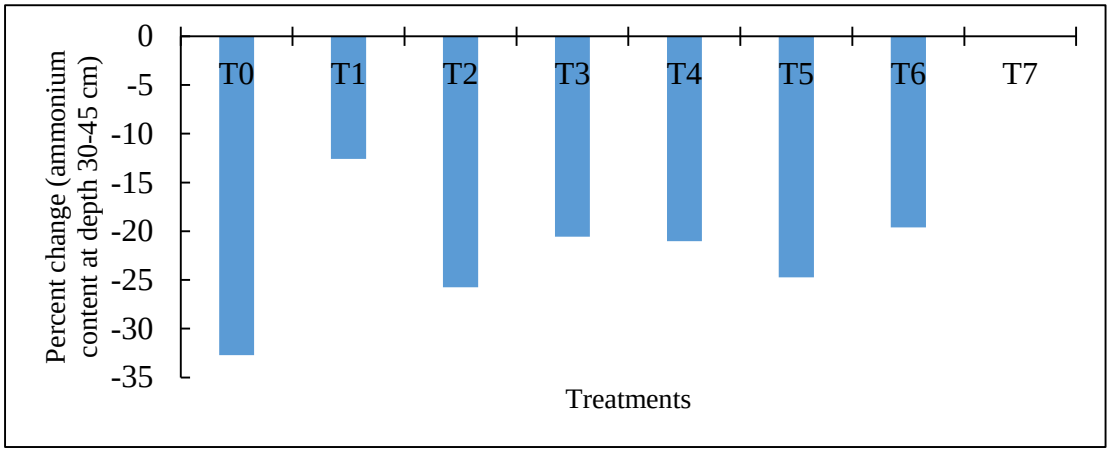


Fig. 4.30 Percentage change in ammonium concentration in soil after harvest with respect to T_7 (recommended dose of chemical fertilizer) at 30-45 cm depth

4.10 Correlation between soil enzyme with organic carbon and available nitrogen content in soil

The Pearson correlation was done in both the crops to determine correlation between the soil enzyme, organic carbon and available nitrogen content in soil. It was found that soil enzymes dehydrogenase, urease and nitrate reductase was positively correlated at 1% and 5% significance level with available nitrogen and organic carbon content in both spinach and baby corn grown field. The R^2 value of correlation is given in Table 4.14.

Table 4.14 Pearson correlation between soil enzyme with organic carbon and available nitrogen content in soil after harvest of spinach crop

Soil enzyme	**Spinach**		**Baby corn**	
	Organic carbon	Available nitrogen	Organic carbon	Available nitrogen
Dehydrogenase	0.48*	0.73**	0.29	0.76**
Urease	0.48*	0.59**	0.31	0.67**
Nitrate reductase	0.54**	0.43*	0.24	0.60**

The organic carbon was positively correlated with nitrate reductase at 1% significance level, while with urease and dehydrogenase positively correlated at 5% significance level in spinach grown field. On the other hand available nitrogen is positively correlated with urease, nitrate reductase and dehydrogenase at 0.01, while available nitrogen was not significant with organic carbon content in soil.

Chapter 5 **DISCUSSION**

The present study explains the phycoremediation potential of microalga (C*hlorella minutissima)* for the treatment of sewage wastewater and to study impact of algae biomass of C. *minutissima* with treated wastewater on soil quality and crop productivity (spinach and baby corn) are discussed under the following heads:

