

Limiting Nutrients in the East River, Korea

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The purpose of this study was to identify the potential limiting factors in the East River based on nutrient ratio and nutrient enrichment bioassay. In addition, physicochemical characteristics including heavy metal concentrations of the East River were identified. Water samples were collected from 16 main streams and 7 branches from May to November in 2008, and the heavy metals and nutrients concentration of the water samples were analyzed. Nutrient enrichment bioassay tests were also carried out with them. *Nitzschia pungens* were used in bioassay tests due to their prevalence in the sampling area. This three-season study showed that the ratio of TN/TP was higher than 65, and phosphorus was the limiting factor for algal growth. i_{\max} was observed at concentration of 1 ppm. The chemical analysis of the water samples showed that the concentrations of Fe and Al were much higher than the others. Based on nutrient ratio measured in the stream data and nutrients enrichment bioassay tests, phosphorus (P) was found to be the limiting factor.

Key words: Limiting nutrients, nutrients enrichment bioassay, heavy metal

1. Introduction

The concept of nutrient limitation has been the main issue of eutrophication studies (Smith *et al.*, 1999). The limiting nutrient for phytoplankton growth was the central theme of eutrophication research in 1970s (Jong, 2006). Phytoplankton biomass yield is proportional to the concentration of the limiting nutrient and growth rate is proportional to the rate of supply of growth-limiting nutrient. It is essential that we understand the quantitative correlation between limiting nutrient concentration and the potential algal biomass yield. We can use this correlation to evaluate the risk for the increased algal biomass, and therefore will be able to decide which nutrient should be removed or controlled in the anthropogenic inputs in order to reduce eutrophication impacts (Jie, 2007).

Phosphorus is an element that stimulates the growth of algae in water bodies. Phosphorus compounds are also found in many types of rocks and soils in the

nature. Inorganic phosphorus is commonly considered as the element most likely to limit the primary production in freshwater ecosystems (Hudson *et al.*, 2000).

Like phosphorus, nitrogen is another potential limiting nutrient to algae, bacteria, and fungi found in the biofilms (Tank and Dodds, 2003). In addition to an adequate supply of N, organisms may be limited by the imbalance of available nutrients (e.g., nitrogen: phosphorus ratio). A comprehensive understanding of the factors controlling N limitation and its uptake in the stream ecosystem is necessary to address problems associated with increased N loading (Hauer and Lamberti, 2007). N limitation has been observed in various streams (Edwards *et al.*, 2000, Francoeur 2001). In some cases, an inadequate supply of total nitrogen (TN) in water bodies has been found to limit the growth of free-floating algae (i.e., phytoplankton). The nitrogen limitation occurs most commonly when the ratio of total nitrogen to total phosphorus is less than 10 (UF/IFAS, 2000).

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Silicate is a bio-limiting nutrient and a major constituent of diatoms which use silicate to encase their cells in a wall impregnated with silica (silica shell) (DWAF, 1995). The processes involved in the silicon cycle are fewer than that of nitrogen or phosphorus and they are composed of only river (through drainage of silicate-rich basins) and ocean inputs, through the removal of dissolved silicon by diatoms and then the following dissolution from these cell walls onto the sea bed upon their death and settling (a slow process) (Tett *et al.*, 2003).

The potential roles of iron as an algal growth limiting nutrient in freshwater habitats are less understood. Phosphorus is considered to be the nutrient which limits freshwater algal growth (Schindler 1978). However, this generalization may not be true for all classes of algae. Van Donk (1983) suggested that iron is a growth-limiting nutrient for chrysophytes, and Sangren *et al.* (1995) proposed that this group of algae may be superior competitors for iron in certain nutrient limited environments.

There is no single method to conclusively demonstrate nutrient limitation. Nutrient ratios have been used as a basis for predicting the nutrient limitation for several decades (Jie, 2007). Nitrogen is considered "limiting" when the ratio of total nitrogen to total phosphorus (TN/TP ratio) is less than 10. When the TN/TP ratio is between 10 and 17, there appears to be a gray area (nitrogen or phosphorus could be limiting) and when the TN/TP ratio is greater than 17, the watershed is phosphorus-limited (UF/IFAS, 2000). In the assessment of stoichiometric limitations in the northern Adriatic Sea and in the northern Gulf of Mexico, Justic *et al.* (1995) proposed a new criteria: (a) N limitation occurs when dissolved inorganic nitrogen: phosphorus (DIN/P) ratio < 10 and Si: DIN > 1 ; (b) P limitation occurs when Si: P > 22 and DIN: P > 22 ; (c) Si limitation occurs when Si: P < 10 and Si: DIN < 1 . Ambient nutrient ratios only suggests a potential for the nutrient limitation, not necessarily an actual nutrient limitation since the actual ambient nutrient concentrations may not be low enough to limit phytoplankton growth (Justic *et al.*, 1995). Nutrient enrichment

bioassay is important for a better understanding of the relationship between nutrient concentrations and planktons (Persic *et al.*, 2005, Graneli 1984). Artificial media are different from a natural growth media since they are typically protected from grazing and scour, and could provide nutrient enrichment through passive diffusion (Ludwig *et al.*, 2008).

The purpose of this study were (1) to investigate the physicochemical conditions, and the concentrations of nutrients and heavy metals in the East River and (2) to identify the potential limiting factors in East River based on nutrients ratio and nutrients enrichment bioassay method.

