

Prevention of Harmful Algal Blooms by Control of Growth Parameters

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Abstract

Harmful Algal Blooms (HAB) were investigated to prevent the outbreak of HAB in both freshwater and seawater. Parameters inducing HAB were volcanic eruption, sunlight, aeolian dust, environmental factors (current, pH, dissolved oxygen, food web, turbulence, growth phase), enzyme, iron, nutrients (carbon, nitrogen, phosphorus, sulfur, silicon, minerals) while the critical growth parameter for the outbreak of HAB was iron (Fe). HAB development was halted in freshwater and seawater due to the sulfur compounds (H₂S, sulfates) inducing the deficiency of the dissolved Fe in the water. The atomic ratio of N/P is commonly known to be 16/1 in freshwater and 176/1 in seawater for HAB. Therefore, nitrogen can be a relatively limiting factor in seawater while phosphorus in freshwater. HAB could be prevented by control of growth parameters such as pH, temperature, sunlight, turbulence, nitrogen, phosphorus, iron, and sulfur compounds prior to reaching the early exponential phase of algal growth.

Keywords

Prevention, Harmful Algal Blooms, Control, Growth, Parameters

1. Introduction

Harmful algae have been the subjects of scientific and societal interest for centuries. There are harmful algal blooms (HAB) in both freshwater and seawater. This is because blooms of toxic dinoflagellates, which are known as “red tides”, cause a variety of deleterious effects on aquatic ecosystems. These include negative aesthetic effects such as beach fouling, oxygen deficiency, clogging of fish gills, or poisoning of various organisms [1], as observed recently in Florida [2]. Red tides of *Chattonella* causing massive fish kills have been recorded in Japan, China, USA (Florida), and South Australia. *Chattonella* spp. has also been observed

in Southeast Asia, New Zealand, Brazil, and Europe (North Sea). Red tides of *H. akashiwo* accompanied by fish kills of salmon and yellowtail have occurred in Japan, Canada (British Columbia), New Zealand, Chile, and Scotland. The mechanism by which *Chattonella* spp. kill fish remains unclear, but suffocation due to gill tissue damage was the ultimate cause of fish death [3]. Kim [4] postulated that HAB occur only if the environmental factors such as light, nutrients, calm water surface layer, temperature, and pH could be simultaneously satisfied with the requirements of the mineral ions supplied by the Asian dust as enzymatic cofactors for the rapid bio-synthesis of the macromolecules during HAB within limited area. The present study examined the prevention of HAB by control of growth parameters including the iron (Fe) in global aeolian dust and water as the key initiator for HAB while sulfur compounds (S) (H₂S, sulfates) induced Fe deficiency in the water due to a chemical reaction as sedimentary insoluble FeS/FeS₂ for prevention of HAB.

2. Experiment

2.1. Distribution of Fe and Chlorophyll-*a* in Freshwater

Samples of the Asian dust were collected at Anmyon Island (36°34'3"N, 126°19'45.6"E) nearby Seoul in South Korea by an air pollution monitoring equipment (Tisch Environmental Inc.). **Figure 1** showed the weekly distribution of Fe concentration in the Asian dust (red color) and chlorophyll-*a* in Daechung Lake (blue color) in South Korea from January 2006 to December 2012. Climate condition was clear along the west coast of the Korean Peninsula. Iron was measured by ICP at Korean Basic Science Support Center while chlorophyll-*a* was determined by method of the standard process test for water contamination. The mean lag times each year between the peak of the Fe concentration and the peak of the chlorophyll-*a* concentration were shown as follows: 2006 (1.7), 2007 (2.1), 2008 (1.3), 2009 (2), 2010 (2.1), 2011 (0.7), 2012 (1). The average mean lag time was 1.6 weeks or 11 days. Thus, it was observed that the concentration of chlorophyll-*a* reached the peak value after Fe supply via Asian dust with a lag time of about 11 days.

The cumulative concentration of Fe in the Asian dust was determined each year by the integration between weekly time intervals defined as below.

$$\begin{aligned}(\text{Fe})_{11} &= (\text{Fe})_1 \\ (\text{Fe})_{12} &= (\text{Fe})_1 + (\text{Fe})_2 \\ (\text{Fe})_{13} &= (\text{Fe})_1 + (\text{Fe})_2 + (\text{Fe})_3 \\ &\dots \\ (\text{Fe})_{1i} &= \sum_{i=1}^n (\text{Fe})_i\end{aligned}$$

where *i* represents the corresponding week. The resultant cumulative concentration of Fe, (Fe)_{1i}, was linearly regressed with respect to the weekly time of "i" each year.

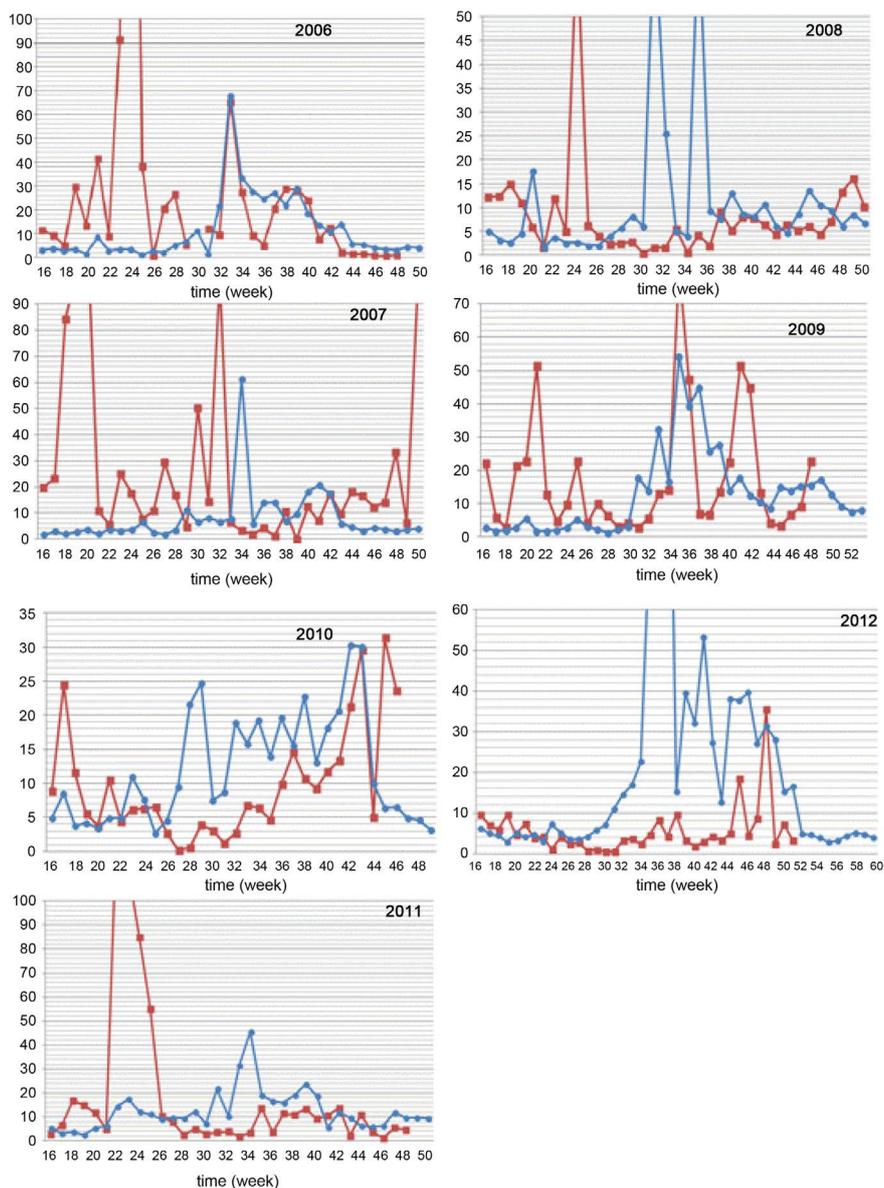


Figure 1. The weekly distribution of Fe concentration of the Asian dust ($\mu\text{g}/\text{m}^3$) (—■—) on Anmyon Island and chlorophyll-*a* ($10\mu\text{g}/\text{l}$) (—●—) in the Daechung Lake of South Korea from 2006 to 2012.

Since algae can not grow without Fe, the correlations between the cumulative Fe concentration slope (x) with respect to either the highest concentration of chlorophyll-*a* (**Figure 2(a)**) or the highest *Cochlonidium* concentration (y) (**Figure 2(b)**) were determined without intercept in **Figure 2**. The resultant correlations were, $y = 26.095x$ with $R^2 = 0.677$ and $p\text{-value} = 0.0239$ for chlorophyll-*a* in the freshwater, and $y = 11,511x$ with $R^2 = 0.389$ and $p\text{-value} = 0.1375$ for *Cochlonidium polykrikoides* in the seawater.

Since the highest concentrations of HAB in the freshwater (**Figure 2(a)**) and in the seawater (**Figure 2(a)**) were proportional to the cumulative Fe concentration in **Figure 2**, it could be possible to predict the outbreak of HAB in both

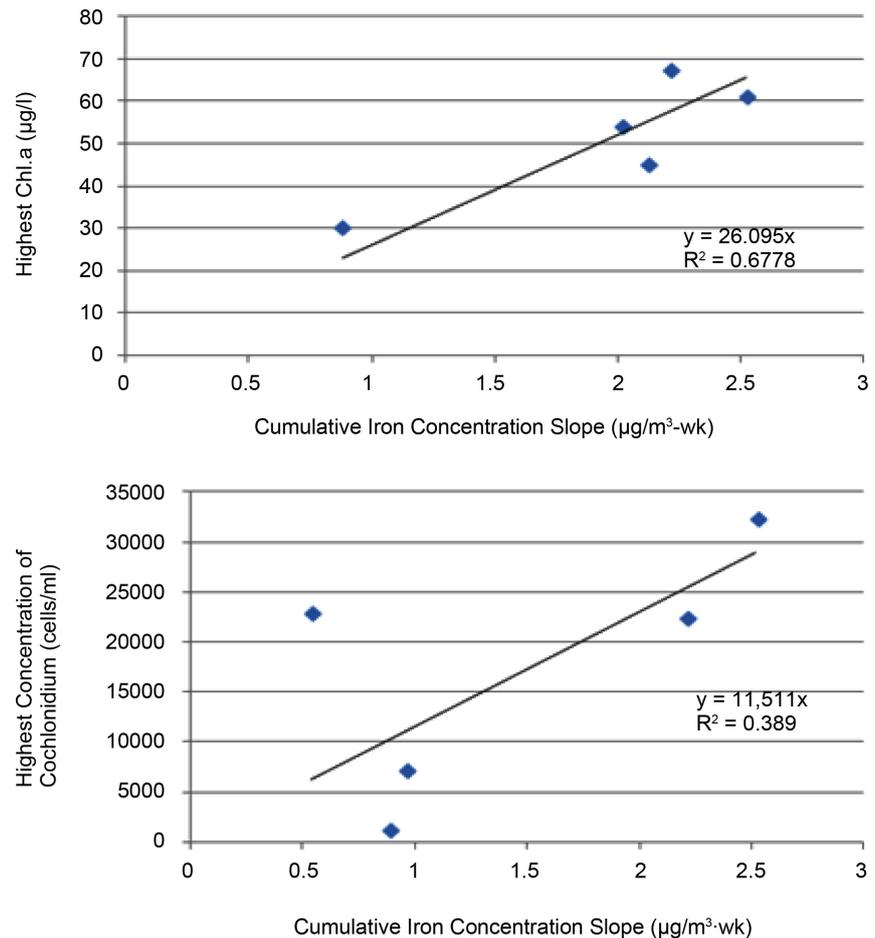


Figure 2. The relationships of the cumulative Fe concentration slope ($\mu\text{g}/\text{m}^3 \text{ wk}$) with (a) the highest chlorophyll-a concentration ($\mu\text{g}/\text{l}$) and (b) the highest *Cochlonidium polykrikoides* (cells/ml) from 2006 to 2012 in South Korea.

freshwater and seawater so long as the weekly distribution of Fe concentration in the desert dust was available in advance.

2.2. Determination of On-Line Cell Concentration of *Cochlonidium polykrikoides*

Indonesia is a good reservoir for the growth of *Cochlonidium polykrikoides*, as shown in **Figure 3**, due to the following reasons;

- 1) Many volcanoes (127) to supply nutrients during volcanic eruptions.
- 2) Strong solar radiation energy at 300 nm (**Figure 4**) near Equator.
- 3) Many islands (18,000) to grow at each seashore.
- 4) Indonesian Throughflow during monsoon (June, July, August) with fast currents of 8 knots (4.1 m/s) for food-webs.
- 5) Wind driven supply of enriched Fe (7% - 18%) desert dust from Australia for the growth of HAB.