5.1 Impact on *Chlorella minutissima* inoculation on sewage water quality

The *C. minutissima* found very effective in removal of salt (EC, TDS), BOD, COD, nutrient (ammonical-N, nitrate-N, P and K) from the wastewater. About more than 85% of EC, TDS, BOD, COD, nitrate and 42 % ammonium were removed by *C. minutissima* after 25 days of inoculation. However, pH and dissolved oxygen was found to be increasing throughout the experiment. The reason for increase in pH might be due to photosynthesis activity performed by microalgae which reduces dissolved CO_2 concentrations and organic molecule. The inorganic chemicals normally used by microalgae are CO_2 and bicarbonate (Borowitzka, 1998), the bicarbonate was converted to CO_2 by enzyme carbonic anhydrase. Furthermore, the pH levels were maintained so that so that the ammonia concentration should not increase. The increase of pH in medium was also reported (Munoz and Guieysse, 2006), which could lead to the precipitation of phosphorus and increasing phosphate adsorption on surface of microalgae (Tam and Wong, 2000). Hammouda *et al*., (1995) reported reduction of BOD and COD by 84 % and 89 % respectively by algae *C. minutissima* and *Scenedesmus* from sewage wastewater. Sharma *et al*., (2013) had reported that microalgae *C. minutissima* reduced TDS, BOD and ammonium-N by more than 90 % and COD and Nitrate-N by more than 80 % and total P by more than 70 % in the raw waste water. Govindan (1984) reported BOD and COD reduction from 75 % to 95 % and from 72 % to 91 %, respectively, from a mixture of dairy and sewage wastewater. The DO decreases initially up to 5 days and later continuously increases, it was so because of high microbial activity and low algal growth in sewage wastewater during initial period. The same trend of DO was also observed by (Senger *et al*., 2011). Choudhary *et al*., (2016) have reported that algae removed COD and nitrate below permissible limit from rural sector wastewater by 87 % and 70 % respectively in the 12 days. Malla *et al*., (2015) stated that *C. minutissima* effectively removed TDS (90–98 %), ammonical-N and nitrate-N (70–80 %), P (60–70 %) and K (45–50 %) from the

wastewater within 12 days. Similarly about (82 %) reduction of TDS, nitrate, ammonia, and P from dairy effluent were also reported by Ummalyma and Sukumaran (2014) within 15 days of incubation. Hena *et al*., (2015) had reported the reduction of BOD up to 82 % from dairy effluent by using consortium of microalga. The reason for increase in DO might be due to absorption of oxygen by algae from atmosphere and photosynthetic activity performed by algae.

The reduction in TDS was mainly due to consumption of dissolved solid from the wastewater for the growth of microalgae. Phosphate and nitrate is mainly require for growth of algae. Phosphorus extracted from wastewater is mainly used for production of phospholipids, ATP, and nucleic acids in algal biomass. (Becker, 1994). A study conducted by (Cho *et al*., 2011) in the municipal wastewater had reported the reduction of both nitrate and phosphate concentrations by 92 % by algae *C. vulgaris*. (Kiran *et al*., 2014) reported the reduction in both ammonical and nitrate nitrogen by more than 95 % by *C. vulagris.*

5.2 Impact of *Chlorella minutissima* biomass application as a manure on agronomic yield of baby corn and spinach

Spinach and baby corn were sown on 20th December 2018 and 2nd February 2019. In both the crops, algal biomass application as manure showed higher or similar growth and biomass yield as compared to other treatments including treatments applied with recommended dose of nutrients by chemical fertilizers. The spinach biomass and leaf length, recoverable root length and root biomass were highest in algae manure applied treatments followed by chemical fertilizer applied treatments however difference was insignificant. Algae manure was also found effective in inducing early leaf maturing for harvesting. The similar results were also obtained in baby corn. The baby corn yield in respect of cob, cob with husk and husk yield was highest in treatment applied with algae manure as compared to other.

The higher yield in algal biomass applied treatments might be due to fact that algae biomass is a slow nutrient releasing manure as compared to chemical fertilizers. Slow-release fertilizers provide gradual nutrient supply to crops for longer period of time. It improves N fertilizer use efficiency and reduces N leaching losses. Renuka *et al*., (2016) reported wheat plot which is inoculated with microalgal biofertilizer recorded a 7.4–33 % increase in plant dry weight and up to 10 % in spike weight.

Mulbry *et al*., (2005) reported that application of algal biomass resulted into release of 3% of total algal nitrogen in to the plant available form at first day of application and gradually reached to 33% within 21 days of application. Our finding is comparable to the finding of Mulbry *et al*., (2005). They reported that shoot dry weight and nutrient content of corn and cucumber (*Cucumis sativus* L.) seedlings treated with algal biomass were equal to those grown with recommended dose of NPK by chemical fertilizers. In other study, Thamida Begum *et al*., (2011) also showed that the yield in rice plots treated with algae manure were statistically identical to the treatment with recommended dose of nitrogen. Mahmoud and Amara (2000) reported that the treatments supplied with fresh and dry algae biomass had significantly higher plant growth as compared to un-treated plant. Several other studies like Verma (1996), Mekki *et al*., (1999), Galal *et al*., (2000) and Ghosh & Mohiuddin, (2000) have also reported similar results. Qualls *et al*., (2000) reported that due to mineralization of organic manures, additional mineral N is released into the soil, resulting in large nitrate residues in the soil. Therefore, application of algal biomass as manure may lead to enhanced plant growth and yield as well as reduction in the use of chemical fertilizer and environmental pollution