2. Materials and Methods

2.1. The study area

The East River is a part of the Han River system that flows at east side of Korean Peninsula. The river is branched into several streams: Bongsan Stream, Golji Stream, Odae stream, Uh Stream, Youngtan Stream, Dongnam Stream, and Seokhang Stream. Water samples were collected from 16 main streams and 7 branches in May, August, and November 2008 (Fig. 1).

2.2. Physicochemical parameter

Water samples for nutrient analysis were collected at mid-depth in the river or stream and directly poured into 5 L acid-washed amber high density bottles.

The measured parameters were: temperature (T), pH, conductivity, dissolved oxygen (DO), light transmission (LT), suspended solid (SS), total nitrogen (TN), dissolved inorganic nitrogen (DIN), nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), total phosphorus (TP), orthophosphate (PO₄), chlorophyll *a* (Chl *a*), Silica (SiO₂), heavy metals (Fe, Al, Ba, As, Cd, Pb, Ce, Co, Cr, Cu, Mn, V, Zn), and Bioassay test. Sample bottles were then kept on ice before being frozen upon returning to the laboratory.

2.3. Nutrient enrichment bioassays (NEBs)

2.3.1. Pre-treatment

The water samples from the field were first filtered

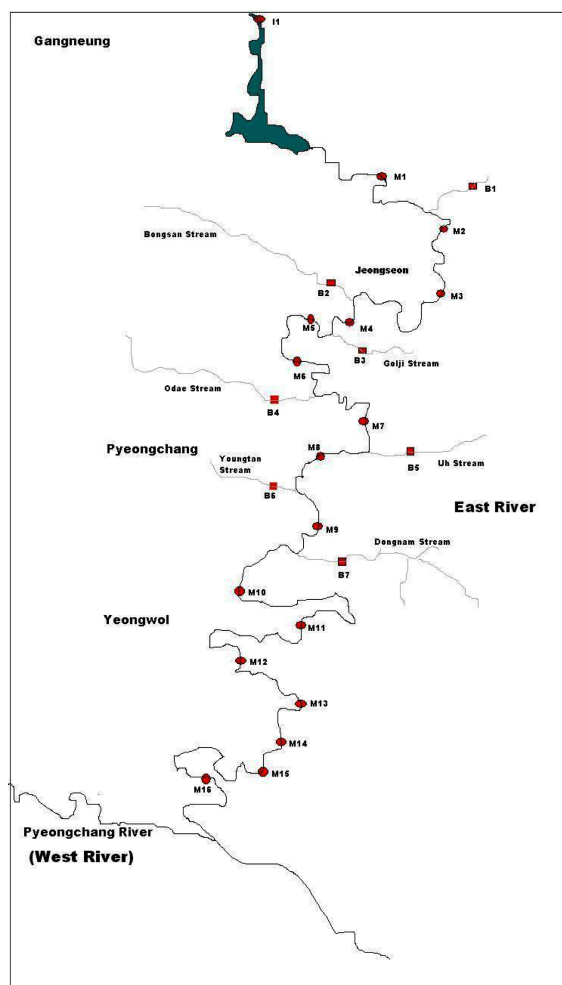


Fig. 1. Map showing the study area.

with filter paper (GF/C Whatman 47 mm), then autoclaved for 15 minutes at 120°C and preserved in the refrigerator. The nutrient concentrations used in the NEB study are 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, and 10 ppm. Nutrients used in this study were NaNO_3 for nitrogen (N), KH_2PO_4 for phosphorous (P), $\text{Na}_2\text{SO}_3 \cdot 9\text{H}_2\text{O}$ for silica (Si), FeCl_3 for iron (Fe), and a standard solution of aluminum (Al). For Algae preparation, *Nitzschia pungens* was used (based on the result from the previous observation, which indicated that the genus was widely found in the sampling area). The algae then was cultured in F/2 media (Guillard, 1975) in a growth chamber SI 2000R at 25°C for 14 hours with and 10 hours without lights until blooming

2.3.2. Bioassay Treatment

The harvested algae were centrifuged at 2500 rpm for 5 minutes. Three repetitions were done with media cleansing using NaHCO_3 . 70 mL of water samples were poured into each 100 mL Erlenmeyer flasks, which contain 0.2 mL of nutrient solution and 1 mL of algae culture (10,000 cells/mL). All Erlenmeyer flasks were incubated at 25°C for 14 hours with lights and 10 hours without lights over 7 days and the chlorophyll concentrations were measured on a daily basis using UV spectrophotometer (Cary 5°Conc, Varian).

A set of algal nutrient bioassay was conducted; the control (no nutrients addition), control + P, +N, +Si, +Fe, +Al, P+Si, N+ Si, Fe+Si, Al+Si, and P+N+Si. The growth rate (μ , day^{-1}) under each set of experiment condition was calculated using APHA equation (APHA, 1995):

$$\mu(\text{day}^{-1}) = \ln(X_2/X_1)/(T_2 - T_1)$$

where, X_1 = the concentration of Chl *a* at the initial incubation stage (T_1 h); X_2 = the concentration T_2 h

Five different algae concentrations were used for this study (0.01, 0.05, 0.1, 0.5, 1, 5, 10 ppm).

Statistical analysis and the comparison of the results were performed with SPSS 13. For each nutrient enrichment experiment, statistically significant difference in treatment effects was determined using one-way ANOVA. If there was a significant response from one or more combination of nutrients, the composite set of the nutrients was considered as limiting factor. Statistical significance was evaluated at the $p < 0.05$ level and performed using SIGMAPLOT 10.

