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Effect of Fertilizer on the Growth Rate of Diatoms

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Abstract: Microalgae including diatoms are the largest group of primary producers. They are responsible for producing 40% of the total organic carbon in the world's oceans and, for the diatoms to grow, a proper ratio of nitrogen, phosphorus and silicon is required. Any deviation from the optimum levels affects their growth. Due to anthropogenic activities, the release of chemicals like fertilizers is causing this hindrance because the nutrients present in the fertilizers such as ammonia are not toxic at normal concentrations in the natural environment, but at increased concentrations it becomes toxic especially at high levels of pH. The increased level of dissolved inorganic nitrogen in the water leads to increased levels of dinoflagellates growth over the diatoms which can induce some unfavorable consequences on the environment. Along with this, the fertilizer run offs can lead to eutrophication leading to the growth of algae causing algal blooms and therefore the depletion of dissolved oxygen. The main focus of this review is to determine what effects does agricultural run offs have on the aquatic ecosystem and the micro algal communities.

***Keywords* – Diatoms, Eutrophication, Fertilizers, Phosphorus, Urea.**

I. INTRODUCTION

Diatoms belong to a major group of algae known as microalgae, which are found in the water streams, oceans and in soil also (Harris *et al*., 2017). Diatoms' cell wall is made up of silica and commonly known as the frustule. They are the organism that not only contributes oxygen to the environment, but also forms a large portion of the biomass (Anbuchezhian *et al*., 2015). They are one of the primary producers in the aquatic ecosystems because of their autotrophic nature (Harris *et al*., 2017). Diatoms are unicellular and they come about either as individual cells or in the form of colonies (Battarbee *et al*., 2001). Their cell size varies from two to two hundred micrometers and their life span is considered around six days. If the diatoms are provided with sufficient nutrients and sunlight, the group of diatoms doubles itself by asexual multiple fission in every 24 hours. Diatoms are generally found in two different shapes i.e. centric diatoms which are radially symmetrical and pinnate diatoms which are bilaterally symmetrical (Battarbee *et al*., 2001).

1.1. Life cycle of diatoms

Diatoms are having the complex lifecycle which includes a peculiar division mode and having the capacity to produce resting stages (Edlund and Stoermer, 1997; Round *et al*., 1990). Diatoms reproduce vegetatively (agamic) by the binary fission, and result into the formation of two new individuals within the parent cell frustule (Tomas, 1996). The vegetative stage in the interaction of diatoms is an exclusive manner with its sexual stage because of the cell estimates planned diatom sex clock which makes it an exceptional property of the life cycle of diatoms (Lewis Jr, 1984). Each new daughter cell receives one valve of the parent cell theca as it epitheca, and then each cell forms its hypotheca after which the cell division is terminated. This type of cell division, with the formation of new siliceous components inside the parent cell, it leads to the size reduction of the offspring (Tomas, 1996). The most distinguishing property of the diatom lifecycle is progressive reduction in cell size during the vegetative phase (Round *et al*., 1990). The decrease in the average size of the population of diatom implies a need for a means of the restoring the cell size. Restoring of the cell size is only possible by the formation of the auxospore. The auxospores are unique to the diatoms. Usually, the auxospores result from the allogamous fusion (Kaczmarska *et al*., 2001). Generally, the cell wall of auxospore consist of two biogenic components that are organic matter and siliceous components (Von Stosch, 1982; Round *et al*., 1990). After this a new diatom frustule of maximal size is being formed within the auxospore sphere called the initial cell takes place (Tomas, 1996). The basic pattern of this sexual lifecycle differs between the pennate and centric diatoms. The centric diatoms were evolved first (Amato *et al*., 2005) and are characterized by the oogamous reproduction, that involves the formation of uniflagellate sperms (male gametes) and larger nonmotile female gametes, and in the pennate (isogamous) produce the nonmotile (Lewin and Guillard, 1963) morphologically identical but sometimes they are different gametes behaviourally (Round *et al*., 1990).

1.1.1 Formation of resting spore

The formation of the diatom resting spore is normally occur as a response to the unfavourable environmental conditions, or when all nutrients have been exhausted, and germination occurs when the conditions improve. The formation of the resting spore is more common in centric diatom but very rare in pennate marine planktonic diatoms (Tomas, 1996). There are three types of which can be distinguished: exogenous resting spore- it is the mature resting spore which is not physically in the contact with the parent cell theca; semi endogenous resting spore- the hypo valve of this spore is enclosed within the one parent cell theca; and the endogenous resting spore- this whole spore is enclosed within the parent cell frustule. The semi endogenous type is the most common type resting spore (Syvertsen, 1979). The morphology of the resting spore some species is similar to the vegetative cells, whereas in some species it differs drastically (Syvertsen, 1979, 1985) (fig 1).