5.3 Impact of *Chlorella minutissima* biomass application as manure on physico-chemical properties of the soil

The result showed positive impact of algal biomass application as manure on the soil physico- chemical and biological properties in both the crops. The N, P, organic-C and enzymatic activity was higher in algal biomass applied soil as compared to other treatments i.e. biogas slurry based manure and chemical fertilizer. However, there was insignificant difference in SOC and available P while significant difference in available N content among the treatments. Among all the treatments, treatments applied with algal biomass showed significantly higher available N content as compared to other treatments and control after harvest of the crops except T_7 (recommended dose of NPK by chemical fertilizer). Available nitrogen content in algae applied soil was higher, however insignificant difference than urea applied soil. Renuka *et al*., (2016) reported that available NPK content in microalgae inoculated treatments were higher compare to other treatments. Further, application of algae with urea significantly decreases available N content as compared to sole algae application. Highest nitrogen content in T_3 (100 % N supplied by algae) as compared to T_7 (recommended dose of NPK by

chemical fertilizer) might be due to slow release of nitrogen after decomposition of algal biomass. While in case of urea, nitrogen get released faster than organic matter which might be taken by crop and lost by leaching and volatilization. The obtained result might be due to the fact that, algae manure act as slow nutrient release manure. Therefore, gradual release of N might had led to poor loss of N and thus higher availability of N to the crops resulting into high crop yield and high available N in the soil. It has reported that, soil application of microalgae leads to locking of some portion of microalgae carbon in to the soil and enhance the organic content of the soil (Bird *et al*., 2012). In other experiment conducted by Swenson *et al*., (2009) grown algae in wastewater and later algae biomass was harvested for manure application and concluded that algae grown in wastewater was effectively works as fertilizer and, algae perform equally as well as the organic, inorganic and commercially available fertilizers. A feasibility analysis of dry biomass obtained by *C. minutisima* was conducted by Khan *et al*., (2019), for a pond having an area of 1 ha (10000m^2). The dry biomass obtained after 25 days of inoculation was 0.44g/L. So 3,91,111 kg of dry algal biomass can be obtained in a year. In their study the NPK content in dry algal biomass after phycoremediation was 5.87 %, 1.15 % and 0.28 % respectively. Accordingly, from this dry weight, one could obtain N, P and K 22,958, 4,498 and 1,095 kg ha^{-1} y^{-1} respectively. Using this biomass in agriculture as biofertilizers, one can save the chemical fertilizers of about USD (United State Dollars) 55,840 ha^{-1} y^{-1}.

The nitrogen content in the treatment having algae manure+ biogas slurry+ gypsum in 100:20:1 and 100:10:0.5 after 15 days of aerobic digestion was 3.9 % and 3.5 % respectively. It was found that final nitrogen content was decreased in resultant manure, compare to initial nitrogen content in algae biomass (6 %). The reason for decrease in nitrogen content was might be due to ammonia volatilisation in alkaline condition. On the other hand, loss of phosphorous in resultant manure obtained by mixing algae manure+ biogas slurry+ gypsum after 25 days were insignificant compare to initial phosphorous content in algae dry biomass.

5.4 Impact of *Chlorella minutissima* biomass application as manure on biological properties of the soil

Enzymatic activity is very important for determining soil health. In our study three enzymes were selected as dehydrogenase, urease and nitrate reductase. It was found that enzymatic activity was higher in all the treatments as compared to initial soil

condition and control. The treatment supplemented with algal manure had better enzymatic activity as compared to other treatments. Dehydrogenase is an extra-cellular enzyme, which gives large amount of information about biological characteristic of the soil (Ross, 1971). Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity. Urease play a role in hydrolysis of urea to ammonia and carbamic acid followed by the chemical hydrolysis of carbamic acid to ammonia and carbon dioxide while nitrate reductase involved in denitrification, which reduces the NO_3^- to NO_2^-. For dehydrogenase activity the obtained results was in agreement with the study of Watts *et al*., (2010) and Scherer *et al*., (2011). According to Scherer *et al*., (2011) and Wolinska and Stepniewska (2012), DHA activity in soil is directly related to the microbial biomass. Ross (1971) also reported that dehydrogenase activity may increase due to NPK+OM (organic manure), OM and NPK application. These results are appeared to be linked with increased microbial population associated with organic manure substrate. The higher OM level can provide enough substrate to support higher microbial biomass, hence higher enzyme production (Yuan & Yue, 2012). Dehydrogenases activity is reported to increase with increase in pH. The optimum range of pH for dehydrogenase activity is in range of 7.4–8.5 as reported by (Nagatsuka *et al.*, 1960). Urease activity in soil may increase with organic matter content, plant residue, microbes and animal waste. Antonius (2016) reported that algae, bacteria, fungi and protozoa present in animal manure release various enzymes, such as ureases, invertases, dehydrogenases, cellulases, amylases etc. Urease activity is mostly controlled by total nitrogen content as reported by (Vahed *et al.*, 2011). Tiwari *et al*., (1989) conducted an experiment in soil treated with earthworm cast and found that increased activity of enzyme in treated plot could be due to higher organic matter and nitrogen content in these plots or because of the presence of higher soil microbial population in treated plots.