3. Results

3.1. Physicochemical Parameter

The mean of chlorophyll *a* concentration in Spring was quite high (14.65 mg/m^3) with maximum value of 36.30 mg/m^3 and minimum value of 5.90 mg/m^3 . In summer, the mean was 4.89 mg/m^3 with maximum value of 12.98 mg/m^3 and minimum value of 1.54 mg/m^3

while in fall, it was in the same range as in spring with 2.54 mg/m^3 . In summer, chlorophyll *a* showed significant correlation with TP and PO_4 . Pearson value of TP and chlorophyll *a* in spring and summer were 0.526 (with *p* value of 0.020), 0.563 (with *p* value of 0.004), respectively. As for chlorophyll *a* and PO_4 , the significant correlation observed only in summer with Pearson value of 0.601 and *p* value of 0.005.

There were seasonal variations in TP and PO_4 concentrations, which was relatively high in spring and fall but lower in summer. Unlike phosphorous, nitrogen concentration (TN, DIN, NO_2 , and NH_4) was high in summer but lower in spring and fall. Mean of DO concentrations in Spring and Fall was higher than in Summer (10.34 mg/L, 10.66 mg/L and 9.21 mg/L, respectively) while temperatures in Spring and Fall were relatively low (13 to 21°C in Spring and 8 to 12°C in Fall). In summer, the temperatures were $18\text{-}25^\circ\text{C}$.

There was significant correlation between DIN and TP both in spring (Pearson value 0.604, *p* 0.002) and fall (Pearson value 0.455, *p* 0.026).

Mean percent value for light transparency (LT) in this watershed area for three seasons (spring, summer, and fall) were 71% to 85%. The SS values in spring and summer showed a higher concentration when compared to SS in fall (3.01 mg/L, 3.93 mg/L, and 1.92 mg/L, respectively). Silicate concentrations at this watershed area in three seasons were 0.59-4.86 mg/L, 1.47-5.86 mg/L, 0.57-7.30 mg/L, respectively. In fall, there is no significant correlation between chlorophyll *a* with other physicochemical parameters except with the conductivity and SiO_2 concentration.

Mean values of TN/TP ratio in three seasons were high; 301.8, 278.7, and 353.8, respectively. The ratios of Si to TP were 29.5-337, 81.8-293, and 28.5-730, respectively. DIN/P ratios were 37-260, 28-304, and 41.3-490.

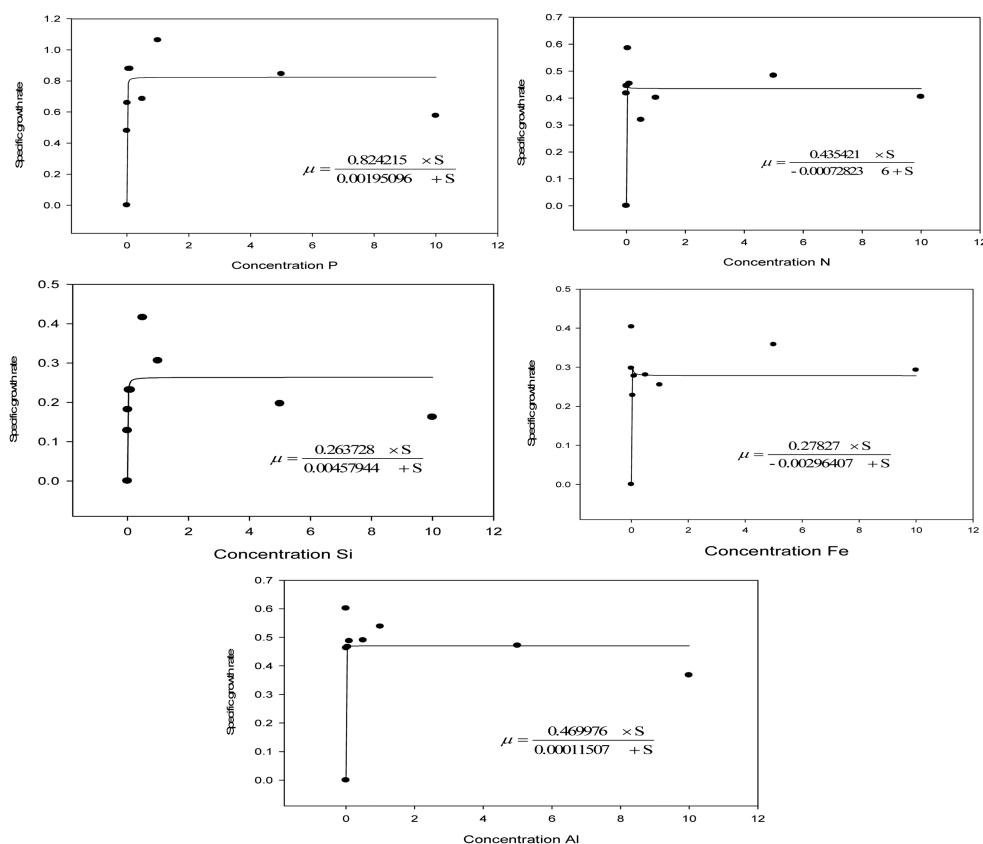


Fig. 2. Specific growth rate of *Nitzschia pungens* treated with P, N, Si, Al and Fe.

Si/ DIN ratio were 0.3-3.7, 0.5-6.7, and 0.26-7.7, respectively. Si/N ratios were 0.06-0.9, 0.2-1.2, and 0.1-1.3, respectively.