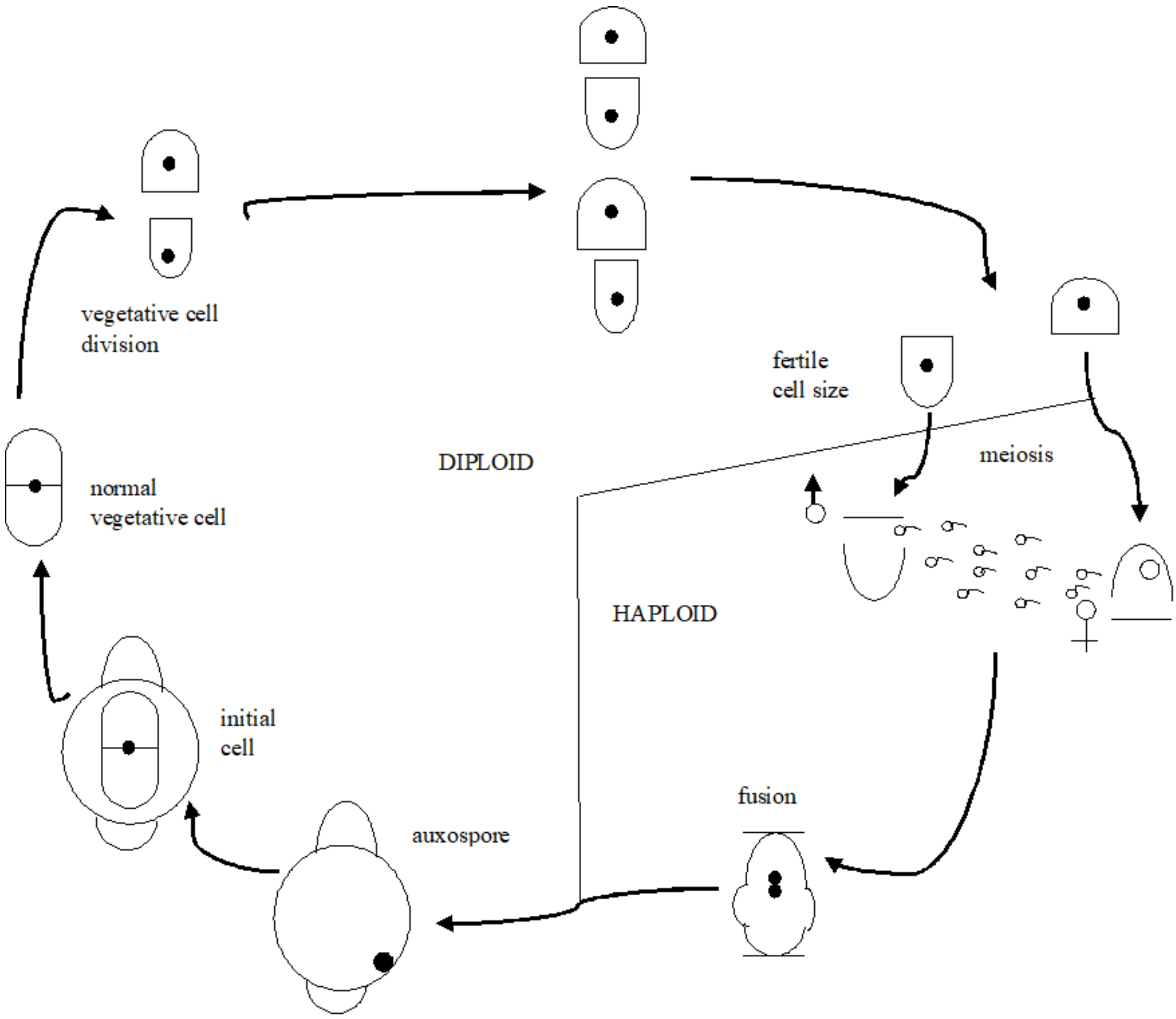

Figure 1. Life cycle of diatom:● -zygote,● -nucleus, ○-pycnotic nucleus (modified from (Tomas, 1997).

