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EFFECTS OF ADDED NUTRIENTS ON DRY MASS, AFDM, CHLOROPHYLL *a* AND BIOVOLUME OF PERIPHYTON ALGAE IN ARTIFICIAL STREAMS[*]

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Abstract – We studied the role of nutrients on various aspects of the periphyton community in artificial streams. Dry mass, ash-free dry mass, chlorophyll *a* and biovolume of the periphyton community were measured under 3 different nutrient regimes, including one from natural streams as a control and two which were enriched with N and N+P. Results of this experiment indicate that the standing crop of the periphyton community in the artificial streams increased with increasing the amount of the nutrient. Also, we showed that diatom diversity was affected by increasing the nutrients concentrations. The periphyton dry mass following 23 days of experiment was 0.96, 1.4 and 3.52 mg/cm^2 for control, N and N+P artificial streams, respectively. Ash-free dry mass (AFDM) of the periphyton community for control, N and N+P treatment were 0.2, 0.98 and 1.04 mg/cm^2, respectively. The experiment also depicted that the periphyton chlorophyll *a* increased with increasing the nutrient content in the artificial streams (for control, N and N+P enriched artificial streams, the chlorophyll *a* content was 2.11, 2.20 and 6.36 mg/m^{2}respectively). The total diatom biovolume as a measure of standing crop in the periphyton community was 6x10^6, 8x10^6 and 48x10^6 μm^3/cm^2 for control, N and N+P enriched artificial streams, respectively. Results of this experiment demonstrated that adding nutrient increases the relative abundance of *Navicula, Achnanthes*, *Nitzschia* and *Cocconies* in artificial streams.

Keywords – Nutrients, periphyton, dry mass, AFDM, chlorophyll *a*, biovolume, diversity, Gamasiyab River

1. INTRODUCTION

The importance of the periphyton community in freshwater environments as a basis for trophic structure is increasingly recognized [1]. In natural communities, periphyton contributes significantly to primary production [2-3]. The periphyton community provides food for many invertebrate animal [4] and fish [5]. It has been shown that added nutrients to water enhance productivity [6]. Phosphorous is probably the key nutrient in becoming a limiting factor, if other factors are sufficiently available, because in most freshwater ecosystems concentration of phosphorous is less than that of nitrogen [7].

Due to difficulties associated with studying periphyton communities under natural conditions, artificial streams of different shapes and dimensions have been widely used to assess the effects of nutrients in lotic systems for the last 30 years [4]. Streeter [8], for example, used artificial streams to study the effects of nutrients on aquatic organisms. Stocker and Shortreed [6] evaluated the influence of the addition of orthophosphate and nitrate on algal biomass in artificial streams. In the mid 1980's,

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artificial streams had played a major role in clarifying nutrient limiting mechanisms in rivers [4]. Recently, Jones et al. [9] appraised the relationships between snails and the periphyton community in artificial streams.

Nutrients, specially phosphate and nitrate have an important role in periphyton community growth in laboratory streams [4]. Added phosphate and nitrate have been shown to increase biomass in the periphyton community [6]. In another experiment, Pringle [10] indicated that the forms of phosphorous have different effects on the growth and composition of algae. High amounts of nutrients affect phytoplankton species composition [11-14]. Phytoplankton growth rates are often limited by nutrients [15]. The biomass of the microbial community has also been shown to be affected by added nutrients in artificial streams [16]. It has also been established that other characteristics of the periphyton community such as periphyton succession, species composition, community structure and species diversity are under the influence of soluble nutrients [17]. Although recent experiments with artificial streams have shown that the general relationships between nutrient concentration and community characteristics are to some extent predictable, the rate by which such communities may react to nutrient manipulation is not clear [18, 4].

Records of research on the effects of added nutrients on the periphyton community in artificial streams in Iran are not known to the authors. Therefore one of the aims of this study is to examine the reaction of a local algal community collected from a pristine stream (Gamasiab River head water) to added nutrient in artificial streams.