3.2. Heavy metals

The mean values of heavy metals in three seasons (spring, summer, and fall) were: Fe (56.94, 63.34, 29.80 mg/L, respectively), Al (75.46, 114.75, 36.38, respectively), and Mn (1.81, 14.53, 3.65, respectively).

Observed concentrations of each metal over the experimental period were below thresholds with iron, aluminum and manganese showing higher concentrations compared to other heavy metals.

3.3. Bioassay treatments and nutrient concentrations ratio

The TN/TP ratio showed values ranging from 86.66-492.3 in spring; 63.8-918.7 in summer, and 39.5-817.6 in

fall. The Si/P ratios were 29.5-337, 81.8-293, and 28.5-730, respectively. Observed ratios of DIN/P were ranging from 37-260, 28-304 and 41.3-490, respectively, while ratios of Si/DIN were ranging from 0.3-3.7, 0.5-6.7, 0.3-7.7, respectively.

Fig. 2 showed the maximum specific growth rates of *N. pungens* under the different growing condition of P, N, Si, Al, and Fe (0.82, 0.44, 0.26, 0.28 and 0.47 mg/L, respectively). Half saturation coefficients were found to be 0.002, 0.001, 0.005, 0.003, and 0.0001, respectively. The maximum specific growth rates of *N. pungens* treated with nutrients combination of P+Si, N+Si, Si+Fe, Al+Si and P+N+Si were presented in Fig. 3. The graphs showed maximum specific growth rates of 0.77, 0.51, 0.56, 0.48, and 0.68, respectively and the half saturation coefficients were 0.005, 0.009, 0.002, 0.001, and 0.001, respectively.

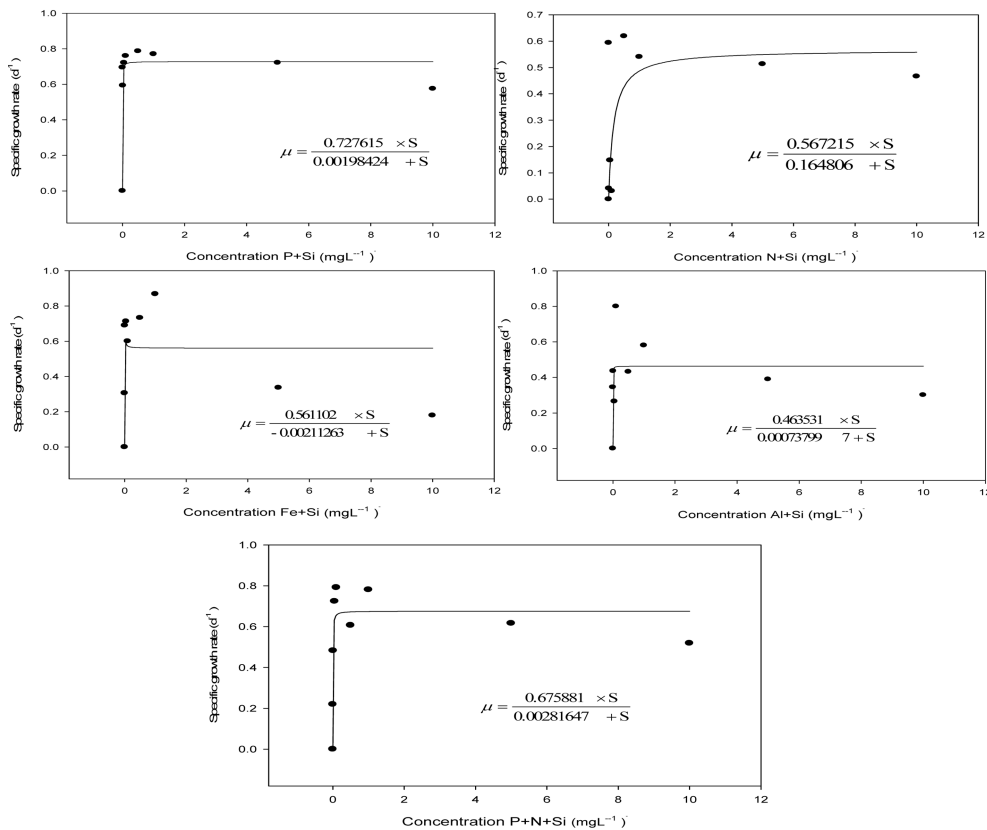


Fig. 3. Specific growth rate of *Nitzschia pungens* treated with composite nutrients of P+Si, N+Si, Si+Fe, Al+Si and P+N+Si.

4. Discussion

4.1. Physicochemical Parameters

Spring and fall are the time when the ambient environment conditions changes. For spring, it is the transition from winter to summer, and for fall it is the transition from summer to winter (Yin, 2002). The nutrient concentrations in spring and summer appear to be relatively high compared to other seasons with the highest concentrations observed in summer. In summer, the amount of rainfall was quite high with an average of 178 mm, while in Spring and Fall, the average rainfall were 58.7 mm and 21.3 mm, respectively (KMA, 2009). Therefore, it is possible that the additional amount of nutrient was introduced into the watershed along with rainfall runoff. During the period of the highest rainfall, the freshwater carries large nutrient loads originated from anthropogenic and natural sources (Jie, 2007). Garratt (2006) stated that in river systems, the concentration of solutes and suspended particles in the water typically increases from source to mouth. Sudden local increases in dissolved substances and suspended particles can occur after heavy rainfall. Fast-flowing runoff from the surrounding land and increased flow and turbulence in the river, produce a pulse of dissolved solutes and suspended sediment and detritus.