Therefore, Indonesia is a good starting point for the warm Kuroshio Current which contains *Cochlonidium polykrikoides* that is driven to fish farmeries in

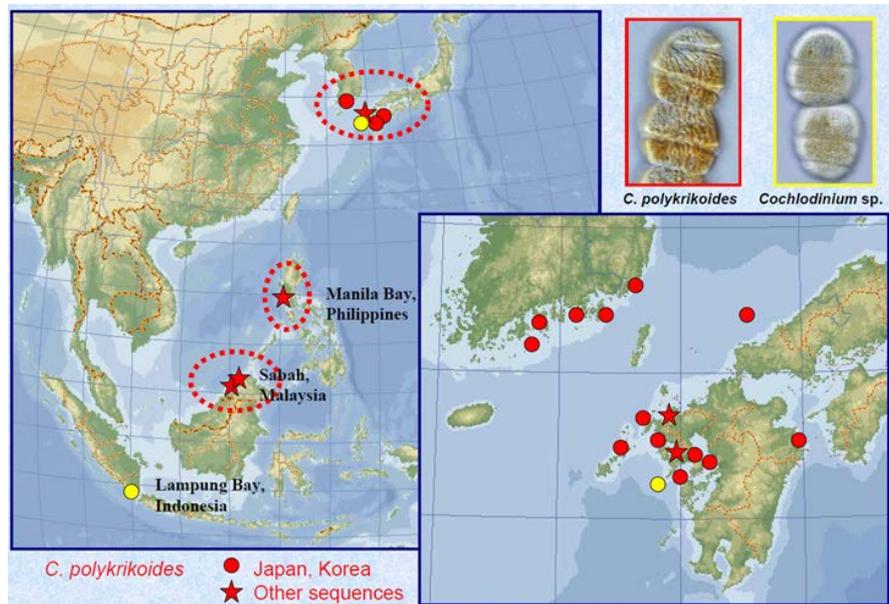
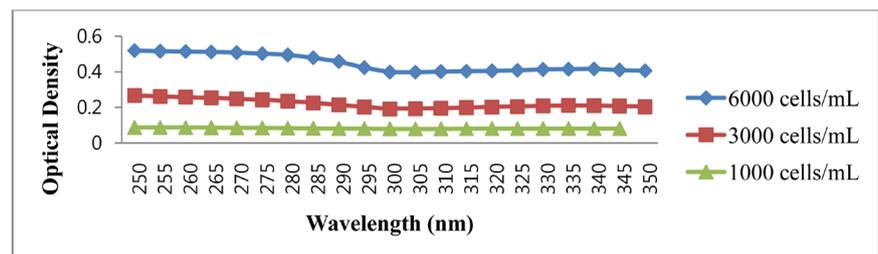
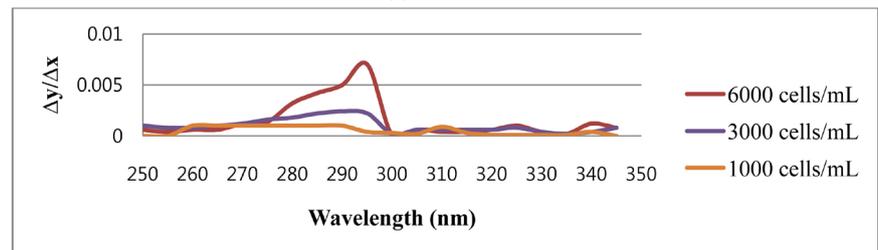


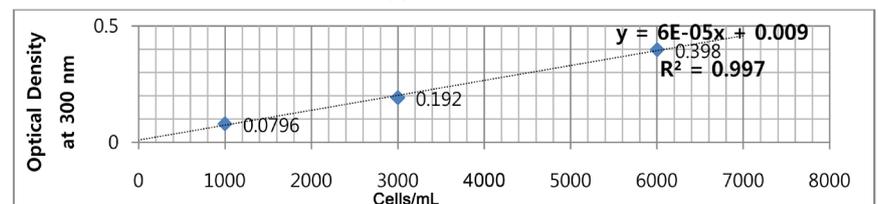
Figure 3. Distribution of *C. polykrikoides* population from Indonesia to Korea and Japan by Iwataki [5].



(a)



(b)



(c)

Figure 4. (a) Scanning data (250 - 350 nm) for *Cochlonidium polykrikoides* selected from Suh [6]. (b) The optimal optical density for the measurement of *Cochlonidium polykrikoides* was determined at 300 nm based on the first derivatives of individual scanings at 1000, 3000, 6000 cells/ml. (c) Distribution of the cell concentrations of *Cochlonidium polykrikoides* at 1000, 3000, 6000 cells/ml showed the high linearity ($R^2 = 0.9972$) with the optimal optical density at 300 nm.

Korea and Japan during summer, as shown in **Figure 3**.

The scanning data (250 - 350 nm) of optical density for *Cochlonidium polykrikoides* [6] at cell concentration of 1000, 3000, and 6000 cells/ml (**Figure 4(a)**) were plotted to obtain the first derivatives for searching the optimal optical density, which suggested 300 nm as the optimal optical density for the measurement of *Cochlonidium polykrikoides*, as shown in **Figure 4(b)**. **Figure 4(c)** showed that the cell concentrations of *Cochlonidium polykrikoides* were linearly ($R^2 = 0.9972$) proportional to the optical densities at 300 nm, which could be caused by its preference for ultraviolet band with high energy in accordance with Einstein-Planck relation. It was thus possible to determine the cell concentration of *Cochlonidium polykrikoides* via an on-line method at 300 nm instead of the present tedious method of cell number counting by microscope after off-line sampling. It was postulated that on-line real-time monitoring of *Cochlonidium polykrikoides* could be possible for the early warning of HAB in conjunction with a smart phone system so long as a portable detector was available at 300 nm.

2.3. Prevention of Harmful Algal Blooms by Sulfur Compounds

Figure 5 showed that iron deficiency (-Fe) inhibited the algal growth while iron enrichment (+Fe) enhanced the phytoplankton productivity. However, fresh 100% Japanese Ontake volcanic ash with enriched sulfur compounds (V100) showed reduced algal-growth.

Hydrogen sulfide (H_2S) is produced by 4 routes; volcanic gas (0.04% - 0.68%), sulfur oxidizing bacteria such as *Beggiatoa*, sulfate reducing bacteria (SRB) such as *Desulfovibrio desulfuricans*, and decomposed microorganism [8].

Standard H_2S gas with 50 ppm (RIGAS in Korea) with a flow rate of 2 L/min

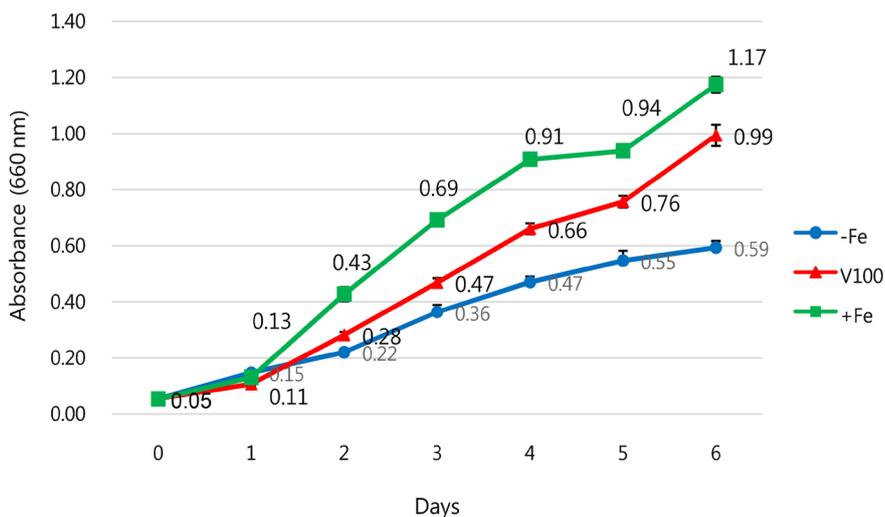


Figure 5. Growth curve of *Chlorella vulgaris* with various JM media; with its own Fe (+Fe, -+-), fresh 100% volcanic ash (V100, -▲-), without its own Fe (Fe, -●-). Standard deviation was expressed by each error bar for three measurements with excellent reproducibility, modified from Kim [7].

for 10 minutes was bubbled into 1 liter of the algal freshwater dissolved 25 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Algae were sedimented due to lack of Fe in freshwater induced by H_2S bubbling. The sedimentary materials were filtered to analyze by X-ray diffractometer (XRD) (Model Dmax2500/PC) for the presence of iron sulfides in the form of Greigite- Fe_3S_4 among sulfur and iron oxide, as shown in **Figure 6**. Quantitative analysis of **Figure 6** implied that the crystallinity of Fe_3S_4 constituted 4.06 wt% of total sedimentary materials among amorphous peaks. It was thus evident that sulfur compounds bind Fe to sediment in black iron sulfide and deprive Fe from the growth of phytoplankton. Decomposed microorganism produced H_2S as high as 0.122% [9] or 1220 ppm while the safe level is below 10 ppm [10]. Therefore, the disposable Biogas with H_2S of 0.2% - 3.5% [10] could be bubbled into both of freshwater and seawater cautiously to prevent HAB typically in Florida [2] during the early algal growth phase.

3. Results and Discussion

3.1. Volcanic Eruption

Volcanic gases are commonly composed in the order of H_2O (37% - 97.1%),

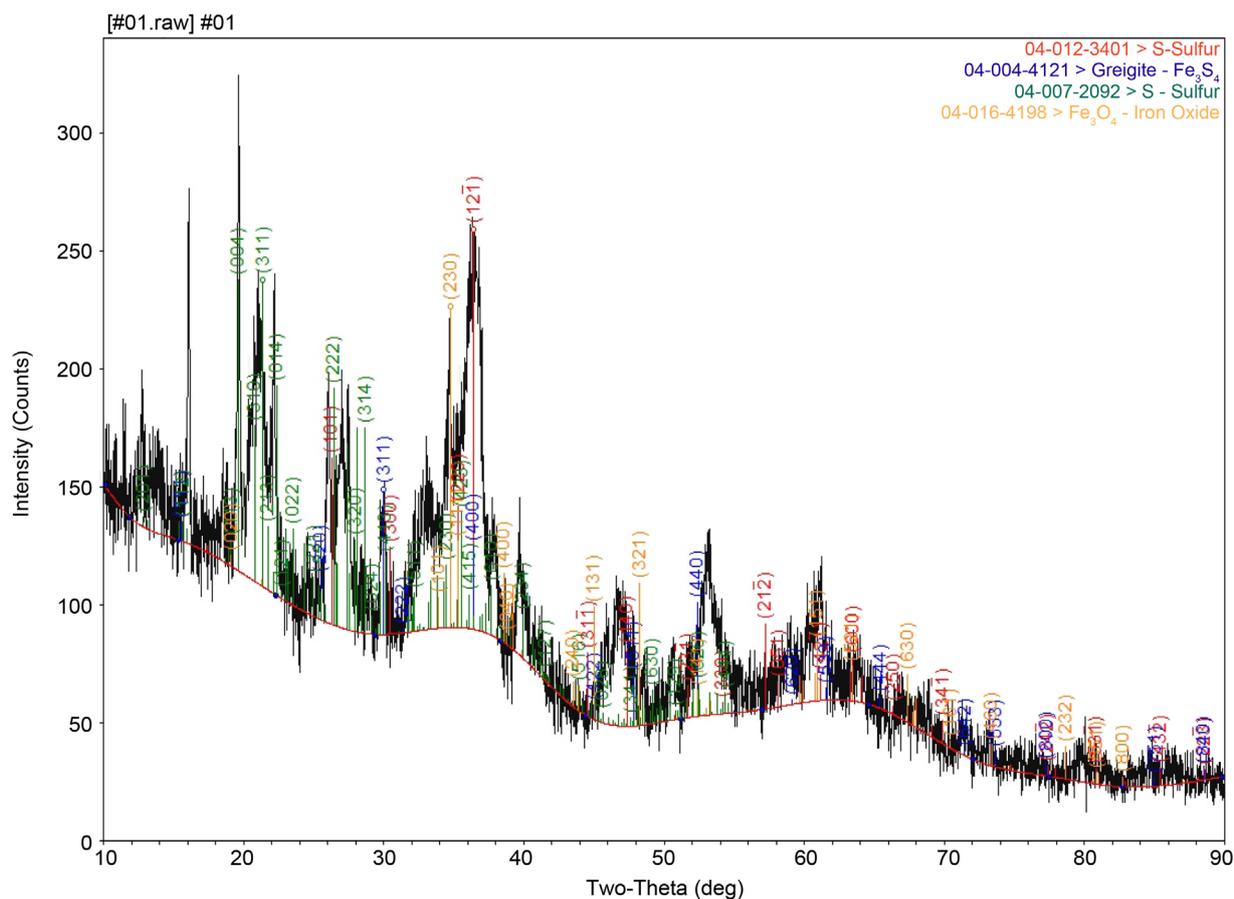


Figure 6. Distribution of X-ray diffractometer (XRD) pattern for the sedimentary materials induced by the chemical reaction between dissolved $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and H_2S gas bubbling in distilled water.

CO₂, SO₂ (0.50% - 11.8%), H₂, CO, H₂S (0.04% - 0.68%), HCl, and HF during volcanic eruptions [11]. Volcanic ash has an iron complex in the forms of Fe₂O₃, Fe₃O₄, FeCl₂, FeCl₃, FeF₂, FeF₃, FeS, FeS₂, FeSO₄ and Fe₂(SO₄)₃ [12]. The primary producers in the ocean that absorb iron (Fe) are phytoplankton or cyanobacteria, while hematite (Fe₂O₃) and goethite (FeOOH) in the aeolian dust are associated with fine (0.3 - 1 μm) particles with long residence times (days) in the atmosphere and thus potentially long transport paths [13]. Fe is essentially required during photosynthesis, electron transport, energy transfer, N (nitrate and nitrite) assimilation, and cyanobacteria N₂-fixation by nitrogenase complex with 32 to 36 Fe atoms [14]. Under aerobic conditions typifying surface waters, Fe exists in ferric (Fe_(aq)³⁺) form.