2. MATERIAL AND METHODS

This study was conducted in three 0-shaped fiberglass streams, each 2 meters in length, 0.5 meter in width and 0.4 meter in depth. Each of the streams has been divided into two equal sections by placing a fiberglass sheet in the middle of the streams. Water from upstream of the Gamasiyab River (34°40′E, 48°20′N) was used in the artificial streams at a depth of about 0.08 m. Using inwater pumps, water velocity in the channels averaged 17cm/s. Light was supplied by 18 metal halide lamps (6 for each channel), which provided a broad spectrum of photosynthetically active radiation. Quantum flux density levels were ≈ 90 $\mu m.m^{-2}.s^{-1}$ when measured at the water surface in the artificial streams. The photoperiod was set at 16L: 8D. Water in the artificial streams was enriched by adding 0.04 mg/L nitrate in the N enriched stream and 0.04 mg/L nitrate plus 0.004 mg/L phosphate in the N+P enriched stream. The water collected from head streams of the Gamasiyab River was used as a control.

Clay tiles (5×5 cm) were placed on the bottom of the channels as substrates for the periphyton colonization. To inoculate the streams with algae, pebble were collected from the Gamasiyab River and scraped with a brush into a water container. The water containing scraped algae was filtered through a mesh and an equal volume of the filtrate containing algae was added to each channel. Water temperature ranged between 20-25°C during the experiment. A sample collected from the artificial streams for the analysis of biovolume and species composition was preserved in a 4% formalin solution.

After 23 days of the fertilization period, four tiles were randomly selected and dried for 24 hours at 60°C and attached periphyton was scraped from the tiles with a razor blade and weighed. For measuring ash free dry mass (AFDM), four tiles were randomly selected and dried for 24 h at 60° C, and periphyton organisms were scraped similar to the dry mass, weighed, ashed in a 500°C furnace for four hours, and reweighed. Representing all organic matter in the periphyton (detritus, algae, bacteria, protozoan, fungi, etc.), AFDM was the difference in the mass before and after ashing. AFDM was expressed as grams per square centimeter of the original substrate.

Four tiles were randomly selected and scraped into an experiment tube containing 10 ml of 95% ethanol. The samples were then stored overnight in a freezer. The light absorbency at 665 nm of the supernatant was determined before and after adding two drops of 0.1 N HCl using a spectrophotometer. The chlorophyll *a* concentration was determined from an absorbency reading using the equation proposed by Nush [19]. The cell counts were converted to cells/cm^2 per taxon, using the formula proposed by Litteral et al. [20]. Densities (cell per square centimeter) were converted to diatom biovolume (cubic micrometers per square centimeter) by multiplying the diatom density of each taxon by estimated cell volume. Total cell volume of a diatom was calculated for all diatom of each taxon with an ocular micrometer using oil immersion at 1000x to obtain mean cell dimensions. Dry mass, ash free dry mass and chlorophyll *a* contents of the samples were compared at different treatments using single factor analysis of variance (ANOVA).

3. RESULTS

a) Dry mass

The effects of added nutrients on the periphyton biomass are shown in Fig.1. It is evident from the current experiment that the dry mass of the periphyton community increases with the nutrient enrichment. Statistical comparison between the dry mass of the periphyton community in 3 different treatments show a significant (P< 0.05, F=71.31) difference using single factor analysis of variance (ANOVA). The extent of changes in biomass due to the nutrient effects in control, P-enriched and N+P– enriched artificial streams in the periphyton algal community were 0.96, 1.4, and 3.52 mg of dry mass per cm^2, respectively (Fig. 1).

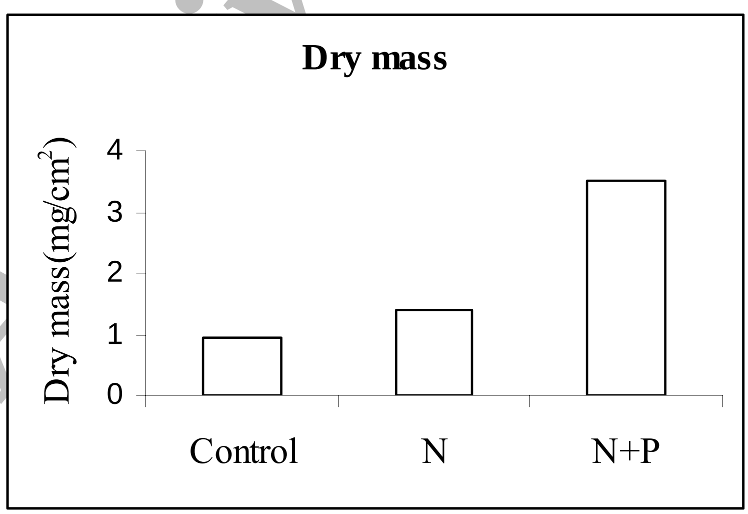

Fig.1. Changes in dry mass for periphyton algae grown in unenriched (control), N-enriched (N) and P+N-enriched in the artificial streams

Periphyton biomass, as indicated by ash-free dry mass (AFDM), increased with enriching the artificial streams. A significant difference in ash-free dry mass is shown (P < 0.05, F=32) using single factor analysis of variance (ANOVA). Periphyton ash-free dry mass (AFDM) were 0.2, 0.98

and 1.04 mg/cm^2 for control, phosphate-enriched and phosphate + nitrate-enriched artificial streams, respectively (Fig. 2).