In spring where the transition between the wet and dry season occurs, the river overflow began to intrude into watershed, but mean values of NO_3 and SiO_2 were still low (1.82 mg/L and 1.79 mg/L). Mean DO value was 10.34 mg/L, which indicated that the oxygen concentration in water was quite high. The temperature began to rise with the range 13-21°C. Light penetration in the river waters were quite adequate for algal photosynthesis (mean value was 81.79%). Meanwhile, the mean value of SS (3.01 mg/L) was still found to be in 'very good' category of healthy stream ecosystem classification of the Ministry of Environment of South Korea. With additional inputs of the nutrients and other particles into the waters, the SS concentration was also increased. In this season, the biomass of algae began to increase because of favorable environmental conditions

for the algae growth.

In summer, chlorophyll *a* concentrations showed significant correlation with TP and PO_4 in the watershed. Pearson value of TP and chlorophyll *a* in spring and summer were 0.526 (with *p* value of 0.020) and 0.563 (with *p* value 0.004), respectively. As for chlorophyll *a* and PO_4 , the significant correlation was observed only in summer with Pearson value of 0.601 and *p* value of 0.005. Results suggested that the increasing concentration of chlorophyll *a* in this watershed was influenced by the presence of phosphorous. Although the amount of phosphorous was smaller compared to nitrogen, the concentration of nitrogen in the waters was higher than that of phosphorous in water. This situation is consistent with Stanley *et al.* (1990) who stated that rapid increases in chlorophyll *a* occurred only when ambient nutrient level was low. Results of conductivity measurements in summer also indicated a negative significant correlation with TP (Pearson value 0.426, *p* value 0.038). This is in agreement with a statement made by Biggs (1996). Conductivity reflects the concentrations of macro-ions in water and therefore the nutrients dissolved from bedrock are assumed to be increasing proportionally with increases in total ions. In summer, the dissolved nutrient concentrations were low because of high algae growth, and it resulted in low conductivity values in these rivers (May *et al.*, 1997). Similarly, the result showed a decrease in DO (mean value of 9.21 mg/L) in this season, compared to the one in spring (mean DO value of 10.34 mg/L).

From the statistical results, it appears that there were significant correlation between DIN and TP both in spring (Pearson value 0.604, *p* 0.002) and fall (Pearson value 0.455, *p* 0.026). Ludwig *et al.* (2008) stated that an increase in agricultural activity around the waters ambient will increase DIN and TP concentration inputs into the rivers. In summer, no significant correlation between DIN and TP was observed due the dilution factor from the heavy rainfall during this period.

According to Fujiki *et al.* (2004), the available P in the system was quickly used up by phytoplankton with excessive N and Si supplied from river outflow. Maiestrini *et al.* (1997) also stated that the rate of

recycling varies with the nature of different elements: for example, phosphorus is faster than nitrogen and silicon. This result is maybe due to the fact that the nitrogen concentration is higher in waters ambient compared to that of phosphorous, due to more phosphorous was consumed by phytoplankton and other organisms.

In fall which was the transition from wet to dry season, the temperature in watershed was low (8–12°C), and the percentage of light in water was also decreased to 71.31%. The average rainfall was low at 21.3 mm (KMA, 2009), causing reduced volume of water in the river. In fall, there is no significant correlation between chlorophyll *a* with other physicochemical parameters except with the conductivity and SiO₂ concentration. The negative correlation between conductivity and chlorophyll *a* in fall may be due to the fact that, ion concentration of macro-nutrients has been reduced by the algae consumption. Positive correlation between the chlorophyll *a* and silicon concentration may relate to the existence of the periphyton, which was abundantly found in the lotic waters. According to Stanley *et al.*, (1990), lotic waters are usually dominated by periphyton. SiO₂ is usually consumed by the periphyton to form the cell wall. In addition, Hauer and Lamberti (2007) reported that diatoms cell walls derived their components from SiO₂ and composed of two overlapping halves. While SiO₂ was used up for algae survival, some was still left in the water. It is because silicon is confined longer since the periphyton frustule recycles silicon much slower.

4.2. Heavy metal in water

The heavy metal concentrations in water column during three seasons were still below threshold. However, iron (Fe), aluminum (Al) and manganese (Mn) showed a higher concentration compared to other heavy metals in each season.

Iron and Manganese are common metallic elements found in the earth's crust. Water percolating through soil and rock can dissolve minerals containing iron and manganese and hold them in solution (Dvorak *et al.*, 2007). Manganese is an essential micronutrient for

plants and animals. It is a functional component of nitrate assimilation and an essential catalyst of numerous enzyme systems in animals, plants and bacteria. Most iron in oxygenated waters occurs as ferric hydroxide in particulate and colloidal form and as complexes with organic, especially humic compounds. Ferric salts are insoluble in oxygenated waters, and hence, iron concentrations are usually low in the water column (Anon, 1996). The impact of freshwater on aluminum concentration depends on several factors inherent to the chemical composition of the water, physicochemical properties and other conditions such as temperature, inorganic salt, and flow speed (Vargel, 2004). The average concentrations of three heavy metals (Fe, Al and Mn) were still in accordance with drinking water standard, except aluminum in summer. In addition, compared to the EPA drinking water standard, observed Fe and Mg concentrations were in compliance with the standards (<0.3 mg/L and <0.05 mg/L respectively), while Al exceeded the standard (>0.05 mg/L). In summer, all the three heavy metals showed an increase in concentration compared to other seasons due to the increase of water volume and the relatively heavy rainfall. Heavy rainfall, mining activities runoff (point source pollution) and farming activities runoff (non point source pollution) may exceed the capacity of the sewer systems, causing them to overflow and contaminate surface waters nearby. River bank erosion occurs due to moderately high flow velocities at peak times of discharge (Ma, 2005).