5.5 Impact of *Chlorella minutissima* biomass application as manure on nitrate leaching through column study

The losses of nitrogen form manures and fertilizers application form soil is mainly in the form of volatilization and nitrate leaching. The nitrate leaching is a major problem after application of nitrogenous fertilizers in irrigated crops. Therefore, in this study potential of algal biomass application to reduce nitrate leaching was done in

through column study. The result showed highest leaching in treatment applied with chemical fertilizer (recommended dose of NPK by chemical fertilizer) while lowest in control and sole algal biomasses applied. The leaching of nitrate is found to be maximum in treatment having chemical fertilizer in all layer i-e- 0-15 cm, 15-30 cm and 30-45 cm. Among different soil layer, maximum nitrate leaching was observed in 30-45 cm layer. Pratt *et al.,* (1972) found that nitrate leaching losses could be 13 – 102 per cent (average 25 - 50 per cent) of added nitrogen. Leclerc *et al*., (1995) demonstrated a low nitrate leaching and efficient nitrogen management through organic manure compare to chemical fertilizers. Powlson *et al*., (1989) conducted a long-term field experiment on arable crops at Rothamsted, and reported that leaching of nitrate from chemical fertilizer is comparatively more that organic fertilizer. Low nitrate leaching in algal biomass applied lysimeter might be due to slow nutrient releasing nature of algal biomass which has been described earlier.

Chapter 6 SUMMARY AND CONCLUSION

6.1 Summary

Decreasing availability of freshwater due to pollution and population growth is forcing reuse of wastewater to enhance availability of fresh water for various use like irrigation. Phycoremediation of wastewater provide an economic and natural way of water remediation. Phycoremediation is the use of green algae for removing an excess nutrient load from wastewater. Generation of wastewater is a severe global issue now a days. Municipal treatment facilities are designed to treat wastewater which produces a liquid effluent of suitable quality that can be discharged to the natural surface waters with minimum impact on human health or the environment. But these processes are expensive and require complex operation and maintenance. Due to improper design, poor maintenance, and lack of manpower, the facilities constructed to treat wastewater do not function properly and remain closed for most of the time. In view of aforementioned limitations, in recent years integrated phycoremediation technology has been receiving greater attention. However, the rate of adoption of integrated phycoremediation technology for wastewater treatment in the developing countries has been neglected and never popularized. Several researchers have already reported the potential role of algae biomass as manure after phycoremediation of wastewater, but the very limited study is available on crops in field condition Therefore to assess the manurial potential of algae biomass as manure on spinach and baby corn after phycoremediation of sewage wastewater by *Chlorella minutissima* an experiment was conducted. The aim of experiment is to a) to quantify temporal dynamics (5 days interval) in physico-chemical quality of sewage wastewater after inoculation of algal culture, b)assess impact of application of recommended dose of N in the form of algal biomass, manure obtained from mixture of (algal biomass+ biogas slurry+ gypsum) and urea on soil quality and crop productivity (spinach and baby corn), and c) to study nitrate leaching in spinach and baby corn grown soil column, irrigated with algal biomass suspension, biogas slurry, gypsum and urea. The experiment was conducted in 1m x 1m plot size with following eight treatments. The experiment was laid out in RBD design with three replication.