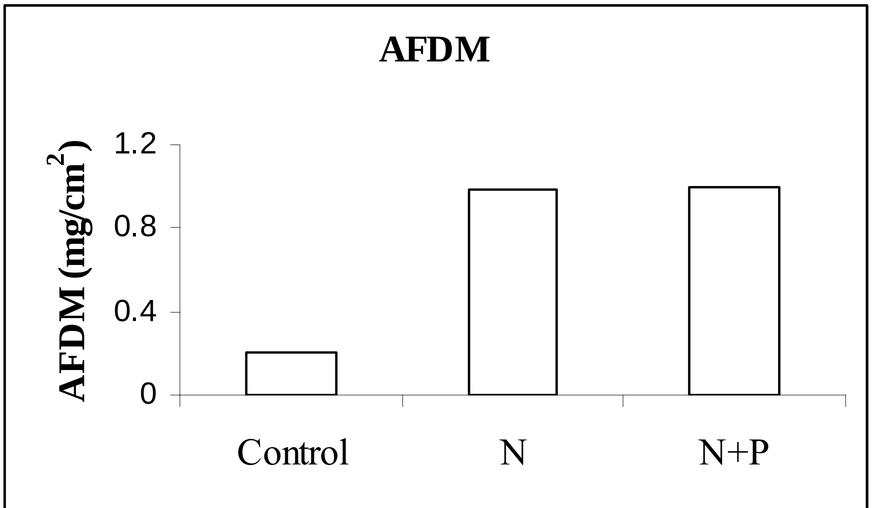

Fig. 2. Changes in the amount of ash - free dry mass (AFDM) of the periphyton community in the artificial streams, Control=unenriched, N=addition of N, and (N+P)=addition of both N and P

b) Chlorophyll a

Figure 3 demonstrates chlorophyll *a* as an indicator of the periphyton productivity studied in the artificial streams for different nutrient regimes. We demonstrated that chlorophyll *a* increased by enriching the artificial streams (P <0.01, F=6.72). Periphyton chlorophyll *a* was 2.11, 2.20, and 6.36 mg/m^2 for unenriched, P-enriched and N+P-enriched artificial streams, respectively.

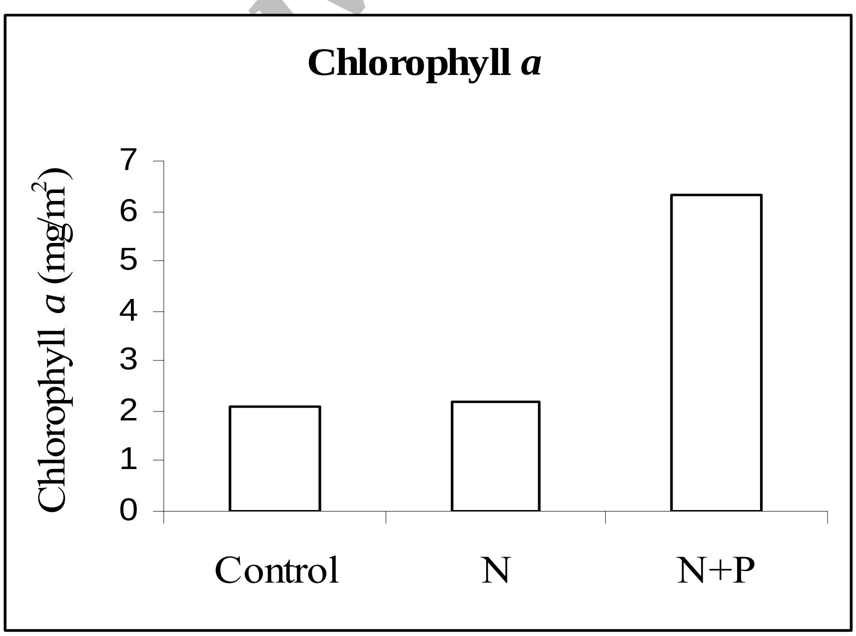

Fig. 3. Changes in the chlorophyll *a* content in the periphyton community in the artificial streams, control=unenriched, N=addition of N and (N+P)=addition of both N and P