The sampling point was located through the Jeongson and Yeongwol area, where there are many ex-mining sites that have been closed. According to Jeongseon government data; there were 23 ex-mining companies, while there was 1 in Yeongwol area. The mining activities from the past operation are considered as possible source of heavy metal contaminants. As Dudka and Adriano (1997) stated, the existence of mining process (metal mining, smelting and other mining) will introduce metal contaminants into the environment. Although mining operations were closed in both cities a few years ago, the impact of mining activities, even the past ones, could still affect the ecosystem. Released

heavy metal from the mining sites could be transported from contaminated soil to the river (Selim and Sparks, 2001).

In addition, there are many farms in both of the sampling areas. From total areas, 9% is used as a farmland, 85% forest, 6% for other activities (Jeonseon and Yeongwol government, 2004). Farming activities are commonly carried out around watershed ambient. Allegedly, the main source of three heavy metal contaminants (Fe, Al, and Mg) derived from this non-point source (farming activities). The major sources of pollution from agriculture are fertilizers containing superabundant nutrients such as nitrogen, phosphorus, and heavy metals (Withers & Lord, 2002). Fertilizers are mixtures of inorganic salts, which certainly can bind Al (Vargel, 2004), Fe, and Mn, with variable proportion of salts of three base elements, N, P, and K. P element adsorbed by the soil fraction is most susceptible to erosion (clays, oxides of Fe and Al) and it is important to reduce soil erosion to control particulate P losses. Phosphorus often accumulates in the upper few centimeters of the soil, particularly under minimum tillage conditions where manures and fertilizers are not incorporated. Hence, dissolved phosphate levels can also be high in the upper few centimeters of soil since they are most interactive with surface runoff. (Ritter and Shirmohammadi, 2001).

When animal manures and fertilizers are applied at rates to meet the crop's N requirements, P levels can build up rapidly in the soil. Increased nutrient demand will certainly be followed by the increasing level of three heavy metals (Fe, Al, and Mg), and will increase the heavy metals concentration in water body with nutrients washed by rainfall from the soil. In the farmland area, high nutrient run-off occurs from the various agricultural practices and eventually washes into the river (Haskins *et al.*, 2000). If it is not managed carefully in the future, the water quality of the river may be altered and would endanger the entire ecosystem and indirectly affect the human life.

4.3. Nutrient ratio and Nutrient enrichment bioassay

Changes in anthropogenic nutrient loading to aquatic ecosystems will likely affect the structure and function of native phytoplankton communities (Piehler *et al.*, 2002). Some effects are easier to predict (e.g. higher rates of primary productivity following the addition of a limiting nutrient), while others such as the community composition shifts and the changes in rates of specific processes (e.g. N_2 fixation) are more difficult to predict. Management strategies designed to ameliorate previous anthropogenic water quality decline must consider unanticipated effects.

The concept of nutrient limitation is not easy to define clearly. There is an important difference in the concept between the limitation of biomass production and of growth rate. The limitation of biomass has been termed "ecological" and depends on the concentration of the limiting nutrient which dictates the final yield of algal biomass. The limitation of growth rate has been termed "physiological" and depends on the supply rate of the limiting nutrient which dictates the growth rates (Paasche and Erga, 1988). The amount of biomass produced may be limited by the ambient nutrient concentrations even if growth rates of phytoplankton are not (Howarth, 1988). According to the University of Florida, his *Beginner's Guide to Water Management Nutrients* (2000), the concept of limiting nutrients is a chemical necessary for plant growth, but is available in smaller quantities than needed for algae growth.

Based on physicochemical parameters results from this study, dissolved phosphorus (PO_4) from most of the sampling was not detected because of its very low concentration in water samples. Due to the low concentration of dissolved phosphorus and dissolved nitrogen, many scientists have used total P and total N as the parameter to estimate the number of P and N in a system (UF, 2000). In the results of physicochemical analysis of the water ambient in the three seasons, TP levels (0.01 to 0.07 mg/L) were a lot lower than TN levels (2.08 to 19.48 mg/L), which suggested that TP was a limiting nutrient in water ambient for the algae growth. This observation agrees with Jie (2007),

implying that a nutrient available in an extreme lower level compared to other nutrients is considered as the limiting nutrient.

According to Fujiki *et al.* (2004), the results of nutrient concentration and ratios suggested P to be a limiting nutrient, if $\text{PO}_4 < 0.2$; $\text{Si/P} > 20$; $\text{N/P} > 20$. University of Florida Guideline stated that the watershed is considered N-limited if TN/TP ratio less than 10, N- or P-limited when the ratio is 10-17, and P-limited when the ratio is greater than 17. Based on University of Florida standard, TN/TP ratio results in this study suggested that P was the limiting nutrient for this water ambient. According to Dzalowski *et al.* (2005), N limited had water column with TN/TP ratios < 18 ; reservoirs that were co-limited by N and P had water column with TN/TP ratios between 20 and 46; and reservoirs that were P limited had water column with TN/TP ratios > 65 . A little different criterion was proposed by Justic *et al.* (1995), in which it stated the system is in: (a) N limitation if $\text{DIN/P} < 10$ and $\text{Si/DIN} > 1$; (b) P limitation if $\text{Si/P} > 22$ and $\text{DIN/P} > 22$; (c) Si limitation if $\text{Si/P} < 10$ and $\text{Si/DIN} < 1$. Based on all the references mentioned above, this study concluded that P was the limiting nutrient in this water environment with Si/P and DIN/P ratios were above 22 in all three seasons.