Increases of reductant under anoxic conditions [15], NADPH-oxidoreductase/ferric reductase [13], iron-reducing bacteria [16], photochemical, superoxide, humic acid and fulvic acid in peat [17], reduce Fe_(aq)³⁺ to Fe_(aq)²⁺, to be transferred into the algal cytosol by Fe_(aq)²⁺ permease [13] for the biosynthesis of macromolecules while excess Fe is stored in ferritin or cellular Fe pool [18]. The only missing ingredient after phytoplankton blooms is the dissolved Fe_(aq)²⁺ in HNLC (High Nutrient, Low Chlorophyll) regions unless there are further Fe inputs from aeolian dust or volcanic ash.

As seen in **Figure 7**, H₂S is produced by 4 routes; 1) directly from volcanic gas (0.04% - 0.68%) with relatively high solubility (0.3 g/100g H₂O) compared to extremely low solubility of FeS (4.4 × 10⁻⁵), 2) from sulfur oxidizing bacteria such as *Beggiatoa*, 3) from soluble sulfate by sulfate reducing bacteria such as *Desulfovibrio desulfuricans*, 4) and decomposed microorganism [8].

The more H₂S available from either the volcanic gas and sulfur oxidation, or soluble sulfates through sulfate reducing bacteria, the more sedimentation occurs in the forms of FeS and FeS₂. Therefore, it can be seen that the volcanic eruption enhances the formation of FeS and FeS₂, which induces less and less Fe available to algae to be the Fe limited condition of LC (Low-Chlorophyll). On the other hand, nutrients such as nitrate, phosphate and silicate are fairly soluble and utilized by algae. However, since Fe was limited, the growth of algae was reduced [19] and thus nutrients were less utilized and further enriched to be HN (High-Nutrient).

3.2. Sunlight with Algae Size

Photosynthesis is the process by which sunlight energy is transformed into chemical energy to produce organic compounds that serve as cellular building blocks and energy reserves. In the first phase of the light dependent reactions, light energy reaching reaction center chlorophyll-*a* molecules, with the formula of C₅₅ H₆₈ O₅ N₄ Mg, is stored in ATP and NADPH. The reverse reaction of photosynthesis is the cellular respiration, which occurs during the night for cells to obtain energy in ATP for maintenance of the cell and its growth. The respiration process produces 38 ATP, passing through the serial metabolic pathways of

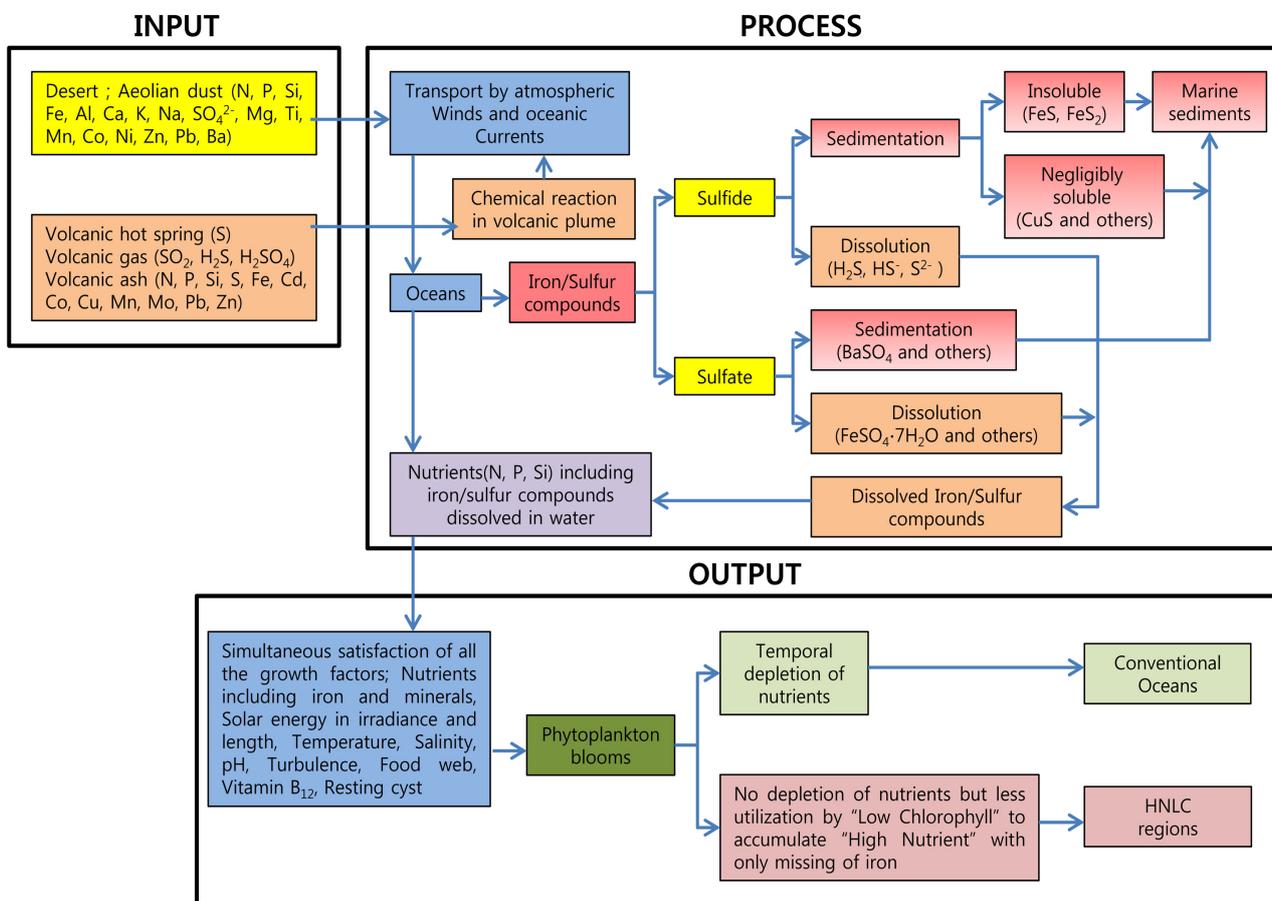


Figure 7. Schematic block diagram of iron (Fe) and sulfur compounds (S) from the input of desert and volcano to the output of oceanic regions via the transport process of atmospheric winds and oceanic current.

glycolysis and citric acid cycle, which require ten and eight individual enzymes along with specific metal ions, respectively.

Photosynthetically Active Radiation (PAR) consists of the light measured from a range of 400 nm to 700 nm [20]. Since the flux is defined as the quantity per unit time per unit area, total light energy is given by,

$$\text{Total light energy} = (\text{Energy Flux}) \times (\text{Area}) \times (\text{Period of Radiation})$$

Table 1 shows the seasonal HAB with various algae sizes. *Anabaena* can bloom during early winter due to its small surface area, requiring small amount of light energy flux (low light intensity and short radiation period). *Microcystis* has a relatively large surface area, requiring a large amount of light energy flux (strong light intensity and long radiation period). As for cyanobacteria, the order of large surface area in **Table 1** is *Microcystis* > *Aphanizomenon* > *Oscillatoria* > *Anabaena*. Since more energy is required for a large surface area, diatoms of *Cyclotella* and *Stephanodiscus* have relatively small surface areas for blooming in spring. As for *Cochlonidium polykrikoides*, they form several cells connected together to get more solar energy during summer and early fall with a preference for the highest energy band at 300 nm, as verified in **Figure 4(b)**. *Cochlonidium*

Table 1. Characteristics of typical species with various algae size causing seasonal HAB.

Species	Type	Diameter (μm)/Length (μm)	Surface Area (μm ²)	Blooming Season	Cell (Number)
Cyanobacteria, <i>Anabaena</i>	Cylinder	7/80	1758	Fall, Early Winter	Single
Cyanobacteria, <i>Microcystis</i>	Sphere	2.6 - 5.4/260 - 540	49,063 (13,800,000 - 123,609,000)	Spring, Summer	Colony (100)
Cyanobacteria, <i>Oscillatoria</i>	Cylinder	10/350	10,990	Fall	Single
Cyanobacteria, <i>Aphanizomenon</i>	Cylinder	2.8/100	70,650 (1,413,000)	Spring, Summer	Colony (20)
Cyanobacteria, <i>Cyclotella</i>	Disk	27/30	2543	Spring	Single
Diatom, <i>Stephanodiscus</i>	Disk	100/20	15,700	Spring	Single
Dinoflagellate, <i>Cochlonidium polykrikoides</i>	Cylinder	35/25 (35/200)	17,172 (192,000)	Summer, Early Fall	Colony (8)

polykrikoides has a diameter of 35 μm with several cells connected together. Therefore, any cloth around the fish farmery with pore diameter less than 35 μm may block the penetration of *Cochlonidium polykrikoides* into the fish farmery. Since traffic signal yellow (590 nm) shows the least absorption (%) for chlorophyll-*a*, artificial LEDs with 590 nm could be installed in the fish farmery to repel *Cochlonidium polykrikoides*, requiring the highest solar energy at 300 nm (Figure 4(b)).

3.3. Desert Dust

Major aeolian dust events arise from the Sahara and Sahel deserts (“African dust”), the Australian deserts (“Australian dust”), and the Taklamakan and Gobi deserts and the Loess plateau (“Asian dust”) [21]. Tanaka and Chiba [22] compared the annual mean global dust fluxes by regions: Africa (61%; North 58, South 3), Asia (30.5%; Arabia 12, Central 7.5, East 11), Australia (6%), and America (2.1%; North 0.1, South 2). Asian dust from northern China and Mongolia [23] occur all year round in the dust source regions and some of them have an adverse impact on environment, eco-systems and human health socio-economic activities not only in the source regions but in the far downwind regions. Iron (Fe) and phosphorus in African dust [24] colimited the nitrogen fixation of phytoplankton in the eastern tropical North Atlantic. Sahara dust storms carry heavy metals, bacteria, and other pathogens to the Caribbean, causing coral death. In Figure 8, two images from NASA’s Total Ozone Mapping Spectrometer (TOMS) instrument showed dust blowing off the Sahara in Africa and crossing the tropical Atlantic.

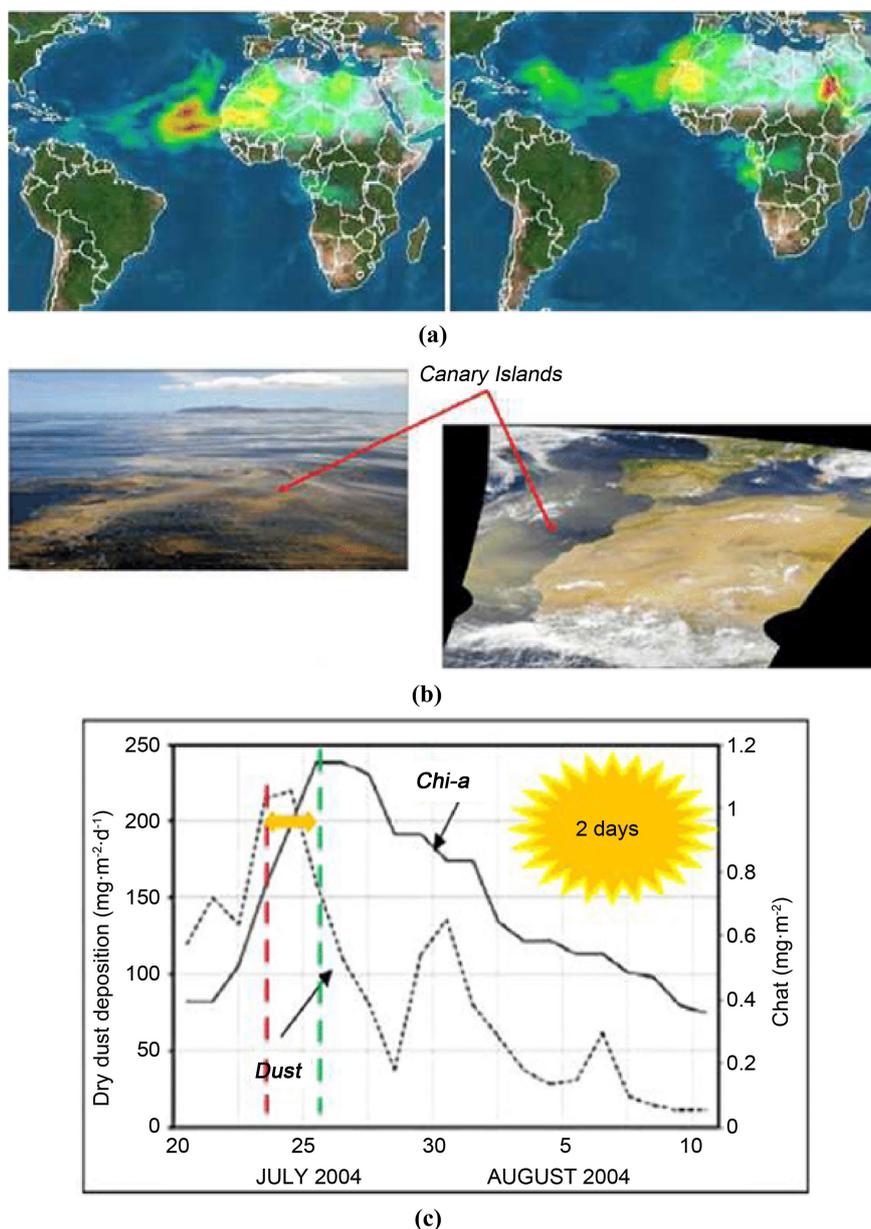


Figure 8. (a) Satellite images of the African dust event on June 17, 1999 (Left) as dust leaves Africa, and on July 2, 1999 (Right) as the dust approaches North America. (b) Bloom of *Trichodesmium*-Canary Islands, August 2004 (Left) and just over w Africa, July 2004 (Right). (c) Chlorophyll-*a* vs. African dry dust deposition (model); no upwelling during the event [25].