Objectives

1. To remediate the sewage wastewater using alga *Chlorella minutissima*
2. To produce manure from algal biomass using biogas slurry and gypsum
3. To evaluate the efficacy of algal manure on baby corn and spinach

Treatments

T_0 Control condition with no nutrient application

T_1 Aerobic digested chlorella manure 1 (algal biomass+ Biogas slurry+ Gypsum in 100:20:1 (w/w/w) ratio)

T_2 Aerobic digested chlorella manure 2 (algal biomass+ Biogas slurry+ Gypsum in 100:10:0.5 (w/w/w) ratio

T_3 Algal biomass on the basis of nitrogen

T_4 75 % dose of N by algal biomass + 25 % by urea

T_5 50 % dose of N by algal biomass + 50 % by urea

T_6 25 % dose of N by algal biomass + 75 % by urea

T_7 Recommended dose by chemical fertilizer

The preliminary treated sewage wastewater were taken from the IARI sewage wastewater stream and characterized their physico-chemical strength. It has been found that the sewage wastewater of IARI could not be directly used for the irrigation as it is having high TDS. So in present study algae C*hlorella minutissima* was inoculated in collected sewage wastewater for remediation. C. *minutissima* was found very effective in the removal of electrical conductivity **(**EC), total dissolved solids, phosphorous (P), potassium (K), ammonium, nitrate, biological oxygen demand and chemical oxygen demand of sewage wastewater. There was significant decrease in the electrical conductivity, TDS, P, K, NH_4^+, NO_3^-, BOD and COD content while increase in pH (insignificant) and DO (significant) of sewage wastewater after the inoculation of alga. The change in the quality of water after treatment with algae was checked at an interval of 5 days for a period of 25 days. pH of the water sample treated with microalgae showed a drift towards alkalinity. pH has been changed from 8.20 to 8.57. The EC,

TDS, P, K, NH_4^+ and NO_3^- value continuously decreased during 25 days of inoculation however, rate of decrease was higher during 0-15 days of inoculation It means *C. minutissima* can effectively reduce the EC, TDS, P, K, NH_4^+, NO_3^-, BOD and COD within 15 days of inoculation. EC, TDS, P, K, NH_4^+, NO_3^- continuously decreased after algal inoculation however BOD, COD and DO followed different trend. BOD and COD initially increased up to 5 days of algal inoculation followed by continuous decrease while DO initially decreased up to five days of algal inoculation followed by continuous increase. After 25 days of inoculation the decrease in EC and TDS was about 93 % and 94.3 %. The BOD and COD was reduced by 93.2 % and 80.5 % respectively, while DO increased by more than twice compare to initial. The decrease in BOD and COD might be due to an increase in DO through photosynthesis of algae that might have led to aerobic decomposition of organic matter in the wastewater. The initial content of nitrate, ammonium, phosphate and potassium in sewage wastewater was 3.06±0.01 mg/L, 5.6+0.18 mg/L, 3.54±0.05 mg/L and 5.5±0.09 mg/L respectively. They get reduced to 0.34±0.05 mg/L, 2.9±0.15 mg/L, 1.15±0.01 mg/L, and 1.85±0.03 mg/L respectively after 25 days of phycoremediation by *C. minutissima*. The percentage reduction was 89 %, 48.2 %, 67.4 % and 66.3 % respectively for nitrate, ammonium, phosphate and potassium

The algae biomass obtained after phycoremediation typically contained 6% N, 1% P and about 0.48% K. Moreover, algal N would have much less potential for leaching or for loss in run-off since only about 5% of the algal N would be available as mineral N at the time of application. This benefit may allow algal biomass to be side-dressed into established crops. After phycoremediation applied dose of N in the form of algal biomass, manure obtained from mixture of (algal biomass+ biogas slurry+ gypsum) and urea on soil quality and crop productivity (spinach and baby corn)

The agronomic parameters like leaf biomass, leaf length, root biomass and root length were measured in spinach crop while in baby corn cob yield, baby corn yield without husk, husk yield and leaf length were measured. The maximum biomass in spinach was obtained in treatment T_3 (7.15 kg) while in treatment T_7 was (6.77 kg) after four cuttings. The leaf length, root length and root biomass were in treatment T_3 and T_7, but higher than control. On the other hand cob yield and cob length was recorded in baby corn. The cob yield was significantly higher in T_3 (100 % N dose by algal biomass), as compare to T_7 (recommended dose of NPK by chemical fertilizer) while

cob length in T_3 was statistically insignificant with T_7.Thus result indicated that, supply of recommended dose of N through algal biomass may lead to higher or equivalent yield of spinach and baby corn as compared yield obtained by supply of recommended dose of fertilizer.

