

Iron released from ilmenite mineral sustains the phytoplankton community in microcosms

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Running title: *Ilmenite mineral sustains phytoplankton community*

Abstract

Natural biotic communities from Kalbadevi bay were monitored in microcosms of one litre flasks to test the postulate that iron released from ilmenite through microbial action contributes to proliferation of phytoplankton. Microcosms containing ilmenite had significantly higher phytoplankton growth compared to controls containing no ilmenite or those containing only ferrous sulphate. Phytoplankton cell numbers in the ilmenite supplemented treatment was 20× higher than in controls. The later stages were marked by the dominance of bottom dwelling *Nitzschia* spp and *Navicula* spp. Iron-preferring *Eunotia* spp and *Pinnularia* spp were also encountered. These results suggest that the increased phytoplankton biomass may be attributed to the steady release of bio-available iron. It is therefore inferred that ilmenite mineral could sustain the phytoplankton community in ilmenite-dominated systems. Increase in density and diversity of phytoplankton over time and significant interrelationship between various parameters in the microcosm containing ilmenite indicate that stimulatory effect of iron released from this mineral could be beneficial to the biota in the system.

Key words: Ferrous sulphate; Ilmenite; Iron; Phytoplankton; Placer

List of non-standard Abbreviations: (ATP, Adenosine tri phosphate; Chl *a*, Chlorophyll a; Ferric, Fe(III); Ferrous, Fe(II); TC, Total counts of bacteria)

Introduction

Nearly 40% of the global photosynthesis occurs in the aquatic environment (Falkowski, 1994). The turnover time for the biomass of marine primary producers is generally considered to be three orders of magnitude faster than that of terrestrial biomass (Jacobs and Worwood, 1974). Thus, phytoplankton play an important role in the global carbon cycle and subsequently on the world's climate. In turn, the nutrients that regulate this primary production also have a role in the process (Ussher et al, 2004). Iron is primarily required by phytoplankton for many of its metabolic reactions and hence is one of the most important trace metals. It is involved in intracellular respiration and oxygenic and non-oxygenic photosynthesis (Falkowski and Raven, 1997). It not only forms a crucial component of the cytochromes, PSI and PSII of the photosynthetic apparatus, but is also involved with ATP synthase (Jacobs and Worwood, 1974). The role of this trace metal in biochemical reactions has been effectively demonstrated in a number of laboratory experiments (Greene et al, 1991, Geider et al, 1993, Sunda and Huntsman, 1997, Kudo et al, 2000, Milligan and Harrison, 2000). Thus, iron is an important micronutrient controlling marine primary production. Its availability to algal cells depends on the species of iron (Rich and Morel, 1990; Wells, 1991; Kuma and Matsunaga, 1995).

In natural waters, iron exists in either the reduced ferrous or the oxidized ferric states (Ussher et al, 2004). Studies show that nearly 99% of the dissolved Fe in seawater is complexed with strong organic ligands (Rue and Bruland 1995, 1997, Wu and Luther 1995). Consequently, there are ecosystems that are limited by the available form of iron.

The coastal area is a key environment in the global iron cycle, where the brackish water environment changes the physicochemical speciation, and thus mobility, of river-introduced iron via aggregation, sedimentation and redox processes (Breithbarth et al, 2010). The transport of iron to the marine environment is usually through three pathways: fluvial input, atmospheric deposition and processes on the seafloor such as diagenesis, sediment re-suspension and hydrothermal venting (Ussher et al, 2004). Thus, iron-containing placer minerals like magnetite and ilmenite released from terrestrial rocks and transported by rivers and streams from catchment areas into the adjoining seas may be a major source of iron especially in the near-shore environments. Although there is a substantial literature on the use of the inorganic form of iron like ferrous sulphate and its role in high-nitrate, low-chlorophyll (HNLC) waters (Harrison et al, 1999), little is known about how iron bound in minerals such as the placer mineral- *ilmenite* is used by the marine biota. Besides, this mineral is widely distributed along the coast of India (Ali et al, 2001).

Kalbadevi Bay, Ratnagiri on west coast of the country is rich in this placer mineral (Krishnan and Roy, 1945; Siddiquie and Rajamanickam, 1979; Siddiquie et al, 1979; Gujar, 1996; Valsangkar and Fernandes, 2009). Interestingly, our observation of the floor of the bay detected widely distributed mats of green algae, which led to the question “could iron leaching out of ilmenite support abundant algal growth?”. In order to answer this question, incubation experiments were set up in microcosms with and without ilmenite and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ over 84 days. Ilmenite-rich sediment from the test site was used as inoculum and changes in the concentration of reduced and oxidised iron generally mediated by indigenous bacteria was followed along with the changes in phytoplankton abundance. We postulated that iron released steadily from the ilmenite mineral through microbial action was bio-available and able to sustain the phytoplankton community in the microcosm for a longer period of time than those supplemented with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. This is the first report of the influence of iron released from ilmenite on the primary producers like phytoplankton and the associated bacterial communities.