c) Diatom biovolume

Total diatom biovolume as an indicator of the standing crop is influenced by the nutrient fertilizations. We demonstrated that diatom biovolume increased with the addition of N and P ($P<0.05$, $F=99.05$). The diatom biovolume for control, P-enriched and N+P-enriched were $6\text{x}10^6$, $8\text{x}10^6$ and $48\text{x}10^6$ $\mu m^3/cm^2$, respectively (Fig. 4). The relationship between biovolume and chlorophyll *a* was determined using regression analysis. Results indicated that this relationship is highly significant ($r^2=0.99$) (Fig. 5). Results of single factor analysis of variance (ANOVA) for various indicators of the periphyton productivity in the artificial streams under different nutrient regimes during 23 days of the experiment are shown in Tables 1, 2 and 3.

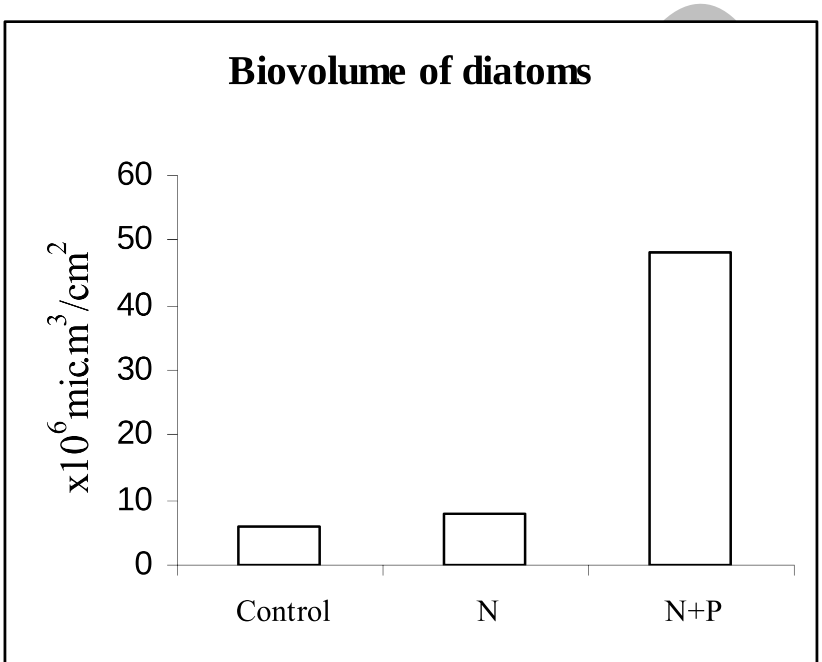

Fig. 4. Diatom biovolume at the artificial streams, control=unenriched, N=addition of N, and (N+P)=addition of both N and P

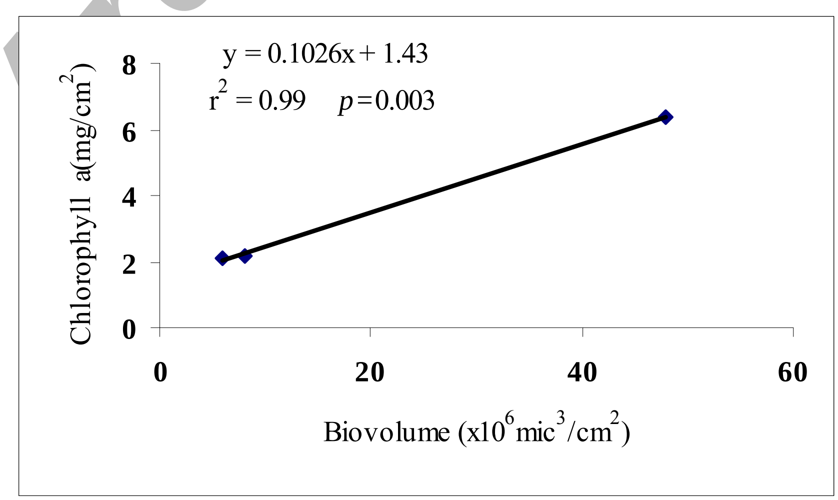

Fig.5. Relationships between chlorophyll *a* and diatom biovolume concentration from the artificial streams