Before sowing and after the harvest of spinach and baby corn, soil was sampled from 0-15 cm from each treatment plots. The sampled soil was used to determine physico-chemical (pH, EC, organic C, available N and P) and enzymatic activity (dehydrogenase, urease and nitrate reductase activities). All the treatments showed higher organic carbon content as compared to initial soil organic carbon in the soil of both the crops. However, difference was statistically insignificant. The maximum SOC content was found in the T_3 (100 % N dose by algal biomass) of the both crops. After harvest of the crop available N, content was varied from 240-361 kg/ha in the soil of spinach and 236-415 kg/ha in the soil of baby corn. The maximum available nitrogen content in spinach and baby corn was found in T_3 (361 kg/ha) and T_5 (415 kg/ha). Statistically in the soil of both the crops, available nitrogen content in treatment (T_3) supplied with only algae and treatment (T_7) supplied with recommended dose of NPK by chemical fertilizer was non-significant. Like available N, available phosphorous content in treatment (T_3) supplied with only algae and treatment (T_7) supplied with recommended dose of NPK by chemical fertilizer was non-significant.

Soil pH and EC were insignificantly different while soil organic-C, available phosphorus and available N content were significantly different among treatments in both the crops. Enzymatic activity determines the quality of soil. In our study dehydrogenase, urease and nitrate reductase were analysed in both the crop. Statistically DHA activity in T_3 was significantly higher than T_7 (recommended dose of NPK by chemical fertilizer) by 42.85 % in spinach grown soil, while DHA activity in T_3 was non-significant with T_7 in baby corn grown soil. Nitrate reductase activity in T_3 was non-significant with other treatments, but it was significant with control in spinach grown soil. On the other hand, T_3 is statistically significant with T_7 in baby corn grown soil. The results indicated that application of algae biomass as manure improves the microbial activity in soil equally or more than that of NPK applied by chemical fertilizer.

The dehydrogenase, urease and nitrate reductase were analysed in both crops after 30 days of sowing. The determination of dehydrogenase (DHA) in the soil samples

provides an information about biological characteristic of the soil. Among all the enzymes in the soil, dehydrogenases are widely used as an indicator of overall soil microbial activity. It was found that dehydrogenase, urease and nitrate reductase activity were found to be maximum in algae grown soil. Although there was insignificant difference in urease activity in both the crops in all treatments. But among the treatments the dehydrogenase activity (0.30 ugTPF$g^{-1}h^{-1}$) in spinach and nitrate reductase activity (0.13 mg NO_2-N produced g^{-1} soil 24 h^{-1}) in baby corn was significantly higher compare to other treatments.

The nitrate content in leachate was determined through a column study in the field. The hollow pipes of PVC (column) of 100 cm length and 12 inches diameter were kept in triplicate. The top part (10 cm) of the column was left for irrigation. At a depth of 15 cm, 30 cm, 45 cm, 60 cm, 75 cm, and 90 cm a plastic syringe of 15 ml capacity was installed for collecting leachate. The cumulative nitrate content in leachate was found to be maximum (24.63 mg/L) in treatment T_7 (recommended dose of NPK by chemical fertilizer) while lowest (15.81 mg/L) in T_0 (control, zero N application) followed by (17.19 mg/L) T_3 (recommended dose of NPK by chemical fertilizer)

6.2 Significant findings

- *C. minutissima* reduced nitrate, ammonium, phosphate, and potassium content in sewage wastewater by 89 %, 48.2 %, 67.4 %, and 66.3 % respectively.
- The algae *C. minutissima* effectively reduced nitrate, ammonium, phosphate, potassium, BOD, COD, and TDS within 15 days of inoculation.
- The algae biomass obtained after phycoremediation (25 days) typically contained 6% N, 1% P and about 0.48 % K, which is higher than most of the available organic manure.
- The application of 100% N dose by algal biomass lead to higher or equivalent economic yield of spinach and baby corn as compared to yield obtained by supply of recommended dose of fertilizer.
- The organic carbon, available P and K content in algae applied soil is more or nearly equal to soil supplied with chemical fertilizer.
- Algae biomass plays an important role in reduction of nitrate leaching, hence reduces the ground water pollution and eutrophication.