Practical aspects of the nutrient enrichment effects studies were generally focused on the total biomass of phytoplankton produced, rather than on the production of any single species. Consequently, the relevant questions are: (1) Can the growth of the natural phytoplankton community be increased (or decreased) significantly by the addition (or deletion) of one or more nutrients? and (2) Which nutrient has the greatest effect? These questions have focused their interests on aggregate variables such as primary production and algal biomass (or a surrogate such as chlorophyll concentration) and their response to nutrient enrichment (Hecky and Kilham, 1988).

Bioassay in this study consisted of two parts; the first was the addition of single nutrient-heavy metal (Al and Fe). The second was the addition of nutrient-heavy metal + silicon (Si). Results from both experiments

showed decrease of algae biomass in nutrient-heavy metal addition above 1 ppm. Bothwell (1989) reported that the rapid increases in *chlorophyll a* occurred only when ambient nutrient levels were very low and increases in *chlorophyll a* were more moderate when ambient nutrient levels were higher. From the results, at concentrations above 1 ppm, the inhibition of algae growth rate is verified by the decrease in biomass, which is observed as the decreases of chlorophyll a concentration. In addition, Richmond (2004) stated that nutrients are often limiting and the oversupply concentration may lead to stress and reduced growth.

Fig. 3 showed that the growth rates of alga biomass were higher compared to treatment without the presence of phosphorus (P). Although other nutrients presented in the mixture, the biggest effect was observed when phosphorus was added to the nutrient solution. In addition, the statistical analysis using one way ANOVA also indicated significant changes indicating the effects of the phosphorus various levels.

High biomass may result from high production or mechanical aggregation of the phytoplankton, while low biomass may be due to low production or mechanical dispersion of the biomass or grazing (Ragueneau *et al.*, 1996).

The results from both methods in this research suggested phosphorous as a potential limiting nutrient. Predictions from the nutrient ratios appeared to be in agreement with the results from the nutrient enrichment bioassays, in which the growth rate is limited by P nutrient. Nitrogen has traditionally been viewed as the nutrient limiting productivity in coastal waters (Oviatt *et al.*, 1995). Recently, P limitation was often observed in river-influenced coastal areas during periods of high river runoff with high N/P loading ratios (Horrison *et al.*, 1990). This report agrees with Scheffer (2001), who stated that the primary production of fresh water systems is mainly phosphorous limited, while nitrogen is less often reported as a limiting nutrient. Hence, potential P limitation is always a possibility in the freshwater-influenced by low salinity at the surface, where thermohaline stratification occurs in the water column, especially during the summer wet season (Jie,

2007).

Nutrients enter the water body through a natural process and also through human activities in the area around the water bodies, especially the agricultural activities. University of Florida Guideline (2000) also stated that the P, N and other nutrients can enter water bodies inadvertently as the results of human activities (landscape fertilization, crop fertilization, wastewater disposal, and storm water runoff from residential developments and commercial areas). However, further research is needed to identify the nutrient source entering the water body that contributed the most in the assessment of the limiting nutrients.