Many large oceanic areas do not have enough dissolved Fe necessary to support large populations of phytoplankton. Dust carries Fe from the land to the open ocean, supplying needed Fe, leading to phytoplankton blooms covering large areas of the North Atlantic and Caribbean. Nitrogen, Fe and phosphorus in Saharan dust fertilized plankton blooms in the tropical eastern Atlantic of the Canary Islands and affected exchange of carbon dioxide between atmosphere and oceans, as shown in **Figure 8(a)** & **Figure 8(b)**. Two days of lag time of algal

blooms appeared on the basis of peak-to-peak value as shown in **Figure 8(c)**, whereas dry dust deposition was obtained by modeling [25]. During the last decade there has been a significant rise in observations of blooms of the toxic cyanobacterium *Lyngbya majuscula* along the east coast of Queensland, Australia. *L. majuscula* productivity was significantly ($p < 0.05$) stimulated by soil extracts, which were high in phosphorus, Fe and organic carbon [15]. It is well-known that Asian desert dust particles can be transported long distances and reach the North American Continent, and oceanic deposition encourages phytoplankton growth in the North Pacific Ocean by natural Fe fertilization [26]. The contributions of Fe, nitrogen, and phosphate by the dust deposition from Asian dust storms promoted the growth of cyanobacterium *Synechococcus* in the Kuroshio Current [27], which is the common prey of red-tide dinoflagellates and heterotrophic nanoflagellates [28]. Asian dust particles were confirmed to be carriers of bacteria such as *Antinobacteria*, *Bacilli*, *Sphingobacteria* [21]. However, residents living nearby the origins of Asian dust such as Aksu, Dunhuang, Zhenbeitai, and Changwu [29], did not have some serious diseases such as cancer or miscarriages although many people have complained of the severe difficulty of breathing and shortage of sunlight due to the Asian dust. On the other hand, people living in the downwind regions passing through industrial complexes such as Beijing and Dailian have suffered severely because of Asian dust particles along with nitrate, ammonia, sulfate, and hydrocarbons from coal combustions [30], which have recently made serious social issues in China.

Many researchers have empirically analyzed the composition of the Asian dust with eight major elements in the parenthesized weight percentage order of Si (27) > Ca (10) > Al (7) > Fe (5) > K (3) > Mg (2) \gg Ti (0.5) \gg Mn (0.1) [29]. **Table 2** shows the necessary elements for algal growth compared correspondingly with components of wind carried particles of Asian dust. HAB could be thus prevented by blocking the deposition of desert dust.

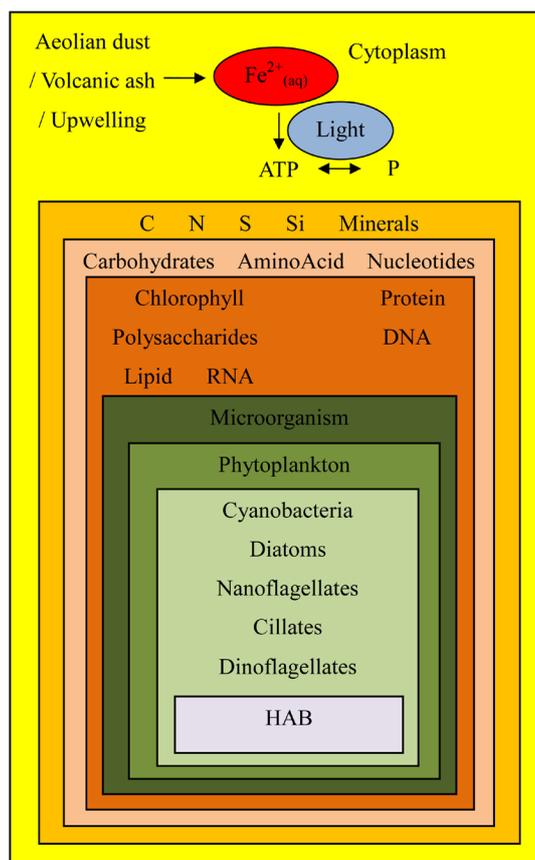
3.4. Iron

Iron (Fe) is the second most abundant metal and the fourth most abundant element by weight in the earth's crust while Asian dust contain iron commonly expressed in iron oxides [31] ranging from 4 to 6 weight percent depending upon different sources [13]. Fe is typically released into the soil or into the ocean through the weathering of rocks or through volcanic eruptions. Fe is an important limiting nutrient for HAB, which is used to produce chlorophyll and protein, as shown in **Figure 9**.

Photosynthesis depends on adequate Fe supply, whose concentration in water is quite low because of low solubility ($<1 \text{ mg}\cdot\text{L}^{-1}$ in freshwater). The primary producers in the ocean that absorb Fe are typically phytoplankton or cyanobacteria. Fe is then assimilated by consumers when they eat the bacteria or plankton, the latter providing a crucial source of food to many large aquatic organisms such as fish and whales. When animals, fishes and plankton die, decomposing

Table 2. Algal growth necessary elements compared with components of particles of Asian dust.

Algal Growth Element [14]		Asian Dust
Element	Major Function	Zhang [29]
N	Amino Acid	o
P	ATP/DNA	o
Cl	Photosynthesis	o
S	Photosynthesis	o
Si	Cell Wall	o
Na	Nitrate Reductase	o
Ca	Alginates	o
Mg	Chlorophyll	o
Fe	Nitrate Reductase	o
K	Enzyme Cofactor	o
Mo	Nitrate Reductase	-
Mn	Photosystem II	o
Zn	Carbonic Anhydrase	o
Cu	Photosystem II	o
Co	Vitamin B ₁₂	-
V	Nitrogenases	o

**Figure 9.** Iron, sunlight, phosphorus, and nutrients for HAB in both of the freshwater and the seawater.

bacteria return Fe to the soil and the water [32]. Haematite (Fe_2O_3) and goethite (FeOOH) tend to be associated with fine (0.3 - 1 μm) desert particles, with long residence times (*i.e.* days) in the atmosphere and thus potentially long transport paths. Haematite content varies between 0.6% and 3.4% (by volume). In wt.% goethite is 2 - 3 times higher than that of haematite. Similar dominance of goethite has been reported for samples from the Sahara and the Sahel [13]. Under oxic conditions typifying surface waters, Fe exists largely in the oxidized ferric (Fe^{3+}) form; as insoluble oxides, hydroxides, and carbonates which readily precipitate and deposit in the sediments. Under anoxic conditions, Fe may be released from the sediments [33] as a more reduced Fe^{2+} prior to HAB, as stepwise shown in Figure 10. It seems that the utilization rate of iron is mainly governed by the dissociation process of iron oxide to aqueous phase Fe^{3+} , its delivery time from the land to the river and coast, following periods of heavy rainfall, and the release of Fe^{2+} from Fe complex. When dissolved oxygen (DO) is so low, the binding of iron oxides with phosphorus sediments is broken-down to release the phosphorus and the oxygen, the latter being utilized by the iron-reducing bacteria. For example, iron-reducing bacteria of *Shewanella* algae BrY reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by the following reactions [34], where maximum concentration of Fe^{2+} in aqueous phase was reached in 15 hours [16].

Figure 10 informs us that $\text{Fe}_{(\text{aq})}^{3+}$ is changed to $\text{Fe}_{(\text{aq})}^{2+}$ for HAB under conditions such as 1) Increase of reductant under anoxic condition, 2) NADPH-

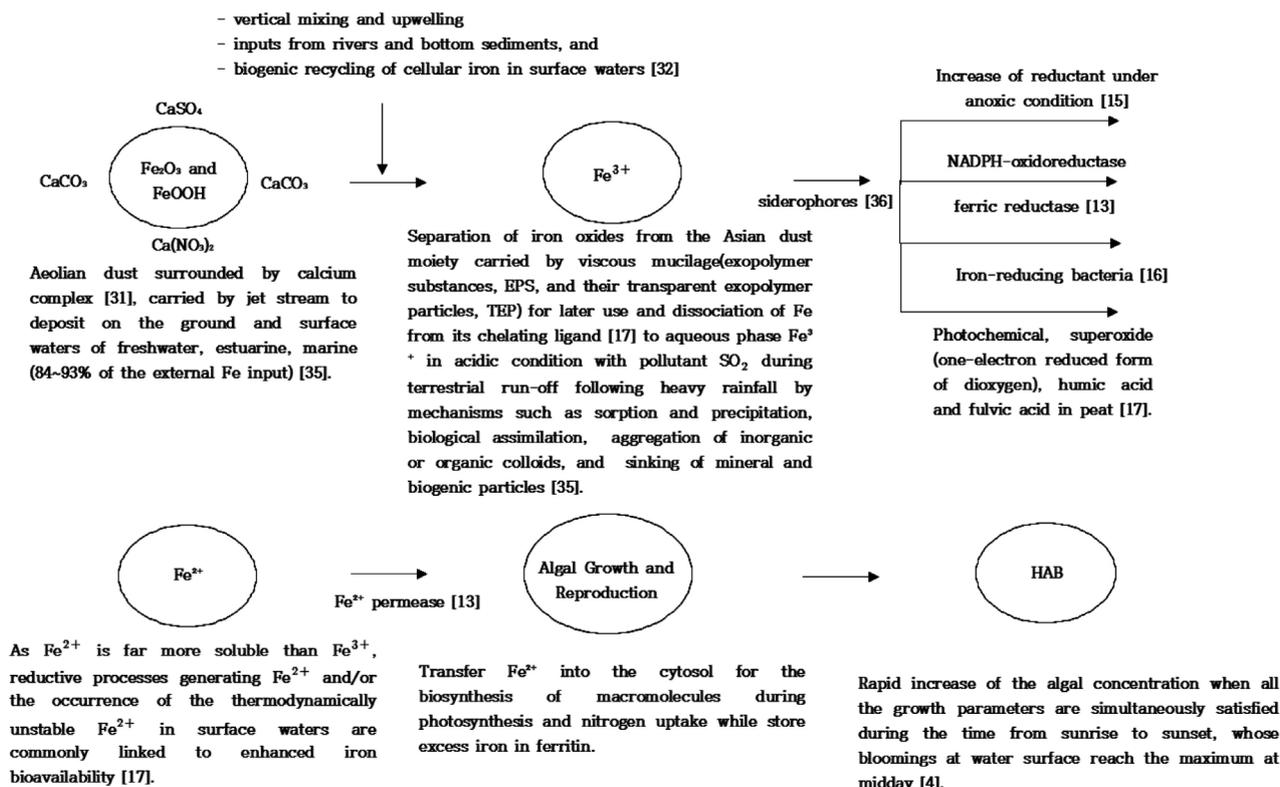


Figure 10. Conceptual stepwise diagram of iron utilization prior to algal blooms [4] [13] [15] [16] [17] [31] [32] [35] [36].

oxidoreductase, ferric reductase, 3) Iron-reducing bacteria, 4) Photochemical, superoxide, humic acid and fulvic acid in peat. It was thus recommended to eliminate Fe by not only blocking Fe^{3+} conversion to Fe^{2+} but also adding sulfur compounds (S) to induce the sedimentary iron sulfides (FeS/FeS_2) for suppression of HAB.

As shown in **Figure 11**, algae utilize the dissolved iron, $\text{Fe}_{(\text{aq})}^{2+}$ in competition with insoluble FeS/FeS_2 , the latter being significant if the volcanic activity is stronger for the sulfur compounds (S) than the desert contribution for iron (Fe).

Soluble Fe sulfates in **Figure 11** are FeSO_4 , $(\text{Fe})_2(\text{SO}_4)_3$, $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ and insoluble iron sulfides are FeS and FeS_2 , while soluble non-Fe sulfates are $\text{Al}_2(\text{SO}_4)_3$, NH_4HSO_4 , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{SO}_3$, BeSO_4 , CdSO_4 , CuSO_4 , MgSO_4 , MnSO_4 , NiSO_4 , KHSO_4 , Pb_2SO_4 , Na_2SO_4 and $\text{Na}_2\text{S}_2\text{O}_3$. Insoluble non-Fe sulfates are Ag_2SO_4 , BaSO_4 , PbSO_4 , Hg_2SO_4 , RdSO_4 and SrSO_4 , while soluble sulfide is H_2S and insoluble sulfides are CdS, CuS, PbS and PoS.

It is important to eliminate the dissolved iron, $\text{Fe}_{(\text{aq})}^{2+}$, with soluble sulfur compounds (S) to induce insoluble iron sulfides (FeS/FeS_2) for suppression of HAB.

The stimulation of diatom growth by Fe addition and the relative abundance of dinoflagellates in Fe limited waters, suggested Fe availability played a key role in the development of HAB [32]. The bioavailable Fe to microorganisms was present in dissolved organic or inorganic, colloidal, and particulate forms, as extensively reviewed by Shaked [17].