Materials and methods

The mineral ilmenite

The sediment samples were collected from Kalbadevi beach, Ratnagiri, India ($17^\circ 04' 03.994 \text{ N}$, $73^\circ 17' 17.557 \text{ E}$) (Fig.1) and subjected to sieve analysis in ASTM sieves. The fractions obtained after sieving were separated into magnetic and non-magnetic fractions using a bar magnet. The non-magnetic fractions were further subjected to heavy liquid separation (bromoform, specific gravity 2.89 g cm^{-3}), following standard procedure. The heavies obtained from the heavy liquid separation were further subjected to isodynamic separation at 0.275 \AA for separation of ilmenite. (Gujar et al, 2010). The mineral obtained was further subjected to thorough washing with acid and MilliQ water. Subsequently, the mineral was dried and autoclaved before use in laboratory experiments. Purity of the ilmenite used was confirmed by energy dispersive X-ray spectroscopy (EDS) using a scanning electron microscope equipped with a energy-dispersive X-ray spectrometer (JEOL-JSM-5800, Japan) (Fig 2). The chemical composition of the mineral was O – 36.22%, Ti – 30.94%, Fe – 23.89%, Si – 3.85%, Al – 2.39%, Mg – 2.08%, Cu – 0.81%, Zn – 0.60%, V- 0.53%, K – 0.32%.

Release of iron over 15 d by indigenous bacteria in microcosms containing Kalbadevi beach sediments in 50ml vials filled to the brim.

Sediment samples were collected from (5-10) cm layer depth from the berm region at Kalbadevi beach and divided into three parts. One part was used to detect the release of iron from ilmenite

sediment and two parts were used as controls. Controls were in the form of heat and azide inactivated sediments. Each set was run in triplicates for 15 d. The experimental set up is described in table 1a. Approximately 1 g of sediment was added to 50 mL modified ferrozine media (Nielsen et al, 2002). The ferrozine media was prepared in artificial sea water containing 20 mM HEPES and 350 μ M ferrozine. pH was maintained at 7.8 ± 0.2 . Sodium molybdate was added at a concentration of 20 mM to inhibit sulphate reduction. Individual sets were maintained and sacrificed for each monitoring day. The vials were incubated in the dark at ambient temperature of $30\pm 0.2^\circ\text{C}$ to avoid photo-oxidation. Production of Fe(II) was indicated as a pink colouration due to the binding to ferrozine reagent (Stookey, 1970; Lovley and Phillips, 1986; Neubauer et al, 2002). Aliquots from the inoculated and control media were taken from each tube and amount of soluble Fe(II) was determined by measuring OD at 562 nm using a spectrophotometer (UV Mini, Shimadzu, Tokyo, Japan). All parameters measured were normalized for one gram dry sediment.

Microcosm experiments to determine the effect of iron released from ilmenite over 84 d in one litre flasks containing 750ml seawater

Experimental setup

The natural phytoplankton community that was present in the Kalbadevi Bay waters during sample collection was used to set up laboratory microcosm experiments. Seawater used to set up the experiment was filtered through 200 μ m mesh to eliminate large grazers like zooplankton. The background concentration of seawater of nitrate in the bay waters ranged from 0.24-0.8 μ M, nitrite from non detectable levels to 0.04 μ M, phosphate from 0.13 to 0.22 μ M and silicates from 0.05 to 1.46 μ M (Anon, 2007). The average total Fe concentration in the waters was $5.4 (\pm 0.1)\text{mg l}^{-1}$.

Treatments

Four different experimental combinations were set up with appropriate controls. Each combination consisted of 12 flasks of 1 l capacity containing 0.75 l of the media (Table 1b). In order to simulate natural the prevailing natural conditions, no additional nutrients or chelating agents were not added. Incubation flasks were randomly assigned in duplicate to the four experimental treatments: Control (“control”), added iron at a concentration of 10 μ M $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (“added Fe”), added mineral source in the form of 100 mg ilmenite (“added Ilm”), and a added combination of mineral (ilmenite) and iron (10 μ M $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$) (“added Ilm and Fe”). Microcosms were sampled by sacrificing one set at 0, 3, 7, 15, 28, and 84 d. However, results presented here are till the 28 d. Parameters monitored

consisted of physico-chemical and biotic variables. Physico-chemical variables monitored were pH, nitrates, nitrites, phosphates, silicates, ferrous [Fe(II)] and ferric [Fe(III)]. Biotic variables measured were total bacterial abundance (TC), adenosine tri phosphate (ATP), chlorophyll *a* (Chl *a*), phytoplankton density, and change in generic composition of the natural assemblage.

Chemical variables

pH was measured with Orion 3star pH meter of Thermo, Beverly, USA.

Sub-samples for inorganic macronutrient analyses such as nitrates, nitrites, phosphates and silicates were performed using a Technicon TACS (Model AA2) autoanalyzer following the methods described by Wurl (2009). Freshly prepared calibration standards were used for each batch. Standards were diluted before use from concentrated stock solutions of the respective salts in double distilled water. The minimum detection limits for the autoanalyzer measurements for silicate was 100nM nitrate was 50nM, nitrite was 10 nM and phosphate was 10nM.

The speciation of Fe was measured using a voltammeter in interface with 797VA computrace (Metrohm, Zofingen, Switzerland). The potential was measured against an Ag/AgCl reference electrode and a platinum rod as a counter electrode. The Fe determination was carried out with SMDE (static mercury drop electrode) in the differential pulse mode. Milli-Q water (MQ; Millipore, USA; resistance 18.3M Ω) was used for reagent and sample preparation. Prior to analysis, 10ml of samples required for the determination of different speciation of iron were placed in acid-washed quartz tubes and then were ultraviolet (UV) irradiated with a 400-W low-pressure Hg lamp for 30min to decompose interfering organic compounds. The speciation of iron was carried out at pH 7 in a measuring cell containing 10ml of supporting electrolyte (0.1M sodium pyrophosphate). The blank was registered before sample addition after 300s degassing with 99.9995% nitrogen (Medgas and Equipments). Depending on the concentration, 0.1 to 1 ml aliquots were appropriately diluted with charcoal treated (to reduce background) and filtered (0.22 μ membrane) Milli-Q water (18.2 Ω resistance) prior to analyses. The sample was directly added to the measuring vessel containing the electrolyte and degassed for another 180s prior to deposition. The analysis was carried out with deposition time 30s, equilibration time 6s, voltage step 5mV and voltage step time of 0.5s. The potential was scanned in triplicates from 0 to -1.3V with sweep rate of 10mVs⁻¹. The analysis was quantified with 2 standard additions of Fe(II) [FeSO₄.7H₂O, 1g l⁻¹ stock solution prepared in 0.1M HCl] and Fe(III) [NH₄Fe(SO₄)₂.12H₂O, 0.5g l⁻¹] in 0.1M phosphoric acid]. The determination of iron was carried out following modified Metrohm VA application work DE4-0191-032007.