6.3 Conclusion

Available fresh water is limited and continuously decreasing due to over exploitation and pollution. Therefore, it becomes necessary to reduce over exploitation and reuse of wastewater for the sustainability of human life on the earth. Phycoremediation is one of the most effective natural methods of reclamation of sewage wastewater by algae. Therefore present study was undertaken to quantify potential of *C. minutissima* for the phytoremediation of sewage wastewater generated in IARI campus. Further, algae has been reported as good source of plant nutrients (NPK), therefore, present study also quantified amount of algal biomass generated during the wastewater treatments as well as effect of algal manure on soil quality and growth of crops (spinach and baby corn). Present study found *C. minutissima* a very effective microalgae for the phycoremediation of sewage wastewater as well as a good source of plant nutrient having positive effect on soil physical, chemical and biological properties as well as on crop growth and yield. . The result of this study showed that *C. minutissima* can reduce 93 % EC, 94.3 % TDS, 93.2 % BOD and 80.5 % COD after 25 days of phycoremediation of the sewage wastewater. Further, it can reduce nitrate, ammonium, phosphate and potassium in sewage wastewater by 89 %, 48.2 %, 67.4 % and 66.3 % respectively within 25 days of inoculation. This reduction was found at faster rate within 15 days of inoculation. In spite of reducing these pollution load of wastewater, *C. minutissima* can also enhance dissolved oxygen content by in the wastewater by more than twice as compare to initial content.

The algal biomass obtained after phycoremediation of sewage wastewater contain higher amount of NPK as compared to other organic manure like FYM, vermicompost, oil cake and biogas-slurry. Therefore, obtained algae biomass after phycoremediation have vast potential for using as manure in agriculture field. The application of 100% N dose by algal biomass lead to higher or equivalent economic yield of spinach and baby corn as compared to yield obtained by supply of recommended dose of fertilizer. The dehydrogenase, urease and nitrate reductase were analysed in spinach and baby corn grown soil. As compared to chemical fertilizer applied soil, *C. minutissima* biomass manure applied soil had higher soil microbial activity and lower denitrification loss of N. The organic carbon, available nitrogen and phosphorus content of algae biomass applied soil were also more or equivalent to the soil having recommended dose of NPK were applied by chemical fertilizer. Apart from

these benefits, algae manure treated soil had less cumulative nitrate leaching loss from 0-45 cm depth compared to chemical fertilizer applied soil. Therefore, it can be concluded that *C. minutissima* is very effective in reducing pollutant content in the sewage wastewater within 25 days. Furthermore, obtained algal biomass after phycoremediation can be used as manure for enhancing crop yield and soil quality as well as reducing leaching loss of nitrate. However, further studies are needed for evaluating the potential effect of algae biomass obtained after phycoremediation as manure on various crops on long term basis. Finally it can be concluded that:

1. Microalgae *C. minutissima* is very effective in removing pollutant load from sewage wastewater.
2. The NPK content in plot grown with 100 % N dose by algae was more or on par with the plot supplied with recommended dose of chemical fertilizer in both the crops.

Evaluation of manure potential of phycoremediated algal biomass on baby corn and spinach

ABSTRACT

Improvement of soil fertility and agricultural productivity is the need of today to meet the increasing demand for food security. To satisfy the need for environmental and food sustainability, we have to recycle and reuse of resources. The wastewater grown microalgae are exceptional in providing the NPK to the soil after phycoremediation and biofuel extraction. In the present study microalgae, *Chlorella minutissima* was selected for phycoremediation. *C. minutissima* reduced nitrate, ammonium, phosphate, and potassium content in sewage wastewater by 89 %, 48.2 %, 67.4 %, and 66.3 % respectively. The reduction in BOD, COD, and TDS was 93.2 %, 80.5 % and 94.3% respectively. Although, *C. minutissima* can effectively reduce the EC, TDS, P, K, NH_4^+, NO_3^-, BOD and COD within 15 days of inoculation. The algal biomass, urea and algal biomass with urea was applied to bay corn and spinach. The application of 100% N dose by algal biomass lead to higher or equivalent economic yield of spinach and baby corn as compared to yield obtained by supply of recommended dose of chemical fertilizer. The enzymes urease, nitrate reductase, and dehydrogenase were analysed in baby corn and spinach grown soil after 30 days of sowing. The soil supplied with 100 N by algae biomass (*C. minutissima*) significantly (P=0.05) increased the dehydrogenase activity in spinach grown soil. While the nitrate reductase activity in soil supplied with alga manure was maximum (0.13 mg NO_2-N produced g^{-1} soil 24 h^{-1}) and significantly higher than other treatments in baby corn grown soil. Nitrate leaching was analysed during the experiment from 0-15 cm, 15-30 cm, 30-45 cm depth soil in column. The cumulative nitrate leaching was low (17.19 mg/L) in algae applied treatment, while highest (24.69 mg/L) in plot supplied with recommended dose of NPK. Hence algal manure is best in terms of complete utilization of N with minimal leaching. Therefore it can be concluded that phycoremediation coupled with manure production from algae biomass is sustainable practice to reduce pollution, recycling of wastewater, and improve the soil quality.