Photosynthesis takes place in chloroplast to capture light energy, whose principal photoreceptor is chlorophyll-*a* with molecular formula of $\text{C}_{55} \text{H}_{68} \text{O}_5 \text{N}_4 \text{Mg}$. The number of nitrogen atoms is thus four per molecule of chlorophyll-*a*, which requires four NH_4^+ ions to be synthesized to chlorophyll by nitrogen reductase. Since 14 Fe atoms are necessary during the process of photosystem and 32 to 36 Fe atoms are required [4] to activate nitrate reductase during N_2 fixation (**Figure 12**), it is thus, $(14 \times 4) + (32 \sim 36) = 88 \sim 92$ Fe atoms can be minimally necessary for the biosynthesis of a molecule of chlorophyll-*a*. Each photosynthetic cell contains 40 - 200 chloroplasts while each chloroplast has grana

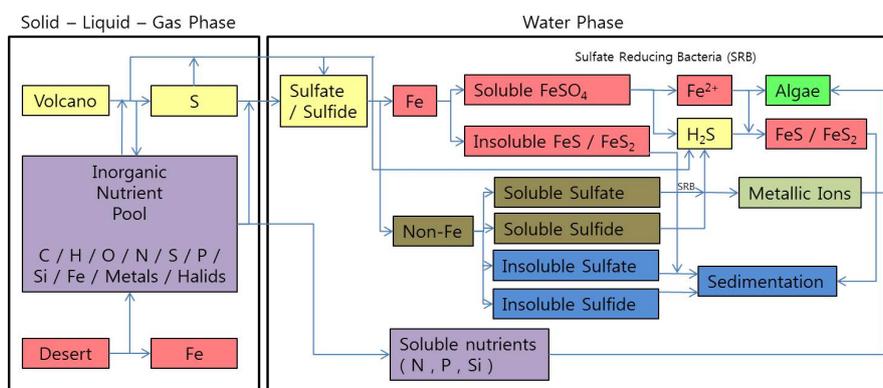


Figure 11. Flow diagram for algal utilization of nutrients, iron, and metallic ions from desert dust and volcano in various phases.

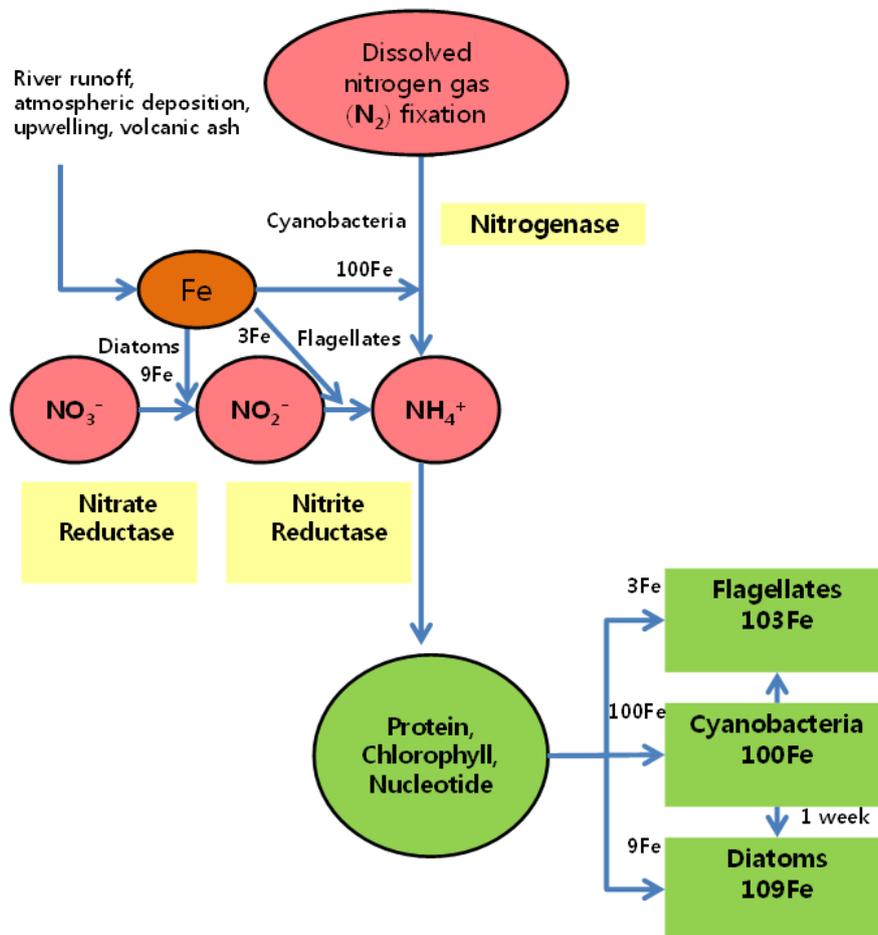


Figure 12. Relative uptake percentage of required Fe for the syntheses of cyanobacteria, diatoms, and flagellates with corresponding nitrogen enzymes while cyanobacteria as main source of Fe were preyed by flagellates and diatoms.

containing 10 - 20 thylakoid and thylakoid membrane is covered with 300 chlorophylls [37].

It is expected that each photosynthetic cell contains $1.2 \times 10^5 - 1.2 \times 10^6$ chlorophylls to be approximated that each photosynthetic cell of algae requires Fe atoms as much as: $(88 - 92 \text{ Fe atoms/chlorophyll-}a) \times (1.2 \times 10^5 - 1.2 \times 10^6 \text{ chlorophyll-}a/\text{algae cell}) = 1 \times 10^7 - 1 \times 10^8 \text{ Fe atoms/algae cell}$, which can be close to experimental observation of intracellular Fe quota for *Synechococcus spp* of $\sim 10^{-18} \text{ mol/cell}$ [38] ($\sim 1 \times 10^6 \text{ Fe atoms/cell}$). Since the algal concentration is in the range of 10^6 cells/ml during algal blooms [39], the resultant Fe atom concentration (atoms/ml) can be $1 \times 10^{13} - 1 \times 10^{14} \text{ (Fe atoms/ml)}$. If the blooming patch is assumed to be 100 meter long, 100 meter wide and 1 meter deep during photosynthesis, its volume can be 10^4 m^3 or 10^7 liter . Thus, Fe atoms in such a volume can be $1 \times 10^{23} - 1 \times 10^{24} \text{ Fe atoms}$ during HAB. Besides, one mole of iron is 55.8 g with $6 \times 10^{23} \text{ atoms}$, the corresponding Fe concentration is 0.001 - 0.01 ppm $\left\{ = \frac{(1 \times 10^{23} \sim 1 \times 10^{24})(55.8 \times 10^3 \text{ mg})}{6 \times 10^{23} (10^7 \text{ l})} \right\}$, which can be a comparable

concentration with 0.0034 ppm (mg/l) in seawater [40] and 0.3 ppm in drinking water. It is expected that Fe ion either in the seawater or in the freshwater can be minimally satisfied to synthesize only chlorophyll-*a* containing algae cells although much more Fe can be required further for protein and cellular reproduction during HAB.

As a result of Fe oxidation to low-solubility compounds and precipitation of Fe with sulfide, (FeS), and phosphorus, (FePO₄), dissolved Fe levels decreased in coastal oceans with sulfate (SO₄²⁻) and phosphate (PO₄²⁻) where dissolved Fe is received from river discharges. In addition, Fe released from the sediments may rise to the surface, along with other minerals. Also, winds carry Fe-rich dust into the oceans from continental land masses, and this dust provides a source of Fe to algae. For example, the cyanobacterium *Trichodesmium* is abundant in the Arabian and Caribbean seas and the Indian Ocean because it is able to capture Fe from dust entering such waters. When Fe is relatively abundant, some algae can store it in ferritin, an Fe-storage protein with 1800 Fe and 640 P per molecule [41]. Kawaguchi [42] have proposed bioavailable Fe as an indicator of ecosystem health in the southeastern USA. Ingle [43] predicted the Florida red tide (dinoflagellate blooms) by means of the Fe index. Alderkamp [44] showed that Fe from melting glaciers fueled phytoplankton blooms in the Antarctic. Langroudi [45] cultured algae of *Tetraselmis suecica* to see that the amount of Fe for its maximum growth was 0.3 ppm while less than 1% of soluble Fe (0.05 ppm) was observed in a Australian creek out of total Fe (43 ppm) [15].

Since the structural formula of chlorophyll-*a* is C₅₅ H₆₈ O₅ N₄ Mg, the most limiting nutrient out of C, H, O, N can be the nitrogen while the oxygen is abundant in the water. However, since algal N₂ fixation and N (specifically nitrate and nitrite) require an abundant supply of Fe²⁺ (Figure 12), HAB can occur only if Fe in Fe²⁺ is fully available for the abrupt cell growth, expressed by the spontaneous increase of the concentration of chlorophyll-*a* during the exponential growth phase of the algae. In many cases, estuarine HAB have been linked to terrestrial run-off, following periods of heavy rainfall [15].

Since dissolved Fe levels decreased by six to seven orders of magnitude due to the formation of low-soluble FeS and FePO₄ in waters, most of the delivered Fe by the Asian dust (60,000 - 120,000 ppm) can be mainly precipitated to the sediments, since Fe in freshwater is only 0.3 ppm and Fe in seawater is barely 0.0034 ppm. Therefore, it can be expected that Fe utilization rate by algae is regulated by the release of Fe²⁺ from Fe complex by reduction, whose reaction is the function of the equilibrium constant and thus the absolute temperature of the freshwater and the seawater. This temperature dependence may be the reason why there is no HAB during the cold season of winter.

Table 3 implied that HAB occurred in August after 4 to 5 weeks of heavy rainfall, exceeding the average 103 mm rainfall on one day in July. Since the cyanobacterial growth in the middle of water was commonly observed a week earlier than the onset of HAB, it could be postulated that the algal growth took about 4 weeks during the lag phase of growth while about one week for the late

Table 3. Harmful algal blooms in freshwater and seawater in South Korea during the years from 2001 to 2012.

Year	Harmful Algal Blooms							
	Freshwater				Seawater			
	Daechung Lake, Daejeon City				Naro Island, Goheung County			
Onset Date	Duration (day)	Species	Heavy Rainfall Date/Precipitation (mm)	Onset Date	Duration (day)	Species	Heavy Rainfall Date/Precipitation (mm)	
2001	9.1	35	<i>Microcystis</i>	7.21/61	8.14	41	<i>Cochlonidium</i>	7.5/119
2002	9.19	16	<i>Microcystis</i>	8.6/175	8.2	57	<i>Cochlonidium</i>	7.2/117
2003	9.9	39	<i>Microcystis</i>	7.9/155	8.13	62	<i>Cochlonidium</i>	7.3/59
2004	9.3	14	<i>Oscillatoria</i>	7.16/131	8.5	30	<i>Cochlonidium</i>	8.22/86
2005	8.2	67	<i>Microcystis/</i> <i>Anabaena</i>	7.11/178	7.19	58	<i>Cochlonidium</i>	6.10/88
2006	8.4	62	<i>Anabaena</i>	7.12/49	8.6	36	<i>Cochlonidium</i>	7.10/151
2007	10.6	14	<i>Aphanizomenon/</i> <i>Microcystis</i>	7.10/63	7.31	50	<i>Cochlonidium</i>	7.1/108
2008	7.25	15	<i>Anabaena</i>	6.18/81	7.30	62	<i>Cochlonidium</i>	6.1/70
2009	8.6	15	<i>Microcystis</i>	7.9/88	10.28	17	<i>Cochlonidium</i>	7.7/164
2010	9.2	43	<i>Aphanizomenon</i>	7.17/71	9.17	3	<i>Cochlonidium</i>	7.16/64
2011	8.12	57	<i>Oscillatorial/</i> <i>Aphanizomenon</i>	7.8/85	-	-	-	5.11/92
2012	9.6	63	<i>Microcystis/</i> <i>Aphanizomenon</i>	7.15/111	7.27	75	<i>Cochlonidium</i>	7.11/119
Average	8.28	37	<i>Microcystis</i> (7)/ <i>Oscillatoria</i> (2)/ <i>Anabaena</i> (3)/ <i>Aphanizomenon</i> (4)	7.13/104	8.12	45	<i>Cochlonidium</i>	7.4/103

log phase of growth, after which it reached the stationary phase forming large surface algal aggregations of HAB. It was known that the required time for the algal reproduction was 6 hours for diatom under no turbulence. Since the bacterial conversion of Fe^{3+} to Fe^{2+} took 15 hours [16] and the photosynthesis was maximized during the 6 hours from dawn (6 AM) to midday (12 PM) [4], the resultant summation (6 + 15 + 6) hours were 27 hours for HAB by diatom, which was in accordance with other studies [24] indicating HAB by diatoms after strong winds of a day or two in the sunlit surface waters.

3.5. Nutrients

The fundamental solution for the demise of HAB was to reduce the nutrients in the form of organic materials. Perhaps the most contentious explanation has been the idea that run-off from land was a major contributor to HAB. When managers released water from the massive inland reservoir as a flood-control measure during heavy rains, the nutrient-rich water has been diverted to the river and on to the sea, laden with nutrient pollution from the cities and farms

[8] [15]. Typical sources of nutrients for algal growth are carbon, nitrogen, phosphorus, sulfur, silicon, and minerals while hydrogen and oxygen are always available within the cell in the form of water to support the cell structure for metabolic functions.

Carbon:

Carbon in the earth's atmosphere exists in two main forms: carbon dioxide and methane. The terrestrial biosphere includes the organic carbon in all land-living organisms, both alive and dead, as well as carbon stored in soils. Once organisms die, bacteria break down their tissues, releasing CO₂ back into the atmosphere or into the soil. CO₂ dissolves in the water and algae while plants and bacteria convert it into organic carbon. Their tissues are ultimately broken down by bacteria and CO₂ is released back into the ocean or atmosphere. Much of the carbon in the earth's lithosphere (about 80%) is stored in limestone, which was formed from the calcium carbonate from the shells of marine animals.