Biotic variables

Chl *a* pigment was extracted in 10 ml of 90% v/v acetone at 4°C for 18 h after water samples were filtered through Whatman GF/F filters (Maidstone, UK). The fluorescence of the extract was measured before and after acidification with Trilogy, Turner Designs fluorometer, Sunnyvale, USA based on the method developed by Yentsch and Menzel (1963) and Holm-Hansen et al (1965). Chl *a* obtained from *Anacystis nidulans* (Sigma) was used as the standard for calibration.

Phytoplankton density was estimated after samples were concentrated from a volume of 100 mL to a volume of ca. 20 mL using settling and siphoning procedure (Hasle, 1978). Abundance of phytoplankton species was determined by enumeration of cells in a Sedgwick-Rafter chamber (McAlice, 1971) using an Olympus Inverted Microscope (IX51, Japan) at 200x and 400x magnification. The entire chamber was examined and possible taxonomic identification was carried out according to Tomas (1997). The identification of the genera was given according to Tomas (1997).

TC was estimated after fixing water samples with pre-filtered formaldehyde (2.5% final concentration), and staining with acridine orange for 5 min. The stained preparation was filtered through polycarbonate Isopore™ Membrane filters (0.22 µm GTBP, Millipore, Ireland) and cells were counted using an epifluorescence microscope (Nikon 50i, Nikon, Tokyo, Japan) (Hobbie et al, 1977).

ATP content was analyzed using the luciferin-luciferase reaction after filtering the water samples through a 0.22 µm nitrocellulose membrane filters (Millipore, Ireland) and boiling the filter paper in 0.1 mM Tris buffer (pH 7.8) for 5min. The extract was centrifuged at 5000rpm for 5 min. About 100 µl of the collected supernatant was mixed with 100 µl of firefly enzyme extract (Firefly lantern FLE 50; Sigma) (Holm and Hansen, 1966). Counts were taken with a Luminometer (BMG Labtech, Lumistar Optima, Hanns-Martin, Germany).

Data analyses

Pearson correlation coefficients were calculated using Statistica version 10 for estimating the relationships between environmental variables and the biotic community in the microcosms. Post hoc correlations were carried out and Bonferroni's adjustment was incorporated according to Legendre and Legendre, 1998. Only significant, relevant and direct inter-relations are shown. The k=13,

$\alpha=0.05$, $\alpha'=0.0038$, where k = number of treatments, α = uncorrected significance level, α' =corrected significance level.

Results

Release of iron over 15 d by indigenous bacteria in microcosms containing Kalbadevi beach sediments in 50ml vials filled to the brim.

Iron release from ilmenite occurred in native and heat-killed and azide poisoned controls at ambient temperature of $30\pm 2^\circ\text{C}$. However, the levels of accumulation of Fe(II) in microcosm with native sediments were considerably higher at 69 and 94% than the two controls, respectively. The biological release of iron from ilmenite started from the 3 d and peaked on 11 d. In the experimental tubes, the average rate of iron released from ilmenite in the form of Fe(II) was nearly 54 and 80% higher than the heat killed and azide poisoned controls (Fig 3). Fe (II) and Fe(III) correlated negatively in control and “added Fe” but positively in “added ilm” and “added ilm and Fe” treatments (Table 2) indicating pulsed release of Fe(II) from ilmenite followed by oxidation to Fe(III).

Microcosm experiments to determine the effect of iron released from ilmenite over 84 d in one litre flasks containing 750ml seawater

Nutrient consumption

At 0 h, the concentrations of the nutrients were $3.9\ \mu\text{M}$ nitrates, $0.02\ \mu\text{M}$ nitrites, $0.02\ \mu\text{M}$ phosphates and $1.46\ \mu\text{M}$ silicates. Nitrate concentrations fell to non detectable levels in all the treatments by 3d (Fig 4a,b,c,d). After 15d, nitrate concentrations increased slightly in “added Fe”, “added ilm” and “added ilm and Fe” treatments. Phosphate concentrations increased to 0.04 - $0.08\ \mu\text{M}$ on 3 d in control and amended flasks (Fig 4a,b,c,d). Increases in silicate concentrations were much more in the amended flasks than the control (Fig 4a,b,c,d).

Initial concentration of Fe(II) and Fe(III) varied from 1.77 to $4.39\ \text{mg l}^{-1}$ and 3.71 to $6.50\ \text{mg l}^{-1}$ respectively. Generally, concentrations of Fe(II) and Fe(III) followed alternate patterns of increase and decrease with time in control and “added Fe” treatment (Fig 4e,f). The scale of the change was much higher in the treated flasks with time especially in “added ilm” flask compared to the unamended control (Fig 4g,h).