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References

1. Anon. b (1996) South African water quality guidelines. 2nd Edition, Domestic Use 1, Department of Water Affairs and Forestry, South African.
2. Biggs, B. J. F. 1996. Patterns in benthic algae of streams. In: *Algal Ecology*. Stevenson, J., M. L. Bothwell, and R. L. Lowe (eds.). Academic Press, San Diego, CA. pp. 31-51.
3. Department Of Water Affairs and Forestry .1995. *South African Water Qualit Guidelines forMarine Coastal Waters*. Vol. 1: the Natural environment.
4. Dudka, S. and D.C. Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. *J. Environ. Qual.* 26:590-602.
5. Dvorak, B. I; Prasai, G.; Skipton, S.O.; Woldt, W. 2007. Drinking water :Iron and Manganese. University of Nebraska-Lincoln Extension. US.
6. Dzialowski, A. R., S.H Wang; N. C Lim; W. W. Spotts and D. G.Huggins. 2005. Nutrient limitation of phytoplankton growth in central plains reservoirs, USA . *Journal of Plankton Research* 2005 27(6):587-595
7. Edwards, A.C., Twist, H., Codd, G.A.2000. Assessing the impact of terrestrially derived phosphorus on flowing water systems. *Journal of Environmental Quality* 29:117-124.
8. EPA. 2000. Nutrient Criteria Technical Guidance Manual Rivers and Streams. United State Environment Protection Agency. Washington DC.
9. Francoeur, S.N .2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society* 20:358-368.
10. Fujiki,T., Toda, T., Kikuchi, T., Aono, H., Taguchi, S. (2004). Phosphorus limitation of primary productivity during the spring-summer blooms in Sagami Bay, Japan. *Marine Ecology Progress Series*. Vol. 283: 29-38.
11. Garratt, R. 2006. Lake and River. Chelsea House Publishers. New York. pp : 21
12. Haskins CA, van Driel D & Siebritz R. 2000. An assessment of the condition of the Bottelaty River based on SASS4 and IHAS methodologies. Scientific Services, Cape Metropolitan Council
13. Hauer, FR and G A. Lamberti. 2007. Methods in Stream Ecology. Academic Press is an imprint of Elsevier. USA.
14. Hecky, R. E and Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environment: A riviewof recent evidence on the effects of enrichment. *Limnol. Oceanogr.*, 33 : 796 - 822.
15. Harrison PJ, Hu MH, Yang YP, LU, X. 1990. Phosphate limitation in estuarine and coastal waters of China. *J Exp Mar Biol Ecol* 140:7987
16. Howarth RW (1988) Nutrient limitation of net primary production in marine systems. *Anu Rev Ecol* 19: 89-110.
17. Hudson, J. J., W. D. Taylor, and D. W. Schindler. 2000. Phosphate concentrations in lakes. *Nature* 406:54-56
18. <http://web.kma.go.kr/eng/index.jsp>. 2009. Korean meteorological
19. Jie, XU. (2007). Nutrient Limitation in the Pearl River Estuary, Hong Kong waters and Adjacent South China sea waters. Thesis. The Hong Kong University of Science and Technology. Jong FD (2006) Marine Eutrophication in perspective. Springer, p 88-94.
20. Justic D, Rabalais NN, Turner RE, Dortch Q . 1995. Change in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. *Estuar Coast Shelf Sci* 40:339-356.
21. Ludwig, A., Matlock, M., Haggard, B. E., Cummings, E. 2008. Identification and evaluation of nutrient limitation on periphyton growth in head water streams in the Pawnee Nation, Oklahoma. *Science Direct* 32:178 - 186
22. Ma, Y. 2005. Monitoring of Heavy Metals in the Bottelary River Using *Typha capensis* and *Phragmites*

- australis. Thesis. University of the Western Cape.
23. May, C. W., E. B. Welch, R. R. Horner, J. R. Karr, and B. W. Mar. 1997. *Quality Indices for Urbanization Effects in Puget Sound Lowland Streams*. Department of Civil Engineering, University of Washington, Water Res. Series Tech. Rep. No. 154.
 24. Oviatt C, Doering P, Nowicki B, Reed L, Cole J, Frithsen J. 1995. An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. *Mar Ecol Prog Ser* 116: 171-179.
 25. Paasche E, Erga SR (1988) Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway). *Sarsia* 73: 229-243.
 26. Persic, V., J. Horvatic & M. Mihaljevic, 2005. Bioassay method in the trophic evaluation of wetland area-a case study in the Danubian region (1426 -1388 r. KM). *Periodicum Biologorum* 107: 299 - 304
 27. Piehler, M.F., Dyble, J., Moisaner, P. H., Pinckney, L. J., Paerl, H. W. 2002. Effects of modified nutrient concentration and ratios on the structure and function of native phytoplankton community in the Neuse River Estuary, North Carolina, USA. *Aquatic Ecology* 36: 371 -385.
 28. Ragueneau O, Quéguiner B, Tréguer P 1996. Contrast in Biological Responses to tidally-induced vertical mixing for two macrotidal ecosystems of western Europe. *Coast Estuar Shelf Sci* 42: 645-665.
 29. Richmond, A. 2004. *Handbook of Micro algal Culture : Biotechnology and Applied Phycology*. Blackwell Science, Australia. Sandgren, C.D.. 1988. Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Carnbridge
 30. Schindler, D.W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnol. Oceanogr.* 23: 478-486.
 31. Smith VH, Tilman GD, Nekola JC (1999). Eutrophication: impacts of excess nutrient input on freshwater marine and terrestrial ecosystems. *Environ Poll* 100: 179-196.
 32. Stanley, H.E., Short, A. R., Harrison, W. J., Hall, R. Weidenfeld, C.R., 1990. Variation in nutrient limitation of lotic and lentic algal communities in a Texas (USA) river. *Hydrobiologia* 206: 61-71.
 33. Tank, J.L and Dodds, W.K. 2003. Nutrient limitation of epilithic and epiphytic biofilms in ten North American streams. *Freshwater Biology* 48:1031-1049.
 34. Tett, P, Hydes, D., and Sanders, R. 2003 Influence of nutrient biogeochemistry on the ecology of northwest European shelf seas. In: Black KD and Shimmield GB (eds.) *Biogeochemistry of Marine systems*. Blackwell Publishing Ltd. pp. 293-363.
 35. UF/IFAS. 2000. *A Beginner's Guide to Water Management Nutrients*. Department of Fisheries and Aquatic Sciences University of Florida. USA.
 36. Van Donk. E., Veen, A., and J. Ringelberg. 1983. Natural community bioassays to determine the abiotic factors that control phytoplankton growth and succession. *Freshwater Biology*. 20: 199-210.
 37. Vargel, C. 2004. *Corrosion of Aluminium*. Elsevier, Netherland.
 38. Withers PJA & Lord EI .2002. Agricultural nutrient inputs to rivers and groundwaters in the UK: policy, environmental management and research needs. *The Science of the Total Environment* 282-283: pp 9-24.
 39. Yin, K. 2002 Monsoonal influence on seasonal variations in nutrients and phytoplankton biomass in coastal waters of Hong Kong in the vicinity of the Pearl River estuary. *Mar Ecol Prog Ser* 245: 111-122.