Nitrogen:

Algae require combined nitrogen to synthesize amino acids, nucleic acids, chlorophyll, and toxins [45]. Nitrogen sources commonly used by cyanobacteria include ammonium, nitrate, nitrite, urea and atmospheric N₂, as summarized in **Figure 12**. Nitrate reductase is membrane-bound while nitrite reductase is both a membrane-bound and a cytoplasmatic enzyme. About 94% of the oceanic N inventory exists as biologically unavailable dissolved nitrogen gas (N₂), which can be made bioavailable through N₂-fixation using Fe-dependent nitrogenase for reduction of N₂ to NH₄⁺. The 6% of biologically available N exists primarily as NO₃⁻ (~88%) and dissolved organic nitrogen (DON, ~11.7%). The remaining 0.3% is found in NO₂⁻, NH₄⁺, nitrous oxide (N₂O) and particulate organic nitrogen (PON) [46]. Dinoflagellates are known to take up substantial amounts of various N forms in the darkness while diatoms can not. This may be caused by their nitrate reductase preference for anaerobic conditions available in the dark. Nitrogen is generally taken up as dissolved inorganic nitrogen (DIN): nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonium (NH₄⁺). DIN is actively transported across the cell membrane via "uptake sites" and metabolised within the cell to form chlorophyll and amino acids and, in turn, proteins and nucleotides [47]. Since uptake mechanism of DIN across the cell membrane is "active transport", it requires external energy such as ATP or NAD(P)H. The conversion of 1 mole nitrogen to 2 mole ammonia requires 25 moles ATP. Therefore, the nitrogen uptake should be followed by ATP synthesis. The atomic ratio of N/P is commonly known to be 16/1 in freshwater and 176/1 in seawater for HAB. Therefore, nitrogen can be the limiting factor relatively in seawater while phosphorus is the limiting factor in freshwater. This may be why cyanobacterial blooms of N₂-fixing *Anabaena* and *Aphanizomenon* are common in freshwaters rich in phosphate. Surplus nitrogen is known to be stored in cyanophycin granules and phycobiliproteins [48]. Dinoflagellates might store their N in the form of uric acid crystals within the cytosol [46]. Although algae typically take up ammo-

nium or nitrate ions directly from the surrounding water with nitrogen ion of 15.5 ppm [40], some algae obtain ammonium that can be used more directly than nitrate to synthesize cellular N-compounds. Algae are generally able to convert nitrate to ammonium by using the enzyme nitrate reductase, which requires the cofactors Fe and molybdenum, that are not always present in sufficient amounts because of Fe formation of insoluble precipitate, FeS associated with active undersea volcanoes.

Phosphorus:

Phosphorus (P) in inorganic phosphate is required during the photosynthesis, as illustrated in **Figure 9**. Energy stored in the phosphate bonds of ATP generated from photosynthesis is mobilized in the form of biomolecules such as polysaccharides, porphyrins, cellulose, proteins, lipids, RNA, and DNA. These macromolecules biosynthesize cell wall (mucopolysaccharides) and membrane (proteins). Inorganic P is found in the soil or water. Plants and algae assimilate inorganic P into their cells, and transfer it to other animals that consume them. When organisms die, their P is released by decomposer bacteria, which convert organic P to inorganic P. Aquatic P follows a seasonal cycle, while inorganic P peaks in the spring causing rapid algae and plant growth, and then declines. As plants and animals die, organic P is re-released into the water. P based fertilizers can cause excessive algae growth in aquatic systems, which can have negative impacts on the environment [22]. In oligotrophic freshwaters, the mineral nutrient that most commonly limits algal growth is P. Blooms of the toxic cyanobacterium *Microcystis* are common events globally, whose intracellular P pool showed a percentage of total cell-associated P (50% - 90%) similar to what has been reported for actively growing algae in marine systems [49]. Surplus phosphate is known to be stored in polyphosphate bodies of cyanobacterial cells [48]. Phosphate levels are usually low in oligotrophic freshwater because this ion readily binds Al^{3+} , Fe^{3+} , and Ca^{2+} , forming highly insoluble complexes in soils and lake sediments. Schindler [50] showed that eutrophication of lakes was not controlled by reducing nitrogen input but limited by phosphorus input. However, where there is sufficient phosphorus available, biologically available Fe becomes a significant limiting factor of biological growth in oceanic systems as well as coastal and estuarine ecosystems [15]. Jun [51] showed that there were four chemically defined sediment P such as adsorbed P, non apatite inorganic P (NAI-P), apatite P and residual P while the release of sediment P was apparent in the NAI-P rich sediments. The phosphorus in calcium phosphate of apatite P was released if pH was low while the phosphorus in (Fe + Al) complexed NAI-P was released if pH was high due to the preferred formation of (Fe + Al) hydroxides. Typically, **Figure 13(a)** showed the pH profile of dissolved inorganic phosphate upstream of the Han River in Seoul in Korea. It shows that dissolved inorganic phosphate could be low if pH in lakes was below 6 while dissolved phosphate levels are relatively high (0.088 ppm), as is in marine waters with pH of 7.8 - 8.4 [40]. An interesting experimental observation was that the level of the dissolved inorganic

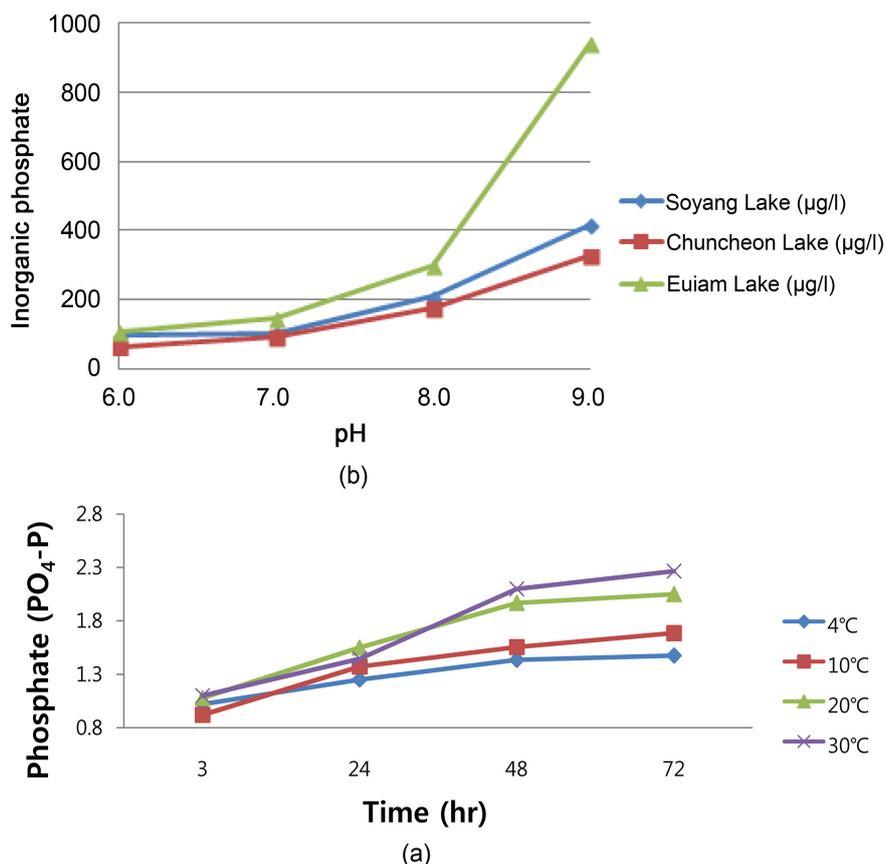


Figure 13. (a) Distribution of dissolved phosphate with variation of pH in Korean Lakes redrawn from Jun [51]. (b) Distribution of dissolved phosphate with variation of temperature in the river redrawn from Min [51].

phosphate was decreased if the temperature of freshwater was below 15°C [52], as shown in **Figure 13(b)**. It was therefore expected that there could be HAB if freshwater temperature was above 15°C.

The discharge allowance of phosphorus from the wastewater treatment plant is 0.2 ppm in Korea. In order to reduce the hazard of HAB in the river, one suggestion is to recycle the wastewater for further removal of phosphorus from the discharge water.

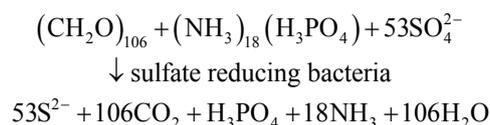
When phosphorus becomes so abundant that it no longer limits algal growth in eutrophic lakes, another nutrient (usually nitrogen, though sometimes Fe) becomes limiting. Moisaner [53] reviewed that phosphorus availability might determine the times and locations for blooms of N₂-fixing harmful cyanobacterium *Nodularia* in the Baltic Sea, Australia, United States, South Africa, and New Zealand although they acknowledged the possibility of other controlling factors such as trace element limitations.

Figure 13(a) showed that dissolved phosphate could be kept minimal if pH was maintained below 6. Since phosphate is used as the energy source during biosynthesis of HAB as shown in **Figure 9**, HAB can be prevented by keeping pH below 6 (**Figure 13(a)**) and temperature below 15°C (**Figure 13(b)**) for

prevention of HAB. Since bubbling with pure CO₂ gas induced the freshwater with pH below 4.2, it was easy to suppress HAB within the small area.

Sulfur:

Sulfur is an essential element for all life and widely used in biochemical processes. In metabolic reactions, sulfur compounds (S) serve as both fuels and respiratory (oxygen-alternative) materials for simple organisms. The oxidation of sulfide occurs in stages, with inorganic sulfur being stored either inside or outside of the cell until needed. Biochemically, reduced sulfur compounds (S) are converted to sulfite (SO₃²⁻) and, subsequently, sulfate (SO₄²⁻) by the enzyme sulfite oxidase [54]. Metabolic mechanism of sulfur within the very surface of the sediment is as follows, which eventually produces hydrogen sulfide (H₂S).



Sulfur is required during the photosynthesis (Table 2) while protein building blocks of amino acids such as cysteine and methionine are composed of sulfur. Marine waters are relatively high (2701 ppm) in sulfate ion (SO₄²⁻). Sulfate-reducing bacteria convert sulfate to sulfide ion (S²⁻), which readily combines with Fe to form an insoluble precipitate, FeS. As a result, less dissolved Fe is available to react with phosphate, so dissolved phosphate levels remain relatively high (0.088 ppm) in marine waters [40]. Decomposed microorganisms from foodstuff disposal produced H₂S gas [9], which could be applied to both of freshwater and seawater to sediment the dissolved Fe as insoluble FeS and FeS₂ for prevention of HAB in Florida's lake and coast, as shown in Figure 14 [2],

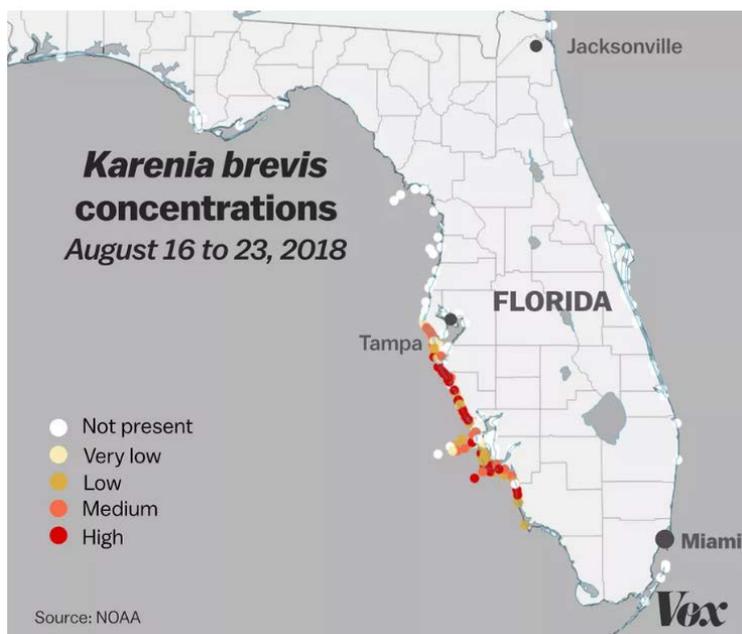


Figure 14. *Karenia brevis* concentrations in Florida (August 16 to 23, 2018) [2].

respectively. Therefore, HAB could be prevented in freshwater and seawater by sulfur compounds (H_2S , sulfates) inducing the deficiency of the dissolved Fe in the water.

Silicon:

The Redfield ratio of Si:N:P shows 16:16:1 while China seas (28:4:1), Sea of Japan (36:7:1), Subarctic Pacific (20:9:1), Eastern Equatorial Pacific(6:7:1), which are on the trajectories of the Asian dust [26]. Unlike the majority of other phytoplankton, diatoms utilize silicic acid $Si(OH)_4$, to construct their cell walls and are controlled by its availability and distribution. The ratio of Si/Fe in Asian dust [29] was approximately 7 while 182 - 526 in algal cell. Limitation of diatom metabolism by the supply rate of silicic acid results in a physiological cascade that affects diatom primary productivity and nitrogen use, potentially altering regional nitrogen and carbon cycling. Diatoms using silicon to make their shells are important bloom-forming phytoplankton that contribute ~40% of global ocean productivity [55]. Lippermeier [56] observed that both diatom cell numbers and chlorophyll-*a* concentration were increased after the addition of Na_2SiF_6 , which showed the importance of silicate for the photosynthetic performance similar to that of other nutrients like nitrogen, phosphate and iron.

Healey [57] found that chlorophyll-*a* synthesis of diatom *Navicula pelliculosa* ceased in 5 to 7 h of silicon (Si) starvation while Kong [58] observed 6 hours for diatom doubling time. Under ammonium limitation the ratio of silica to chlorophyll-*a* was 22.7 - 21.0 in *Skeletonema costatum* [59]. The Weddell Sea has been deemed by scientists to have the clearest water of any sea with a Secchi disc visible at a depth of 79.86 meters (Weddell Sea-Wikipedia, <https://en.m.wikipedia.org>). The Weddell Sea showed the lowest biogenic silica accumulation rates in the Southern Ocean [60]. Therefore, the clearest water of the Weddell Sea could be induced by the high content of silica. It was thus recommended to spread the silica powder (~74% Si) over the water surface prior to the early exponential phase of algal growth for prevention of HAB in freshwater or in seawater.