Effect of added ilmenite and Fe on biotic variables

Total living microbial biomass – ATP

ATP concentration exhibited different temporal patterns for the different treatments (Fig 5 a,b,c,d). ATP presented maximum concentration on 3, 7, 28 and 15 d for “control”, “added Fe”, “added ilm” and “added ilm and Fe”, respectively. Generally, in flasks containing no amended ilmenite, ATP concentration increased during the first few days of incubation, followed by a decrease. However, in the flasks containing only ilmenite i.e. “added ilm” supplementation, ATP increased significantly with time and was 50 times higher than in “added ilm and Fe” (Fig 5c,d, Table 2)

Total abundance of bacteria (TC)

Generally, bacterial abundance increased significantly with time in ilmenite-unamended flasks (Fig 5a,b). Exponential increase was recorded in “added Fe” treatment (Fig 5b) while numbers were static at 10^9 cells l^{-1} in both the ilmenite amendments (Fig 5c,d). The abundance on peak days were 8.8×10^9 cells l^{-1} in “control”, 16.7×10^9 cells l^{-1} in “added Fe”, 5.7×10^9 cells l^{-1} in “added ilm”, 5.6×10^9 cells l^{-1} in “added ilm and Fe” on 28, 28, 7, 15 d respectively.

Chlorophyll a (Chl a)

Chl *a* concentration began to increase in all treatments by 3 d (Fig 5 e,f,g,h). It reached maximum values at 7 d in the control and “added Fe”, at 28 d in the “added ilm”, and at 15 d in “added ilm and Fe” treatment. Peak concentrations were 0.14, 0.22, 0.80 and 0.84 μg l^{-1} in “control”, “added Fe”, “added ilm” and “added ilm and Fe”, respectively. The decline in chlorophyll was sharper in the control and “added Fe” treatments than in “added ilm and Fe” treatment. Chl *a* concentrations correlated significantly with phytoplankton in both the control and experimental flasks (Table 2).

Phytoplankton abundance

Phytoplankton cell abundance showed a mixed response in all the treatments (Fig 5 e,f,g,h). Initial cell number was $8(\pm 0.4) \times 10^3$ cells l^{-1} . The highest abundance of $33.9(\pm 9.8) \times 10^3$ cells l^{-1} was recorded on 7 d in “control”, and decreased thereafter. Similarly in “added Fe” treatment, peak abundance was attained on the seventh day and the abundance was $70.9(\pm 13.4) \times 10^4$ cells l^{-1} . Phytoplankton cell numbers increased significantly with time in ilmenite amended flasks and maximum abundance was recorded at 28 d in both the treatments. Further, phytoplankton abundance correlated significantly with ATP in “added ilm” and “added ilm and Fe”. Phytoplankton related

positively with Fe(II) and Fe(III) in both “added ilm” and “added ilm and Fe” flasks (Table 2). Random counts of ciliates showed that the average numbers were 12×10^2 , 8.8×10^2 , 14.4×10^2 and 12.8×10^2 cells l^{-1} for “control”, “added Fe”, “added ilm” and “added ilm and Fe”, respectively.

Distribution of phytoplankton assemblages

Concerning the phytoplankton assemblages, some common trends were observed in all treatments. Diatoms dominated in terms of the number of genera and abundance. Centric diatoms consisting of *Coscinodiscus* spp and pennate diatoms comprising of *Navicula* spp., *Nitzschia* spp and *Thalassiothrix* spp contributed to the chlorophyll maximum (Fig 6).

At the start of the incubation period, the phytoplankton assemblage comprised of 14 genera namely *Asterionella* spp., *Biddulphia* spp., *Coscinodiscus* spp., *Ditylum* spp., *Grammatophora* spp., *Navicula* spp., *Nitzschia* spp., *Phaeocystis* spp., *Prorocentrum* spp., *Rhizosolenia* spp., *Synedra* spp., *Thalassionema* spp., *Thalassiothrix* spp., and *Triceratium* spp. (Fig 6a). During the course of the experiment, 29 genera were identified. Highest numbers of genera were recorded by the end of 3 d. It was 16 in “control”, 17 in “added Fe” and 28 in “added ilm and Fe” flask. However, maximum genera of 27 were recorded at the end of 15 d in “added ilm” treatment. *Nitzschia* spp. dominated followed by *Navicula* spp in most of the treatments (Fig 6b,c,d,e).

Though *Coscinodiscus* spp. was present in large numbers in the controls, their abundance was much lower in microcosms containing ilmenite amendments. However, *Eunotia* and *Pinnularia* spp. increased in ilmenite amended flasks. These species were conspicuously absent in the “control” and “added Fe” treated flasks during the later stages of incubation (Fig 6d,e). Highest Shannon diversity index was recorded in ilmenite amended flasks.

Visual observations showed that there was massive development of green algae at the bottom of the flasks in ilmenite amended treatments after 84 days of incubation. However, in treatments containing only iron, the phytoplankton biomass was less and the mats were translucent and distributed throughout the flasks.

Discussion

We compared the effect of readily-available iron in the form of inorganic $FeSO_4 \cdot 7H_2O$ with that of the iron released from ilmenite. The experiments showed that iron released from ilmenite is

stimulatory, but slower-acting, presumably because of the slower release of iron. Incubation times required for measurable effects were much shorter with ferrous salt supplementation.