1.2. Impact of nutrients on growth of diatoms

Zooplanktons use them as their primary source of food so they are considered as the base of the food chain in the marine communities; this also effects the food availability for the larger sea animals such as salmon. Their abundance and distribution patterns can help in the detection of the ecosystem (Facca *et al*., 2004). Every year, they produce 40% of the total organic carbon in the world's oceans. But, due to increasing anthropogenic activities, toxic contaminants like domestic wastewater, agricultural fertilizers, pesticides etc. are released into the aquatic ecosystems is a large amount. An optimal ratio of nitrogen, phosphorus and silica is required for the proper growth of diatoms and any disturbance in the ratio of these elements hinders their growth, according to a study conducted at Huanghai Sea (Fu *et al*., 2012). In those environments, where silica is a limiting factor, non-silica dependent phytoplankton dominates over silica dependent diatoms which further leads to algal blooms (Howarth *et al*., 2011). Due to presence of siliceous cell wall diatoms are quite easy to get sampled and preserved, thus they provide a permanent record for assessing short-term or long-term change (COX, 1991; Smol, 1992). Decreased ratios of silicon & nitrogen and silicon & phosphorus can lead to an increase in the dinoflagellate's growth over diatoms and the diatom/dinoflagellates shift may have certain different consequences in the ecosystem (Davidson *et al*., 2012; Egge and Aksnes, 1992; Ren *et al*., 2020; Smayda, 1997; Zhou *et al*., 2017).
Due to the increase in the human population day by day, food demand has increased a lot, which results into the excessive use of the agricultural fertilizers for more yield. These fertilizers move to the water streams, rivers and finally to oceans and show their adverse effects on the flora and fauna of the aquatic systems**.** Benthic diatoms are the marvelous indicators of this water pollution (Stevenson *et al*., 1999). Diatoms are in demand increasingly as bio-indicators because their communities give a quick and strong response to the environmental changes (B. A. Whitton *et al*., 1991). The release of the fertilizers increases the nitrogen and silica in the natural environments that stimulates the growth of the benthic diatom species and more nitrogen consumption**.** In the Bay of

Brest, a biologically active silicate pump helps the Bay to deal with the increased inputs of the nitrogen (Ragueneau *et al*., 2002). A new silicate had up surged from deep water onto the surface by an unstable water front in the North eastern Atlantic. It was able to enhance the export of diatom carbon and also lengthen the diatom bloom (Allen *et al*., 2005). So, it would be quite interesting to know whether the addition of silicate (independently or as a supplement can favor the growth of diatoms and the diatom food chain thereby reducing eutrophication (Wassmann *et al*., 1996).

The use of the diatoms in pollution monitoring would be highly valuable in the remote areas. Such a location or area exists in the Himalayan region in Nepal, where use of diatoms has recently employed for the very first time to collect data regarding the natural and anthropogenic influences on the biodiversity present in the river (Ormerod *et al*., 1994).

1.3. Evolutionary Relationships of diatoms

In diatoms plastids is derived from the algae (Rhodophyta) by endosymbiosis. Recent studies have shown that the nuclear genes in diatoms and few closely related connected protista's have been descended from the algae (Chlorophyta). Eventually some studies have shown that the OUC (Ornithine Carbamide Cycle) in metazoans are linked with diatoms as well. The OUC performs the removal of fastened nitrogen in metazoans, whereas in diatoms it helps in distribution and repackaging of inorganic carbon and nitrogen. Earlier it was thought that OUC is only evolved in metazoans but later it was discovered that it is found in diatoms too (Bruder and Medlin, 2007).

1.4. Diversity and Distribution

Diatoms is considered as one of the finest taxa of photosynthetic unicellular entity. They have around 200 genera along with 10-12 many beneficial known species. It is a direct result of their variety they are next to no known. Diatoms present in jungles are investigated less, even diatoms are found in outrageous living space too from marine, freshwater to a portion of the earthbound natural surroundings also. Not many heterotrophic diatoms live in dull conditions, some of them live as endosymbionts while some as non-obtrusively on the body surface of other life forms. In fact, every diatom has their own particular natural resistance. Concerning the climate diatom's structure species arrays. Anyway, numerous diatoms show worldwide appropriation and a large portion of them are contamination lenient species. Notwithstanding, an enormous number of endemic taxa have been accounted for from different districts across the world, for the most part from old lakes like African Rift Valley lakes, lake Baikal, etc (Seckbach and Kociolek, 2011). Investigations of diatoms began for the most part in nineteenth century. Various scientist came to know diatoms in India. In India nearly around 7000 diatoms taxa are available and mostly from freshwater, some from marine climate and fossils too. Around the world mostly from jungles numerous novel diatoms have been isolated. The ongoing projects in the Western Ghats of India have discovered new types of diatoms. In India, there are many areas that are still pending to carry out the further studies on the various varieties of the diatoms (Karthick *et al*., 2013).