Table 1. Results of single factor analysis of variance (ANVOA) for various characteristics of the periphyton community during 23 days of the experiment

Characteristics	unit	Control	N-enriched	N+P-enriched	F
Dry mass	mg/cm2	0.96	1.4	3.52	****73.31
AFDM	mg/cm2	0.2	0.98	1.04	****32
Chlorophyll a	mg/cm2	2.11	2.2	6.36	**6.72
Biovolume	µm3/cm2	6×10^6	$8x10^6$	$48x10^6$	***176.5
$P > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$					

Table 2. Diatom biovolume (10^6 µm^3/cm^2) in control and the enriched artificial streams, values are means

Diatom	Control	N-enriched	N+P-enriched	F
Navicula	0.07	0.1	0.2	3.46
Achananthes	0.3	0.7	1	3.2
Nitzschia	0	0.7	2	40.1**
Cocconies	0	0.01	0.3	
$P > 0.05$, $^{**}p < 0.01$				

Table 3. Density (10^3 cell/cm^2) of diatoms in control and enriched the artificial streams. Values are means

Diatom	Control	N-enriched	N+P-enriched	F
Navicula	8.8	21.74	57.9	*19
Achananthes	12.59	18.1	35.4	3.3
Nitzschia	00.16	0.24	12.9	****904.9
Cocconies	0	0.89	6.24	*22.72
$P > 0.05$, $^{*}p < 0.05$, $^{****}p < 0.0001$				

The added nutrients (P and N) have affected relative abundance in the diatom community in the artificial streams (Figs. 6 & 7). Total biovolume has increased from $0.37*10^6$ µm^3/cm^2 in control artificial stream to $3.5*10^6$ µm^3/cm^2 in N+P artificial stream. In the control stream, which was filled with water from the head stream of the Gamasiab River, the most abundant species in the control stream is *Achnanthes sp*. However, by enriching the relative abundance of this species decreases to 28 percent in the N+P enriched treatment (Figs. 6 & 7). *Nitzschia* sp and *Cocconies sp* have also been affected by the addition of N and P. These taxa responded significantly to water enrichment. ANOVA results of the effects of nutrients on specific diatom biovolume and density were shown in Tables 2 and 3.

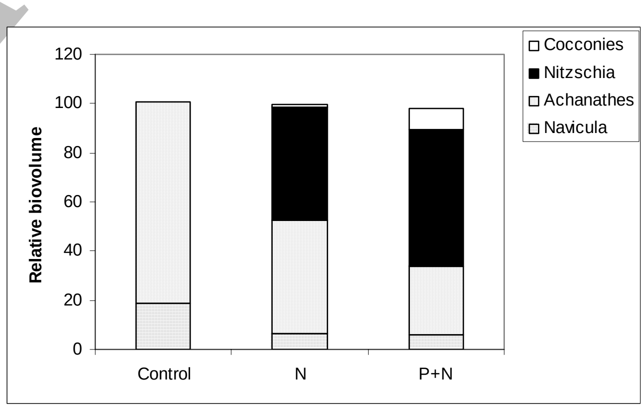

Fig. 6. Effects of nutrients on diatom relative biovolume in artificial streams, control=unenriched water, N=addition of N, and N+P=addition of both N and P