Preliminary experiments carried out to test whether the native bacterial population could release soluble Fe(II) iron from the mineral ilmenite were positive. Concentration of Fe(II) in the experimental sediments was generally higher than the controls at 69 and 94% at the end of the incubation period. This indicated that the microbial rate of release of iron from the ilmenite was higher than the controls. Reductive dissolution is known to occur in natural ilmenites which also contain significant amounts of Fe(III) (White et al., 1994). However, the microbial participation acts as a catalyst and increases the reaction several fold. Thus, the accumulation of iron in the form of Fe(II) and Fe(III) in the “added Ilm” of 84 d microcosms, even without any added $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, suggest that there was a microbial participation. These results are also complemented by the results from 15 d microcosms where the release of iron by indigenous bacteria was evident (Fig 3). The accumulation of the two species of iron is also noted in the “added Ilm and Fe” treatment. Thus, the increased rates in the presence of bacteria and phytoplankton in the microcosms suggest their contribution in the recycling of this element i.e. uptake and release of iron. Although, there was an alternate increase and decrease of iron which can be attributed to chemical oxidation and reduction, the rate gets accelerated 0.2 to 2.76 times in the presence of bacteria and phytoplankton community over the period of incubation.

The influence of iron released from ilmenite on living organisms was measured as ATP flux. ATP is a measure of total living biomass and in this experiment it acts as a proxy for both bacteria and phytoplankton community. In nature, the release of iron not only supports bacteria but other living organisms as well. Iron plays a major role in the reduction of oxygen for synthesis of ATP, photosynthesis and respiratory electron transport (Geider and La Roche 1994; Neilands, 1995). Our experiment clearly demonstrates that iron released from the ilmenite contributed to elevated ATP concentrations (Table 2). This is corroborated by high phytoplankton in “added Ilm” but steady bacterial densities.

Furthermore, in the presence of ilmenite, bacterial numbers were more or less static at 10^9 cells l^{-1} suggesting that the input is equal to the removal. This lack of increase in bacterial biomass could be due to control by the protozoans. When the bacterial abundance was maximum, the protozoan abundance was minimum and vice versa suggesting that the “top-down” control was operative (Fig 7). Ciliates were generally encountered at 10^2 cell l^{-1} in these samples. They are also known to prey on bacteria (Bernard and Rassoulzadegan, 1990). The equivalent responses in “added Ilm” and

“added Ilm and Fe” flask suggests that bacterial numbers were maintained steady by higher number of ciliates. Higher number of bacteria in the “added Fe” is due to immediate response of bacteria which have shorter generation times. Besides, lower number of ciliates are also responsible. Lower number of ciliates in “added Fe” compared to “added Ilm and Fe” could be due to higher availability of Fe made possible by relatively lower numbers of phytoplankton. Thus, the concentration of Fe in the former could be above the optimal limit restricting the numbers of ciliates. However, van Wambeke et al. (2008) showed that after stimulation of heterotrophic bacteria with Fe, the number of heterotrophic flagellates also increased which leads to enhanced bacterial predation. Alternatively, the difference in our study could also be due to the difference in the response time of ciliates.

Iron is required not only in the respiratory cycle, but also in photosynthesis. Chl *a* was used as a proxy for live phytoplankton biomass because of the difficulty in differentiating between live and dead phytoplankton. During the course of the experiment, both phytoplankton density and Chl *a* increased in the treatment flasks. In these, it was much higher than in “control” and “added Fe” flask, and peaked on 14 d. In “control” and “added Fe” flask, the Chl *a* concentrations peaked by 3 d, and there was a gradual decrease thereafter. The early and low peak in the “control” may be due to the low concentration of ambient iron. On the other hand, the early and high peak in “added Fe” flasks may relate to the higher bio-availability of iron in the form of added $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The delayed peaks in “added Ilm” and “added Ilm and Fe” flasks are attributable to the slower release of iron from ilmenite. The concentration of Chl *a* was high in “added Ilm and Fe” and higher still with “added Ilm” alone. This is probably because of lower and optimal release of iron from ilmenite alone. Probably the lower and steadier release of iron from ilmenite prevented the system from crashing. Fig 5g and 6d clearly shows a further increase in Chl *a* and phytoplankton which supports our inference. Thus, our experiment clearly demonstrates that the iron released from the mineral ilmenite is used in the photosynthetic cycle and thus stimulates the growth of phytoplankton. In mesoscale experiments conducted elsewhere, phytoplankton biomass and productivity, especially in large-celled phytoplankton such as diatoms, increased several fold after Fe enrichment in the surface mixed layer (Martin et al, 1994; Coale et al, 1996, 2004; Boyd et al, 2000; Tsuda et al, 2003). Chl *a* correlated significantly with phytoplankton abundance, suggesting high cell viability.

The average abundance of phytoplankton in “added Ilm” and “added Ilm and Fe” flask increased significantly with time and abundance was >20-fold higher than in “control” flask. Both ilmenite amended treatments supported 27 and 28 genera, respectively, whereas “control”, “added Fe” flasks

supported 16 and 17. The “added Ilm” flask supported the highest phytoplankton count and diversity index (Fig 8).