Key words: Phycoremediation, *Chlorella minutissima*, Algae manure, Nitrate leaching, Spinach, Baby corn

बेबी कॉर्न और पालक पर फाइकोरेमेडिएटेड अल्गल बायोमास की खाद क्षमता का मूल्यांकन

सारांश

खाद्य सुरक्षा की बढ़ती मांग को पूरा करने के लिए मिट्टी की उर्वरता और कृषि उत्पादकता में सुधार आज की आवश्यकता है। पर्यावरण और खाद्य स्थिरता की आवश्यकता को पूरा करने के लिए, हमें संसाधनों का पुनर्चक्रण और पुन: उपयोग करना होगा। अपशिष्ट जल को विकसित करने वाले माइक्रोएल्गी, फाइटोकेमेडिएशन और जैव ईंधन निष्कर्षण के बाद मिट्टी को एन पी के प्रदान करने में असाधारण हैं। वर्तमान अध्ययन में माइक्रोएल्गी, क्लोरेला मिनुटिसिमा को फाइकोरेमेडिएशन के लिए चुना गया था। क्लोरेला मिनुटिसिमा ने सीवेज अपशिष्ट जल में नाइट्रेट, अमोनियम, फॉस्फेट और पोटेशियम की मात्रा क्रमशः 89%, 48.2%, 67.4% और 66.3% कम की। बीओडी, सीओडी, और टीडीएस में कमी क्रमशः 93.2%, 80.5% और 94.3% थी। हालांकि, क्लोरेला मिनुटिसिमा ईसी, टीडीएस, पी, के, एनएच 4 +, एनओ 3-, बीओडी और सीओडी को टीकाकरण के 15 दिनों के भीतर प्रभावी ढंग से कम कर सकता है। यूरिया के साथ एल्गी बायोमास, यूरिया और एल्गल बायोमास को बेबी कॉर्न और पालक में लगाया गया था। रासायनिक उर्वरक की अनुशंसित खुराक की आपूर्ति द्वारा प्राप्त उपज की तुलना में, एल्गी बायोमास द्वारा 100% एन खुराक के आवेदन से पालक और बेबी कॉर्न की उच्च या समकक्ष आर्थिक उपज होती है। बुवाई के 30 दिनों के बाद बेबी कॉर्न और पालक उगाए गए मिट्टी में एंजाइम यूरेस, नाइट्रेट रिडक्टेस और डिहाइड्रोजनेज का विश्लेषण किया गया। शैवाल बायोमास (क्लोरेला मिनुटिसिमा) द्वारा 100 एन के साथ आपूर्ति की गई मिट्टी काफी (पी=*0.05*) ने पालक में विकसित मिट्टी में डिहाइड्रोजनेज गतिविधि को बढ़ा दिया। जबकि शैवाल खाद के साथ आपूर्ति की गई मिट्टी में नाइट्रेट रिडक्टेस गतिविधि अधिकतम थी (0.13 मिलीग्राम एनओ 2-एन उत्पादित ग्राम$^{-1}$ मिट्टी 24 घंटा$^{-1}$) और बेबी कॉर्न उगाई मिट्टी में अन्य उपचारों की तुलना में काफी अधिक है। स्तंभ में 0-15 सेमी, 15-30 सेमी, 30-45 सेमी गहराई मिट्टी से प्रयोग के दौरान नाइट्रेट लीचिंग का विश्लेषण किया गया था। शैवाल लागू उपचार में संचयी नाइट्रेट लीचिंग कम (17.19 मिलीग्राम/एल) था, जबकि एनपीके की अनुशंसित खुराक के साथ आपूर्ति की गई साजिश में उच्चतम (24.69 मिलीग्राम/लीटर)। इसलिए कम से कम लीचिंग के साथ एन के पूर्ण उपयोग के संदर्भ में एल्गल खाद सबसे अच्छा है। इस प्रकार यह निष्कर्ष निकाला जा सकता है कि शैवाल बायोमास से खाद उत्पादन के साथ युग्मित फाइटोरैमिडिएशन प्रदूषण को कम करने, अपशिष्ट जल के पुनर्चक्रण और मिट्टी की गुणवत्ता में सुधार करने के लिए स्थायी अभ्यास है।

मुख्य शब्द: फाइकोरेमेडिएशन, क्लोरैला मिनुटिसिमा, शैवाल खाद, नाइट्रेट लीचिंग स्पिनच, बेबी कॉर्न

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