Minerals:

Algal growth requires the assimilation of mineral nutrient from the water. Such mineral nutrients (K: 390 ppm, Mg: 1295 ppm, Ca: 416 ppm, Si: 3 ppm, Fe: 0.0034 ppm, Mn: 0.0004 ppm in seawater [40]) are commonly taken up by means of transport proteins located in the plasma membrane, though some algae also obtain minerals by engulfing particles such as bacterial cells and picoplankton [28]. Major chemical elements required by green plants are N_2 , P, K, S, Ca, and Mg while minor ones are Fe, Cu, Zn, Mn, and Mo. If molybdenum is deficient in plants, the plant cannot make proteins [61]. Molybdenum in drinking-water has a mean of 0.0859 ppm and a range of 0.003 - 1.024 ppm in the USA [62] while 0.0105 ppm in seawater [63] and 1.57 ppm in aeolian dust [64]. Combined (fixed) nitrogen, iron, phosphate, and sometimes silica (SiO_2) are essential nutrients needed by algae in particularly large amounts. The mineral

nutrients most likely to limit algal growth are important because additions of these minerals are most likely to cause HAB and other undesirable changes in aquatic systems [34]. Inorganic chemical composition of a typical algal cell is normalized with respect to nitrogen in atomic basis, C/N (6.6/1), Si/N (1/3.3 - 1/5.7), S/N (1/10 - 1/20), P/N (1/16), Mo/N (1/232 in river - 1/1476 in seawater), and Fe/N (1/600 - 1/3000 in *Synechococcus*) [65]. Thus, Fe and molybdenum can be limiting factors for algal growth. In order to prevent HAB, it is important to limit mineral ions as primary sources and not secondary ones.

3.6. Environmental Factor

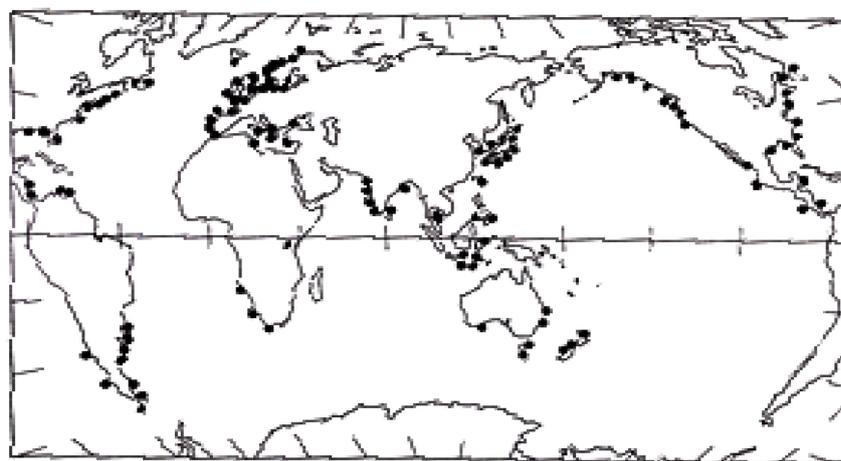
Water quality parameters and meteorological conditions influencing HAB, have been extensively studied by many investigators [4] [33] [53]. Growth factors for HAB are summarized in **Table 4** while important parameters were stepwise discussed as below.

3.6.1. Current

Currents are unidirectional water flows that are strongly influenced by winds and coastal topography, which bring a constant supply of inorganic nutrients to algae. **Figure 15** shows the area where in-land algal blooms in rivers or lakes have occurred with animal damage by harmful cyanobacteria such as *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria* with toxins of Anatoxin-a (*Anabaena*), Microcystin (*Microcystis*), and Nodularin (*Nodularia*) [66]. Moisaner [53] concluded that phosphorus availability may determine the times and locations for blooms of N₂-fixing harmful cyanobacterium *Nodularia* in the Baltic Sea, Australia, United States, South Africa, and New Zealand although they acknowledged the possibility of other controlling factors such as trace element limitations. Hallegraeff [45] studied the transport of toxic dinoflagellate cysts via ship's ballast water to explain not only the apparent global increase in the geographic distribution of paralytic shellfish poisoning but also the distribution of Australian ports with establishment of toxic dinoflagellate populations. Although the ballast water can be one of the causes of HAB, it may not explain the global in-land HAB where there are no port and the water streamline is not inwards to the land but outwards to the coast. It is important to consider the facts that major aeolian dust events arise from the Sahara and Sahel deserts ("African dust"), the Australian deserts ("Australian dust") and the Taklamakan and Gobi deserts, the Loess plateau, and the northern India and Bangladesh

Table 4. Growth factors for HAB in physical, chemical and biological processes.

Process	Growth Factors for HAB
Physical	Current, Upwelling, Temperature, Light, Tubulence, Rainfall,
Chemical	Nutrients, pH, Dissolved Oxygen
Biological	Cyst, Food web, Growth phase, Algae size, Enzyme



(a)



(b)

Figure 15. (a) Global distribution of red tide occurrence, (b) Global distribution of harmful cyanobacterial bloom occurrence country where the animal damage (●) and the occurrence (▲) were reported by Watanabe [67].

(“Asian dust”). **Figure 15(a)** (Global red tide occurrence region) and **Figure 15(b)** (Global harmful cyanobacterial blooms occurrence country) show that aeolian dusts carry the micronutrients in the atmosphere or by oceanic currents to cause global HAB in both on the coast (**Figure 15(a)**) and in-land (**Figure 15(b)**).

3.6.2. pH

Abundance of cyanobacteria as a function of pH was shown in various rice soils from India [68] in **Figure 16**. Cyanobacteria had the maximal growth at a pH of 7.15. Therefore, cyanobacteria growth can be retarded if $\text{pH} < 5$ or $\text{pH} > 10$ as less as 1000 folds from 10^8 units/g soil to 10^5 units/g soil. Low pH 5 can be achieved by bubbling atmospheric air with CO_2 of about 400 ppm or pure CO_2 gas for fast response. There are CO_2 gas tanks in every drinking water purification plant to adjust the pH of aluminium sulfate, $\text{Al}_2(\text{SO}_4)_3$, as a coagulating agent in the purification of drinking water. High pH 10 can be reached by caustic soda

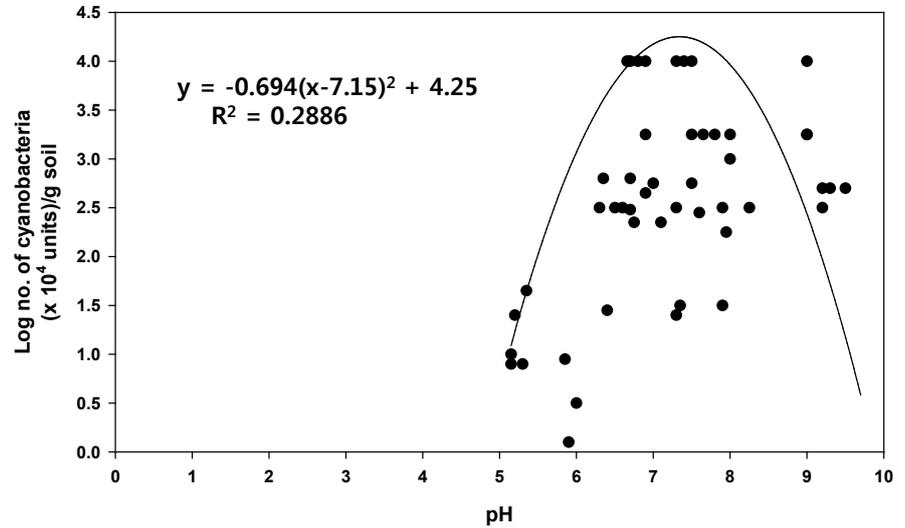


Figure 16. Abundance of cyanobacteria as a function of pH in various rice soils from India (redrawn and correlated from Nayak [68]).

(NaOH) with dilution of more than 1000 folds. Since algal growth is strongly dependent upon pH, it is possible to prevent HAB in the freshwater by pH adjustment, especially during the early growth phase with a small blooming area.

Figure 17 showed the algal growth curves in the seawater with various pH in the bench scale. The least cell growth was observed at pH of 5 and 6 although it was impossible to adjust the pH of seawater in the field.

3.6.3. Temperature and Salinity

Since temperature promotes the enzymatic activity of algal, temperature is an important parameter to prevent HAB in freshwater and seawater. From 2010 to 2012 the Han River in Korea showed HAB when water temperature was greater than 25°C. On the other hand HAB induced by *C. polykrikoides* showed the reduced cell growth curve when the seawater temperature was less than 10°C, as seen in **Figure 18** & **Figure 19**. Stable stratification with thermocline induced HAB in the seawater [58]. Low salinity did not extensively decrease the growth of *C. polykrikoides*. HAB in the seawater after the heavy rainfall (**Table 2**) could be associated with bulk inputs of nutrient, iron, nitrogen, and minerals, run-off from land by heavy rainfall into the sea. Diluted salinity of seawater after the heavy rainfall might not be the main reason of HAB in the seawater.

3.6.4. Dissolved Oxygen

In aquatic systems, the concentration of dissolved oxygen (DO) is related to water temperature and the speed at which the water moves.

Mass balance DO in the water is given by,

$$\frac{dm}{dt} = (\dot{m})_{in} - (\dot{m})_{out} + (\dot{m})_{gen} - (\dot{m})_{cons} \quad (1)$$

where

$(\dot{m})_{generation}$ = Oxygen produced by algal photosynthesis as,

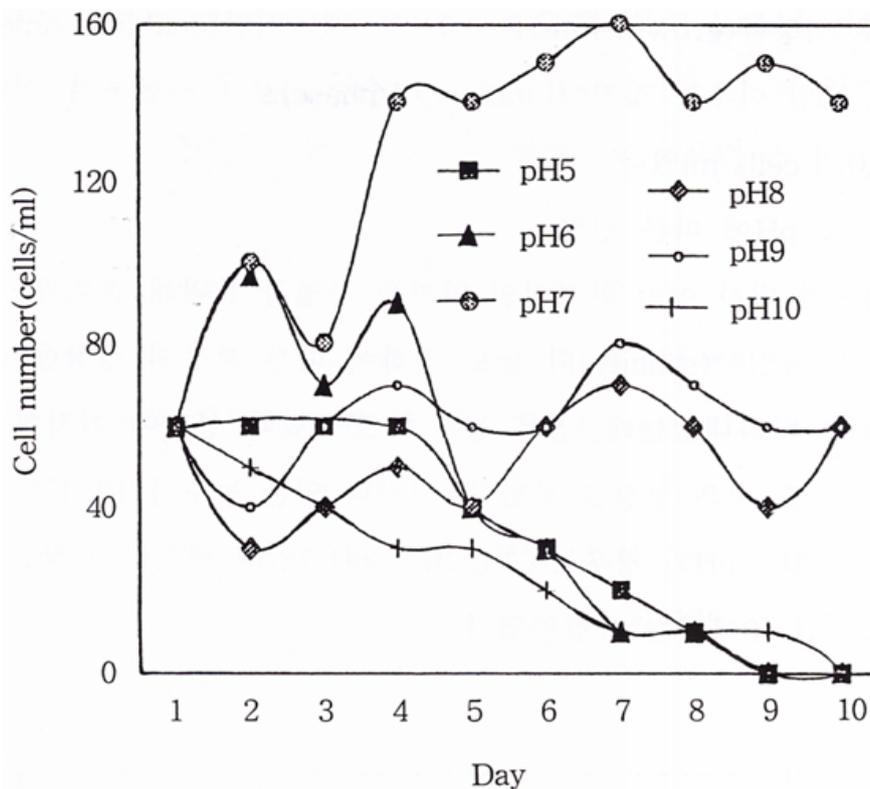


Figure 17. Growth curves of *Tamarenses* with various pH from National Institute of Fisheries Sciences [69].

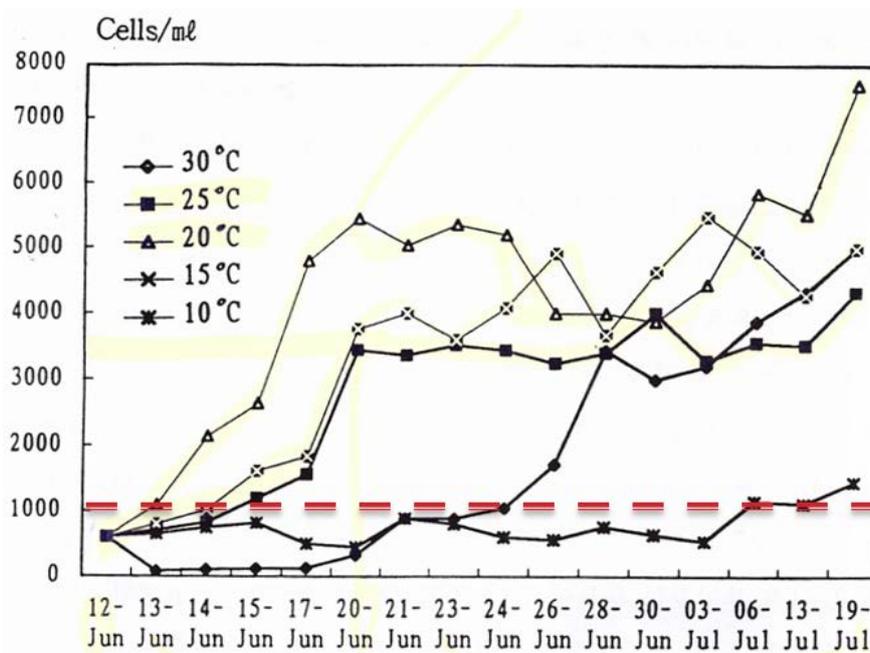
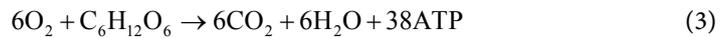


Figure 18. Growth curves of *C. polykrikoides* at the various temperatures from National Institute of Fisheries Sciences [69].