The temporal distribution of phytoplankton assemblages of natural communities in microcosms indicated that diatoms were dominant in control as well as the experimental microcosms. Percentage of centric diatoms, were observed to increase by >90% of the original assemblage. This initial blooms of fast-growing centric diatoms have also been reported in other microcosm experiments (Grice et al. 1977, Davis 1982, Estrada *et al*, 1988). Earlier studies have shown that factors that were likely to induce the initial bloom include reduction of physical losses, and predator exclusion (Estrada *et al*, 1988, Berdalet et al, 1996). Bottom dwelling genera such as *Nitzschia* spp and *Navicula* spp. proliferated at the later stages of the experiment in all microcosms suggesting they had tolerance to a wide range in iron concentrations. However, in the presence of ilmenite, percentage occurrence of two genera namely *Eunotia* and *Pinnularia* spp. increased during later stages of the experiment. Both these species occur in iron enriched waters of moderately high conductivity (Czarnecki and Cawley, 1997), thus suggesting their preference for higher concentrations of iron. Strands of filamentous algae and bubbles were also especially prevalent in “added Ilm” flask indicating active algal and plankton growth. In contrast, “control” had no visible growth throughout the experiment. Growth in “added Fe” was marked by white floccules of diatomite indicating early loss of viability. Such observations were made by Sutherland et al, (1998). “Blister” or “bubble” mats have been usually attributed to oxygen trapped in the microalgal mucilage (Jørgensen et al, 1983; Yallop et al, 1994).

During the course of the experiment, the change in the nutrient levels were monitored for understanding the dynamic response of biotic variables. The fall in nitrate levels could be attributed to the uptake by phytoplankton and associated bacteria. Subsequent increase of this nutrient on 15 d, could suggest the recycling through ammonification, nitrification from dead and senescent cells. Nitrates were consumed relatively faster than phosphates and silicates. It may be possible that the diatoms forming the bulk of the initial population peak possibly consume the available nutrients. Increase in phosphates during the later stages of incubation suggests release of this nutrient from bacteria and phytoplankton. Net increase in the silicate concentrations could possibly be due to the higher rate of release by silicate solubilising bacteria than its uptake by the phototrophic community.

Conclusion

We demonstrated the influence of bio-available iron released from ilmenite by microbial action on the abundance and diversity of phytoplankton. Ilmenite enrichment led to higher diversity. The interrelationship between different parameters, abundance and diversity of phytoplankton over time all suggest the maintenance of a stabler microcosm in “added Ilm” system under the influence of iron released from ilmenite for a longer duration of time. Thus, in the presence of biotic community, there is a possibility of reductive dissociation of the mineral ilmenite which possibly provides a source of bio-available iron.

Thus, our study suggests that the stimulatory effect of iron released from ilmenite could be beneficial to the biota in the microcosms. The bay sediments have been known to harbour ilmenite. Most probably such an effect could be expected in bay waters of Kalbadevi. However, further in situ time series measurements of the processes mediated by bacteria and phytoplankton would lend support to the present study.

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Figure legends

Fig. 1. Map of the study area

Fig. 2. SEM and EDS spectrum of the mineral ilmenite

Fig. 3. Release of iron by indigenous bacteria in microcosms containing Kalbadevi beach sediments in 50ml vials filled to the brim.

Fig. 4. Temporal changes in nitrate (open circles), nitrite (filled circles), phosphate (open squares), silicate (closed squares), ferrous (open triangles), ferric (filled triangles), and pH (closed stars) in different treatments

Fig. 5. Temporal changes in total bacterial counts (open circles), ATP (filled circles) phytoplankton (open squares) and Chl *a* (filled squares) in different treatments

Fig. 6. Genera composition of phytoplankton assemblage during different day in different treatment in control, added Fe, added ilmenite and added combination of ilmenite and Fe.

Fig. 7. Response of phytoplankton and bacterial abundance to presence of ciliates.

Fig. 8. Temporal distribution of phytoplankton assemblage in different microcosms

a. Diversity index b. Number of Genera

Table 1: Experimental set up for microcosm studies

a) Release of iron by indigenous bacteria in microcosms containing Kalbadevi beach sediments in 50ml vials filled to the brim in soluble form from ilmenite

Experimental set up	Set I	Set II	Set III
Kalbadevi sediment with native bacteria	√	√	√
Ferrozine medium I (50ml)	√	√	√
Autoclaving at 121 °C for 15 min (heat killed)	-	√	-
Sodium azide (20 mM)	-	-	√

b) To determine the effect of iron released from ilmenite in microcosm

Experimental set up	Seawater	Ilmenite (100mg)	Fe 10μMFeSO ₄ .7H ₂ O
control	√	-	-
added Fe	√	-	√
added Ilm	√	√	-
added Ilm and Fe	√	√	√