II. FERTILIZERS AND ALGAL GROWTH

Diatoms are microalgae which have been used fantastically as water quality assessment. Refined of microalgae as sustenance for business raising of marine animals is of essential importance inside the hydroponics business. Made refined microalgae have a fundamental influence in marine creature culture. As the most wellspring of food in larval developmental phase of various marine animals in maritime climate microalgae can additionally push ahead the creatures' dietary regard. Marine benthic diatoms as the basic sustenance of sea cucumber and abalone hatchling in arrange of tally calories change can start the larval settlement just as change. Benthic diatoms can be refined and fill rapidly in specific conditions identifying with the central natural parts, for example, lighting up, water temperature, etc (Guedes and Malcata, 2012). Without nitrogen, phosphorus, press and silicon, which are advantage for the diatom assimilation framework, diatoms can't grow conventionally in the midst of the mass culture. The availability of assorted green development measure and their compound creation is identified with their healthy regard, especially for the raising hatchlings of a couple of shellfish and point species. For huge scope social orders of microalgae, it will be imperative to use less expensive food sources to decrease the age caused significant damage (Gonzalez-Rodriguez and Maestrini, 1984; Elías and Voltolina, 1993; Gracida-Valdepeña, 1999). Lately, for huge scope social orders of benthic diatoms the use of provincial composts as enhancements must be extensively used (Simental-Trinidad *et al*., 2001).

III. DIFFERENT NITROGEN SOURCES OF FERTILIZERS AND THEIR EFFECT ON DIATOMS

3.1 Ammonia

Almost every fertilizer has ammonia as its component, which we know are useful for the plant growth but these compounds are also known to have toxic effects at certain concentrations in the aquatic environment. In oceanic or aquatic environment, ammonia is a considerable constituent that influences the composition of phytoplankton species. As the organisms living in the aquatic environment are well known to use up some complex organic nitrogen sources, nitrate, nitrite, ammonium as well as inorganic nitrogen. But most of the photosynthetic marine phytoplankton mainly depends upon nitrate and it is therefore one of the most crucial factors which determine the primary productivity whereas the remaining phytoplankton better utilizes ammonium and it is well suited to them (Glibert, 2016).

3.2 Urea

Due to the continuous use of the agricultural fertilizers and overproduction of the urban wastes, the concentration of both N and P is increasing very rapidly in the water bodies (Reid, 2005). It is more costly to eliminate the N from the wastewater because N has direct effect on the primary production (Schindler *et al.,* 2008). Eutrophication of the water bodies due to the presence of the excessive amounts of nitrogen results not only in the decline of the primary productivity, but also degrades the water quality (Leavitt *et al*., 2006). With every passing year, the use of fertilizers all around the world is increasing at a faster rate, especially of the nitrogen containing fertilizers (Galloway and Cowling, 2002). It was found that the worldwide usage of industrially important Nitrogen was greater than 170 Megatons every year (Glibert *et al*., 2014). The effect of the N on the primary production highly depends upon the source of the N used in the agricultural fertilizer, which runoffs to the lakes or other water bodies (Berman and Chava, 1999; Finlay *et al*., 2010). And, ever since 1970's there has been 100 times more usage of urea and urea then became the most used component in the agricultural fertilizers. More than 50% of the composition of fertilizers used worldwide, now consists of urea. Therefore, it ultimately led to more exposure of urea to the aquatic and the marine phytoplankton including diatoms (Glibert *et al*., 2006).