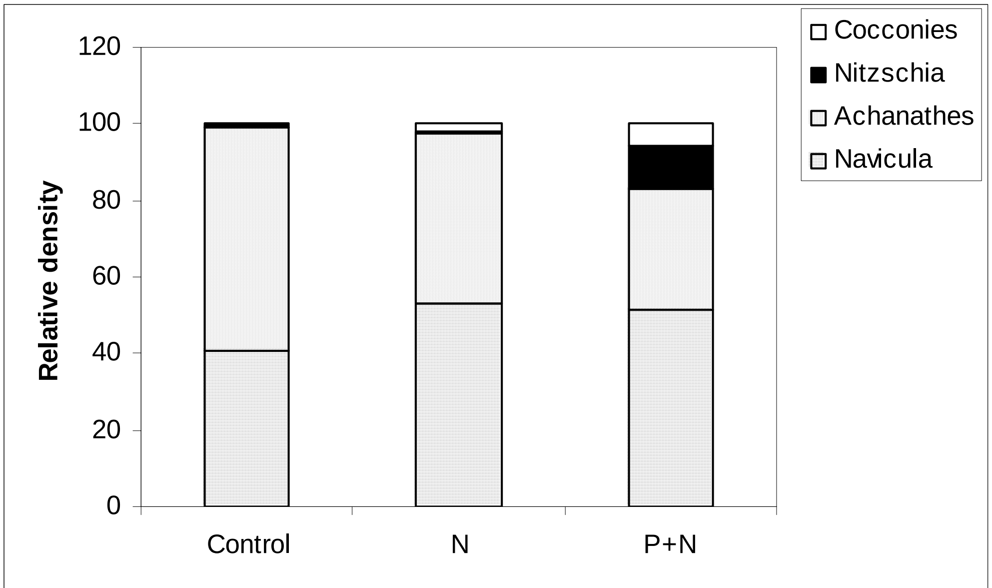

Fig.7. Effects of nutrients on diatom relative density in artificial streams, control=unenriched water, N=addition of N, and N+P=addition of both N and P

4. DISCUSSION

Results obtained from the current experiment indicated that the dry mass of the periphyton community developed in the artificial streams have significantly increased in the added N and N+P treatments (Table. 1). Results also showed that ash-free dry mass (AFDM) was higher in the enriched treatments (Fig. 2). Similar results have been obtained by other investigators. Rosemond et al [21] demonstrated that the greatest values of AFDM observed in the treatment to which N and N+P were added compared to unenriched treatments. These authors have also shown that chlorophyll *a* as an indicator of productivity in the artificial streams has been affected by the addition of nutrients. Also, Hershey et al [16] documented that algal chlorophyll *a* was higher in N+P addition, compared to unenriched treatments. Total biovolume of diatoms was also affected by the addition of nitrates. We showed that the nutrient addition had significant positive effects on diatom biovolume (Fig. 4). Rosemond et al. [21] demonstrated that the addition of N and P increased diatom biovolume, compared to unenriched treatments.

Studies carried out in artificial streams shows that algal biomass often reaches a plateau after considerable periods of time [4], although in most investigations, added nutrient has caused a positive response by the periphyton community. This effect is performed with different strengths across various experimental conditions [22]. The positive effects of nutrients enrichment on the algal biomass obtained in the current experiment were inconsistent with pervious experiments in freshwater productivity [23-25].

A comparison between the amount of bivolume and the chlorophyll *a* content in the periphyton community developed in the artificial streams indicates a strong correlation between these two factors (Fig. 5). The high correlation between biovolume and chlorophyll *a* indicated that chlorophyll *a* can adequately represent the algal productivity in the artificial streams.

The current experiments have shown that the same taxa growing on various nutrient regimes responded differently to water enrichments. Results indicated that the addition of N and P had positive effects on *Achnanthes sp*, *Cocconies sp*, *Navicula sp* and *Nitzschia sp* (Figs. 6 & 7). Results showed that density and biovolume of *Nitzschia* was significantly higher in enriched treatments. Pringle [23] also demonstrated that compared to unenriched treatments *Nitzschia sp* was higher in enriched water treatments. Results of single factor analysis of variance (ANVOA) indicated that the addition of N and P also caused a significant positive effect on biovolume and density of *Achnanthes sp* and *Navicula sp* (Table 2-3). In a similar study performed by Pringle (1990) [23], water enrichment is shown to have an insignificant positive effect on *Achnanthes sp* and *Navicula sp*. Instead, the *Cocconies* showed a significantly greater response to the added nutrient.

Field investigations on the relative abundance of various species of diatoms have been conducted to evaluate changes in species composition, obtained following enrichment experiments with artificial streams. For example, it is well known that *Achnanthes ssp* are dominant in unenriched water. Such results have been obtained by several authors [26-28]. It is also known that these species of diatom have high oxygen requirement, therefore, *Achnanthes sp* abundance has been used as an indicator in assessing water reoxygenation after pollution [29]. *Nitzschia sp* were abundant in our enriched treatments (Figs. 6 & 7). Similar results have been previously obtained [30-31]. *Nitzschia sp* generally reaches greatest abundance in eutrophic water. Also it is shown to be the most pronounced development of heterotrophic capabilities among diatom [32]. Pringle [23] indicated that the motile taxa are much larger than sessile taxa, which may allow them to store large quantities of nutrient. Smaller sessile diatom that are dominant in unenriched treatment (*Achnanthes sp)* appear to be better adapted to exploit relatively low levels of dissolved nutrient in flowing water, possibly by virtue of their surface to volume ratio and growth habits [23].

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