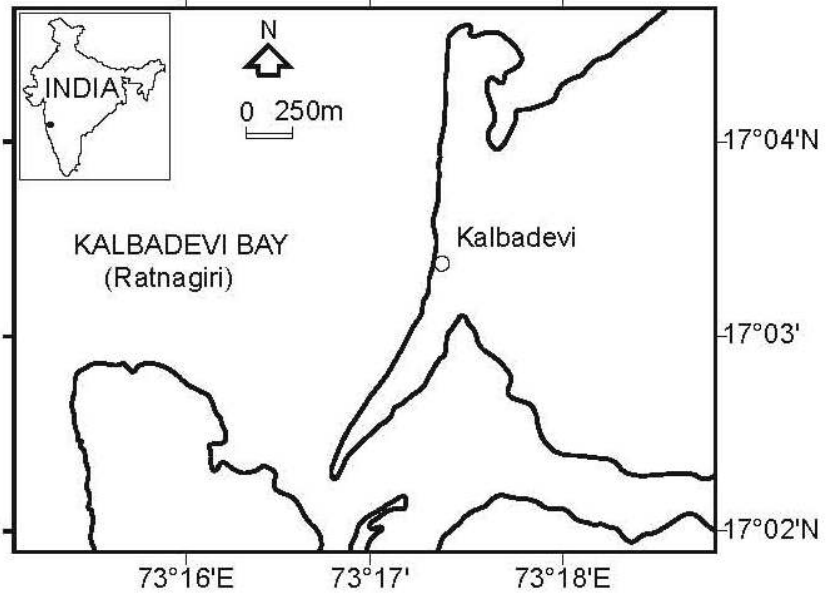
* √ - addition

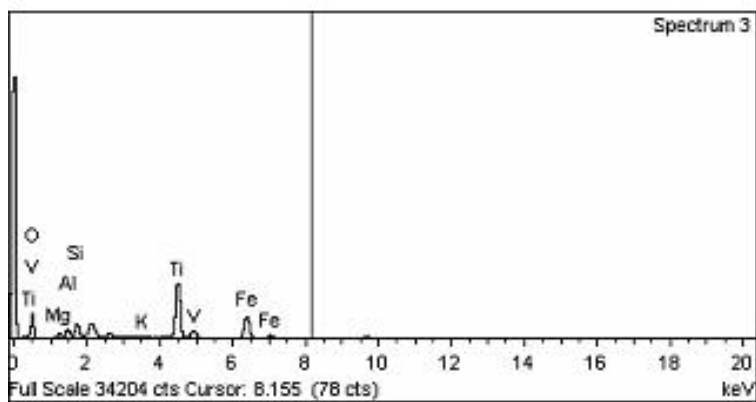
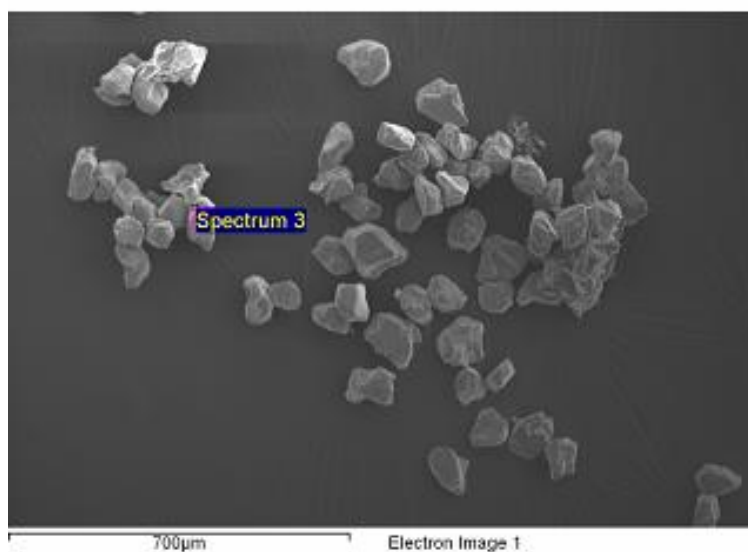
Table 2: Post hoc treated (Bonferroni's adjustment) Pearson's correlations between various parameters in the microcosm

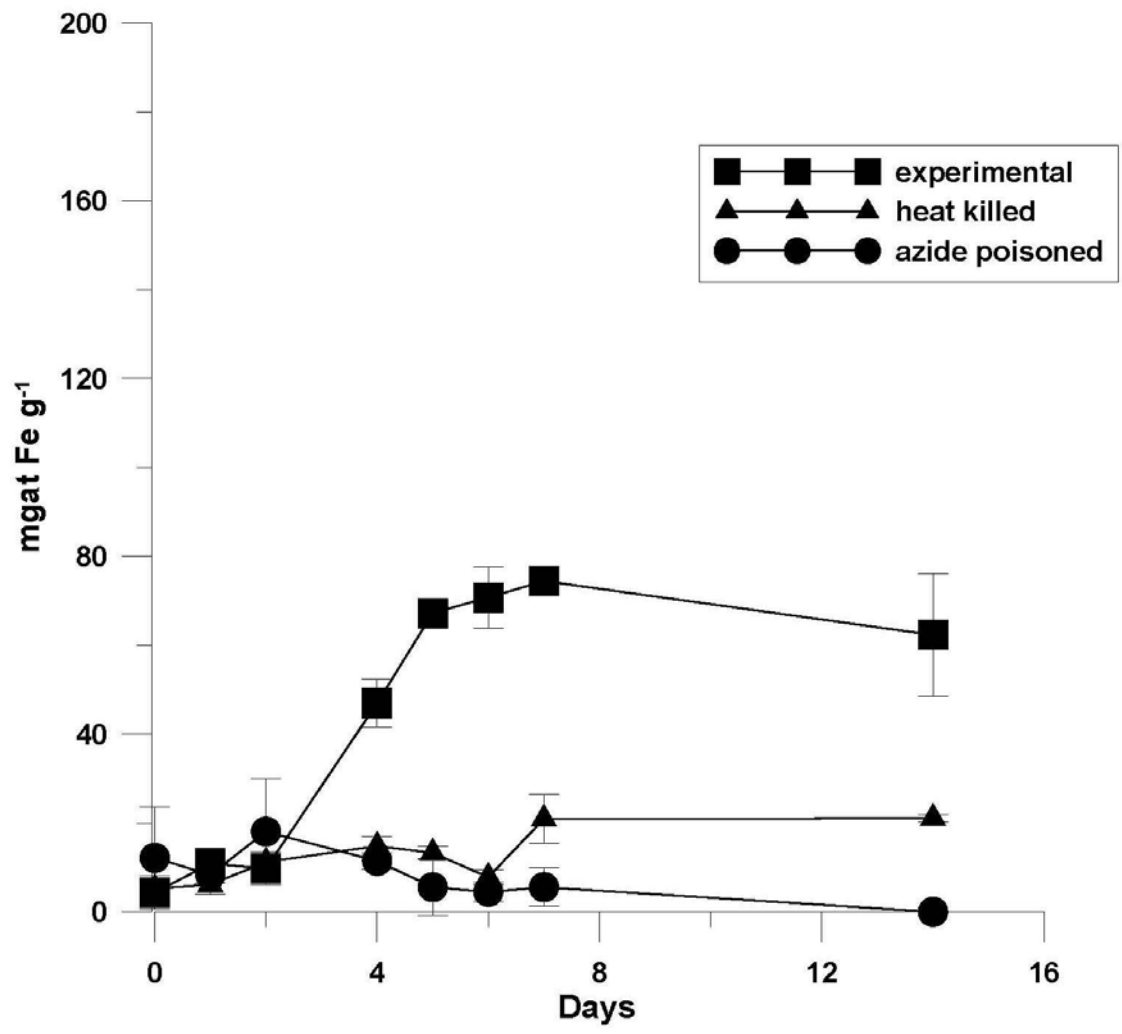
Interrelating parameters	Correlation coefficients (r) in microcosms			
	control	added		
		Fe	Ilm	Ilm and Fe
Fe(II)/Fe(III)	-0.757	-0.742	0.938	0.940
Phyto/Fe(II)	0.744	-	0.919	0.826
Phyto/Fe(III)	-0.746	-	0.958	0.929
Phyto/ATP	-	-	0.892	0.912
Phyto/Chl <i>a</i>	0.859	0.957	0.900	0.886
TC/ATP	-	-	0.744	0.922