Urea, a very good source of nitrogen is in excessive use in the form of the fertilizers that accounts for approximately 50% of the entire N fertilizer usage all around the world. Out of this, 5-40% of the urea that is added to the fields lost to the runoff (Glibert *et al.*, 2006). Some of the whole lake experiments have showed that N can increase the biomass in the lakes (Barica *et al*., 1980; Levine and Schindler, 1999). It is because the reduced nitrogen species like urea can be efficiently and energetically assimilated by the diatom species as compared to the atmospheric nitrogen (Herrero *et al*., 2004; Turpin *et al*., 1985). In the year 2016, an approximate of 4750 tons of the liquid N-containing fertilizer was collapsed in Denmark**.** The majority of that fertilizer contained only urea (Markager, 2016), but some part of the ammonium and nitrate was also present in it. In the fertilizer, there were two isotopes of the N (N16 and N32). N16 was in the amount of 750 tons and contained urea only. N32 spill was 2000 tons and was having 50% urea, 25% each of nitrate and ammonium (Markager, 2016). As a result, urea has become a great source of N for the marine and coastal diatoms and phytoplankton's (Glibert *et al*., 2006). In general, most of the diatoms prefer nitrate as a source of the nitrogen (Glibert et al., 2006). In some of the diatom strains like *P. multiseries*, *P. calliantha* and *P. fraudulenta*, decrease in the growth rate has been found when they were allowed to grow on urea as the only source of nitrogen as compared to nitrate and ammonium (Thessen *et al*., 2009). As urea contains two N atoms and is important for the Domoic Acid (DA) production and cell growth, its different concentrations can have impact on both domoic acid content and cell growth. Like, for strain 19 of *P. seriata*, a positive correlation was found between the concentration of urea and maximum growth rate and on the other hand, another strain of *P. seriata* i.e., strain 11 showed low cell densities and even the low growth rate. *P. delicatissima* and *P. obtusa* showed similar growth rate on both urea and nitrate as N sources, although showed high growth rate when were allowed to grow on urea only. Some studies have shown that some diatoms cannot utilize urea well or not at all (Solomon *et al*., 2010). Yamaguchi and Itakura, 1999 suggested that *K. mikimotoi* could utilize NO_3^-, NO_2^- and NH_4^+, but not uric acid and organic form of urea**.** Once ammonium reaches inside the cell, it can be directly used in amino acids, but urea can't be assimilated as such and requires the use of enzymes like urease as the final N product (Antia *et al*., 1991)**.** Due to the increased usage of urea in the fertilizers, many cases of Paralytic Shellfish Poisoning i.e., PSP, caused by the HAB dinoflagellates i.e. Harmful Algal Bloom (Glibert *et al*., 2006) species have been reported.

IV. EFFECT OF PHOSPHORUS ON THE GROWTH RATE OF DIATOMS

A balanced ratio of the nitrogen, phosphorus and silicon is required for the optimal growth of diatoms required (Harris *et al*., 2017). But when this ratio is disturbed, the significant growth of diatoms gets hindered. Generally, the nitrogen, silicon, phosphorus and iron are required for the growth of benthic diatoms (Yang *et al*., 2014). When phosphorus was not added in the culture there was not any growth of diatoms. But with the concentration of phosphorus going up, the growth of benthic diatom was getting better (Yang *et al.,* 2014). But the extra amount of phosphorus which can be added by the fertilizer, leads to the increased growth of the diatoms, which can result into the eutrophication, and can also leads to production of toxins.

Phosphorus can be used to reduce the blooms of the benthic diatom, *Didymosphenia geminata* (James *et al*., 2015). *Didymosphenia geminata* is a stalk producing benthic diatom with oligotrophic waters (Spaulding and Elwell, 2007). *Didymosphenia geminata* produce stalk (bloom) at very low concentration of phosphorus (B. Whitton *et al.*, 2009). As the low concentration of phosphorus concentration is proximate cause of the bloom formation by this diatom (Kilroy and Bothwell, 2012). Increasing the concentration of phosphorus with *D. geminata* might diminish the blooms (James *et al*., 2015) and for the increment of phosphorus concentration in the water, fertilizers were used and the fertilizers provided the phosphorus and elevated the phosphorus concentration in the water. The enrichment in the phosphorus concentration by adding the phosphorous fertilizer resulted in the reduction of the biomass of the diatom (*Didymosphenia geminata*). There is a temporarily reduction in *D. geminata* mats could be done due to the enrichment in phosphorus. The enrichment in phosphorus can lead to the undesirable eutrophication (James *et al*., 2015).