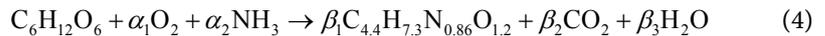


$(\dot{m})_{\text{consumptionI}}$ = Oxygen consumed by respiration of aerobic microorganism

as,



$(\dot{m})_{\text{consumption2}}$ = Oxygen consumed by algae and microorganism during biosynthesis,



Case 1; $(\dot{m})_{\text{gen}} > (\dot{m})_{\text{cons.}}$ $\frac{dm}{dt} > 0$ O.K.

Case 2; $(\dot{m})_{\text{gen}} < (\dot{m})_{\text{cons.}}$ $\frac{dm}{dt} < 0$ not O.K. but require external air bubbling

Therefore, DO is one of the important parameters for HAB not to suffocate fish.

Water mills and boat turbulence for the enhanced DO can inhibit HAB in freshwater and in seawater.

3.6.5. Food Web

Algae play an important role in the aquatic food web, as shown in **Figure 20**.

Algae growth and reproduction decreased so long as their preys of the particulate or dissolved in organic materials in aquatic system are limited. One of the best ways to prevent HAB is thus to increase their grazer, lytic bacteria, and virus [28].

3.6.6. Turbulence

HAB was inhibited by shear in the freshwater and in the seawater. Therefore, suitable nutrient, temperature, light, and shear are the initial growth requirements to prevent HAB [70]. Boat turbulence and water mills on the surfaces of the freshwater (river, lake, reservoir) and the seawater (fish farmery) disturb the water surfaces for turbulence to prevent HAB. Long hydraulic retention time without turbulence or shear induce HAB in the waters.

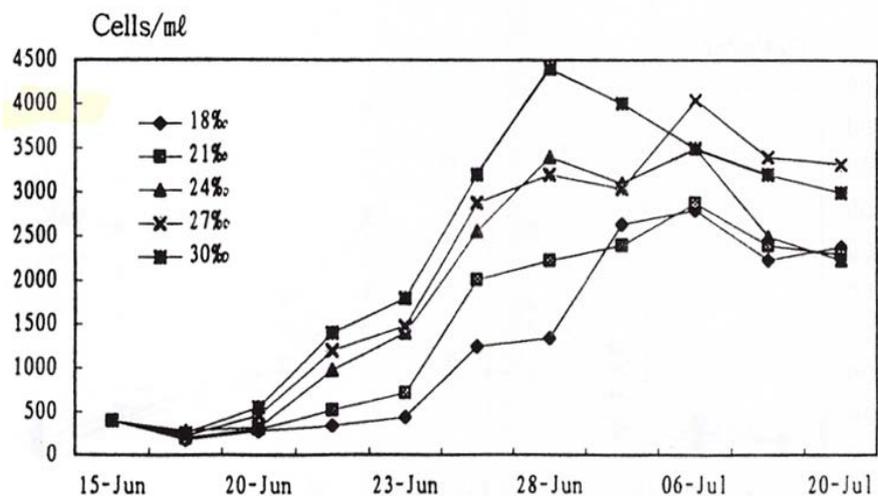


Figure 19. Growth curves of *C. polykrikoides* at 15°C with various salinity of the seawater from National Institute of Fisheries Sciences [69].

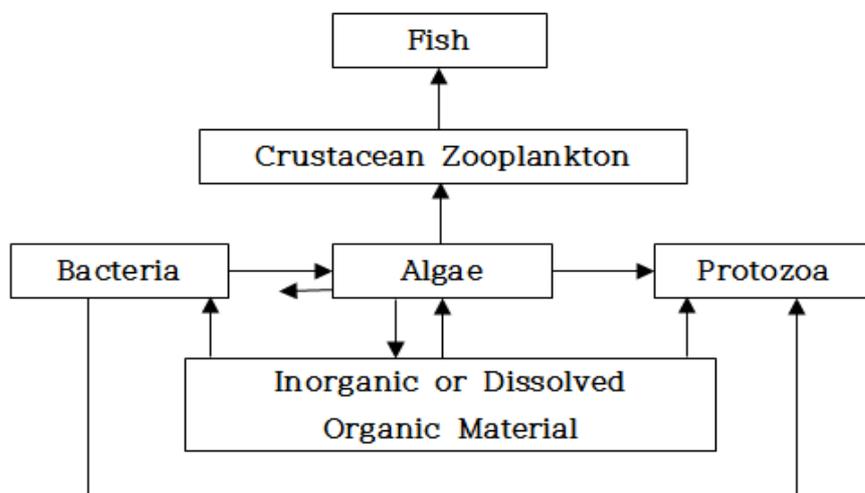


Figure 20. Conceptual diagram of aquatic food webs with algae (redrawn from Graham [14]).

3.6.7. Enzyme

Enzymes as protein molecules possess complex structures with a variety of reactive amino acid side chains in enzyme active sites to provide many kinds of catalytic activities. Especially, metal ion requirements for an enzyme are usually specific, with little or no enzyme activity observed if the specified metal ion is absent or if another metal ion is substituted for the natural one. Metal ions such as Fe, magnesium, manganese, cobalt, copper, nickel, molybdenum, zinc, and others are required for enzyme catalytic action. As with the organic cofactors, metal ions are required in only very small amounts, so they are considered essential trace elements or micronutrients. It can be seen that all the biological processes including HAB can be manipulated by their relevant enzymatic activities with micronutrients of metal ions. **Figure 21** shows the schematic flow diagram of HAB with enzymes. Since enzymes accelerate specifically the biosynthesis of macromolecules such as polysaccharides, lipids, and proteins to be made as algal membrane, cell wall, DNA, and RNA, only if the cofactors of mineral ions are available, the initial step of algal growth is fully dependent upon the availability of metal ions. It is thus necessary to keep certain levels of minerals free of aeolian desert dust not only for proper enzymatic reaction at an optimal temperature and pH but also for algal growth and reproduction during HAB.

3.6.8. Growth Phase

The algal growth curve is typically shown in **Figure 22(a)**. It is difficult to determine the timing of the onset of logarithmic growth phase (**Figure 22(b)**) to prevent HAB.

An index (θ) was proposed as control parameter of HAB defined as,

$$\tan \theta = \frac{d \log n}{dt}$$

$$\theta = \tan^{-1} \left\{ \frac{d \log n}{dt} \right\}$$

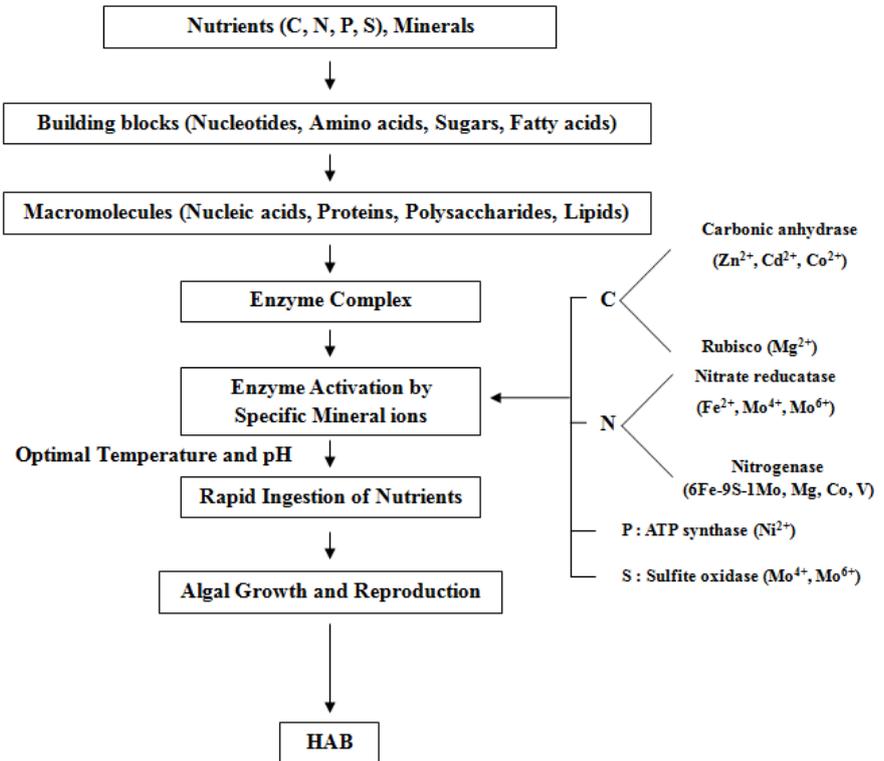


Figure 21. Schematic flow diagram of HAB with enzymes and minerals.

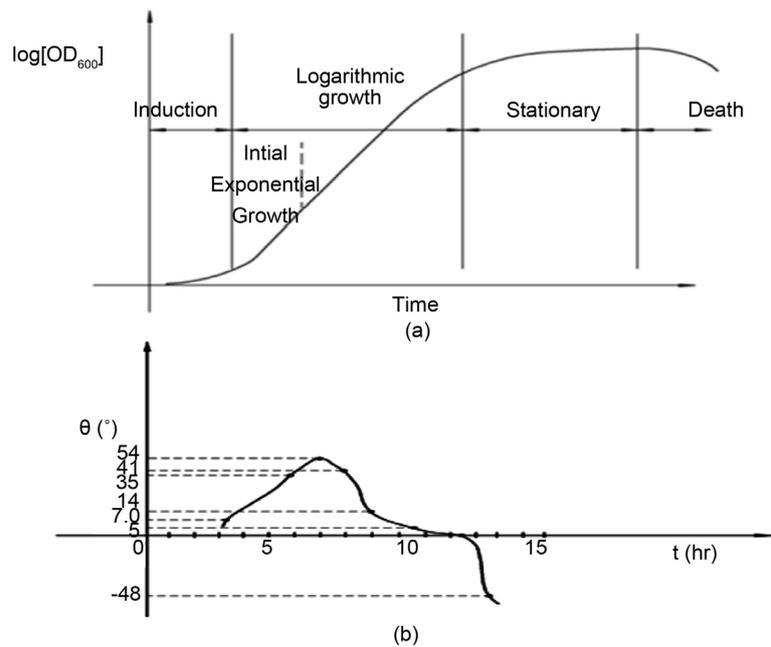


Figure 22. (a) Algal growth curve for various phases. (b) Determination of the onset time of logarithmic growth phase by index during HAB.

where, n : algal cell concentration (cells/ml), \tan^{-1} : slope of logarithmic algal growth curve $\left(\frac{d \log n}{dt}\right)$, t : time.

Figure 22(a) is a typical algal growth curve while **Figure 22(b)** is the index (θ) for determination of the onset of logarithmic growth phase during HAB. **Figure 22(b)** indicates that the onset of the logarithmic growth phase begins at the typical index of 7 until 54. It is therefore necessary to suppress HAB before reaching such a maximal index by various control measures of growth factors in **Table 4**. Therefore, HAB can be prevented if the algal growth is limited by the key parameters prior to reaching the early exponential growth phase, after which the algal toxins are produced to be superior to other competing species and reaching the exponential growth phase and the stationary phase where no further prevention tool is possible and we can only observe the resultant damage of HAB.

4. Conclusion

Harmful algal blooms (HAB) were initiated by the iron (Fe) from volcanic ash, river, bottom sediment, and desert dust to be converted as $\text{Fe}_{(\text{aq})}^{2+}$. The concentration of chlorophyll-*a* reached the peak value after Fe supply with a lag time of 11 days. It could be possible to predict the outbreak of HAB in both freshwater and seawater so long as the weekly distribution of Fe concentration in the desert dust was available in advance. HAB occurred in August after 4 to 5 weeks of heavy rainfall, exceeding an average of 103 mm rainfall in one day of July. Heavy rainfall ran off the nutrient-rich water from the river to the sea with occurrence of HAB in the seawater. Diluted salinity by heavy rainfall might not be the direct cause of HAB in the seawater. HAB could be prevented if the algal growth was limited by the key parameters prior to reaching the early exponential growth phase determined by an index (θ), after which no outcome is possible except HAB. Seasonal HAB was predicted by the surface area of algae size associated with the total sunlight energy. As for cyanobacteria, the order of large surface area was *Microcystis* > *Aphanizomenon* > *Oscillatoria* > *Anabaena* with seasonal HAB due to various light energy flux. Dissolved inorganic phosphate in lakes was low when pH was below 6 and temperature below 15°C while recycling the wastewater from the urbine wastewater treatment facility was suggested for further removal of phosphorus to reduce HAB in the river. Boat turbulence and water mills on the surface of the freshwater (river, lake, reservoir) and the seawater (fish farmery) disturbed the occurrence of HAB. The cell concentration of *Cochlonidium polykrikoides* in the seawater was linearly proportional ($R^2 = 0.9967$) to the optical density at 300 nm for on-line real time measurement. Prevention of HAB could be achieved by the decrease of pH to below 5 with CO_2 gas bubbling in the small area, a black curtain to block the sunlight during photosynthesis, and finally the Biogas (H_2S) from decomposed microorganisms could be bubbled into the water to prevent HAB in the early growth phase to remove the bioavailable iron $\text{Fe}_{(\text{aq})}^{2+}$ as insoluble iron sulfides (FeS/FeS_2). It was recommended to spread the silica powder (~74% Si) over the water surface for sedimentation of algae for prevention of HAB in freshwater or in seawater.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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