The use of phosphorus helps in the reduction of bloom production but it cannot be considered as a long-term solution for the bloom production and does not eliminate the *D. geminata* from the streams, but the biomass is reduced as long as phosphorus enrichment is continued (James *et al*., 2015). So, instead of phosphorus the light reduction can be used as alternative approach for the *D. geminata* bloom management (James *et al*., 2014). As it is well known that due to the fertilizer, there can be nutrient enrichment. Nutrient enrichment caused an increase in the total density of diatom and decrease in the diversity and species richness, and mainly in the *Nitzschia* species there was change in the taxa proportion of diatom and favoring motile forms (Licursi *et al*., 2016). Diatom species have shown the decrease in the taxa in response to the nutrient enrichment were considered as mesotrophic and they have a recognized sensitivity to increased conductivity, organic matter and nutrient concentration (Gómez and Licursi, 2001; Licursi *et al*., 2010; Van Dam *et al*., 1994). *Nitzschia frustulum*, *N. inconspicua*, and *N. palea* were more tolerant to nutrient enrichment and after adding the fertilizer there was increase in the cell density of these species of diatoms because of nutrient enrichment (Licursi *et al*., 2016). The increase in the relative abundance of the *Nitzschia* species has been associated with the high sediments and the phosphorus enrichment (Kelly, 2003; Licursi and Gomez, 2009).

V. EFFECT OF TRACE ELEMENTS OF FERTILIZERS ON THE GROWTH OF DIATOMS

Trace elements play an important role in the ecology of phytoplankton (Tadros *et al*., 1990). They are non-biodegradable pollutants and a result of many industrial processes that are released into the water bodies affecting the microorganisms at different concentrations (Manimaran *et al*., 2012). Trace elements are considered as essential in determining which species are present to use the nutrients in the algal community (Patrick, 1978). Trace elements such as lead (Pb), copper (Cu), aluminum (Al), cadmium (Cd), boron (B), selenium (Se), chromium (Cr), manganese (Mn), cobalt (Co) and arsenic (As) are considered important for the microorganism (Manimaran *et al*., 2012) but they can be toxic at certain concentrations (Tadros *et al*., 1990). Iron (Fe) is an important micronutrient for the growth of phytoplankton and it exhibit the control of primary productivity in large oceanic region (Annett *et al*., 2008). Copper is also an important micronutrient for as it is a component of various proteins and enzymes which are involved in various metabolic pathways (Manimaran *et al*., 2012). A study was conducted to check the effect of presence of copper (Cu) and iron (Fe) on the growth rates of diatoms. So, eight species of centric diatoms which includes both coastal and oceanic strains were taken. It was shown that by decreasing the availability of iron, there was significant reduction in the growth of both coastal and oceanic diatoms. Significant difference was observed in the growth of both coastal and oceanic diatoms in response to the availability of iron as compared to copper and coastal strains are observed to have larger depletion in the growth rates than oceanic strains with iron limitation (Annett *et al.,* 2008). Another study was conducted to check the effect of chromium and selenium on the growth rates of diatoms. It was shown that in different concentrations of chromium, the diatoms were considered dominant in the control and at the lower concentrations. It was found that at the highest concentration of selenium the diatoms were diminished although the diatoms tolerated well at other concentrations (Patrick, 1978).

VI. CONCLUSION

Diatoms are defined as a group of unicellular aquatic microorganism that belongs to a specific group of algae called the golden-brown algae. Diatoms they acquire their energy directly from sunlight and they also use organic matter such as carbon dioxide as one of the major sources of foods. They are one of the major oxygen producers in the world, nearly producing 20% of earth's oxygen and are also the primary food producer among the aquatic ecosystem thereby a significant member of the food chain. Water contamination with inorganic nitrogen and phosphorus as the chemical fertilizer components is a growing concern. Many studies were performed and according to those studies, the water quality is highly affected due to these fertilizers as it is responsible for an increase in algal growth (Frink, 1967; Ryan *et al*., 1972; Sikka and Pramer, 1968). These algal blooms cause problems in water quality such as changed taste and odor which leads to increase in costs for water purification. Also, these prove to be dangerous for other species in the aquatic environment because they exhaust the dissolved oxygen supply in the water (An and Park, 2002). Agricultural run-off is one of the major contributors of pollution worldwide (Conley, 1999). Due to the excessive release of fertilizers or nitrogen and phosphorus along with some other nutrients into the aquatic ecosystems, there is a sudden population explosion in the growth of algae which then ultimately leads to the production of low oxygen zones due to reduction in the amount of dissolved oxygen (Beman *et al*., 2005). This happens because, more and more nutrients from the agricultural fertilizers in the form of nitrogen and other nutrients are available for the algal growth.

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