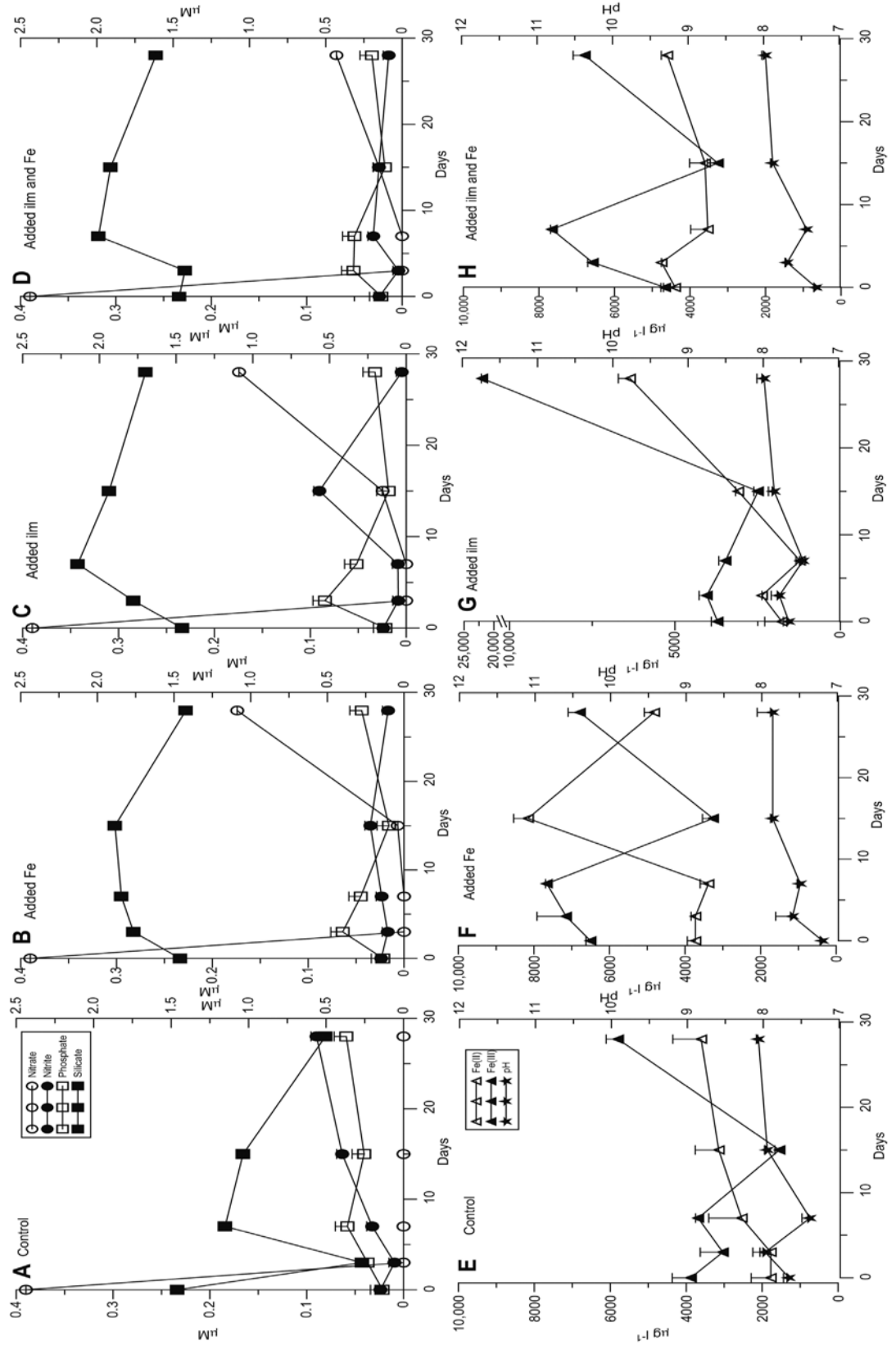
Fe(II) (ferrous), Fe(III) (ferric), ATP (Adenosine triphosphate), Chl *a* (Chlorophyll *a*), TC (Bacterial abundance), Phyto (Phytoplankton abundance).

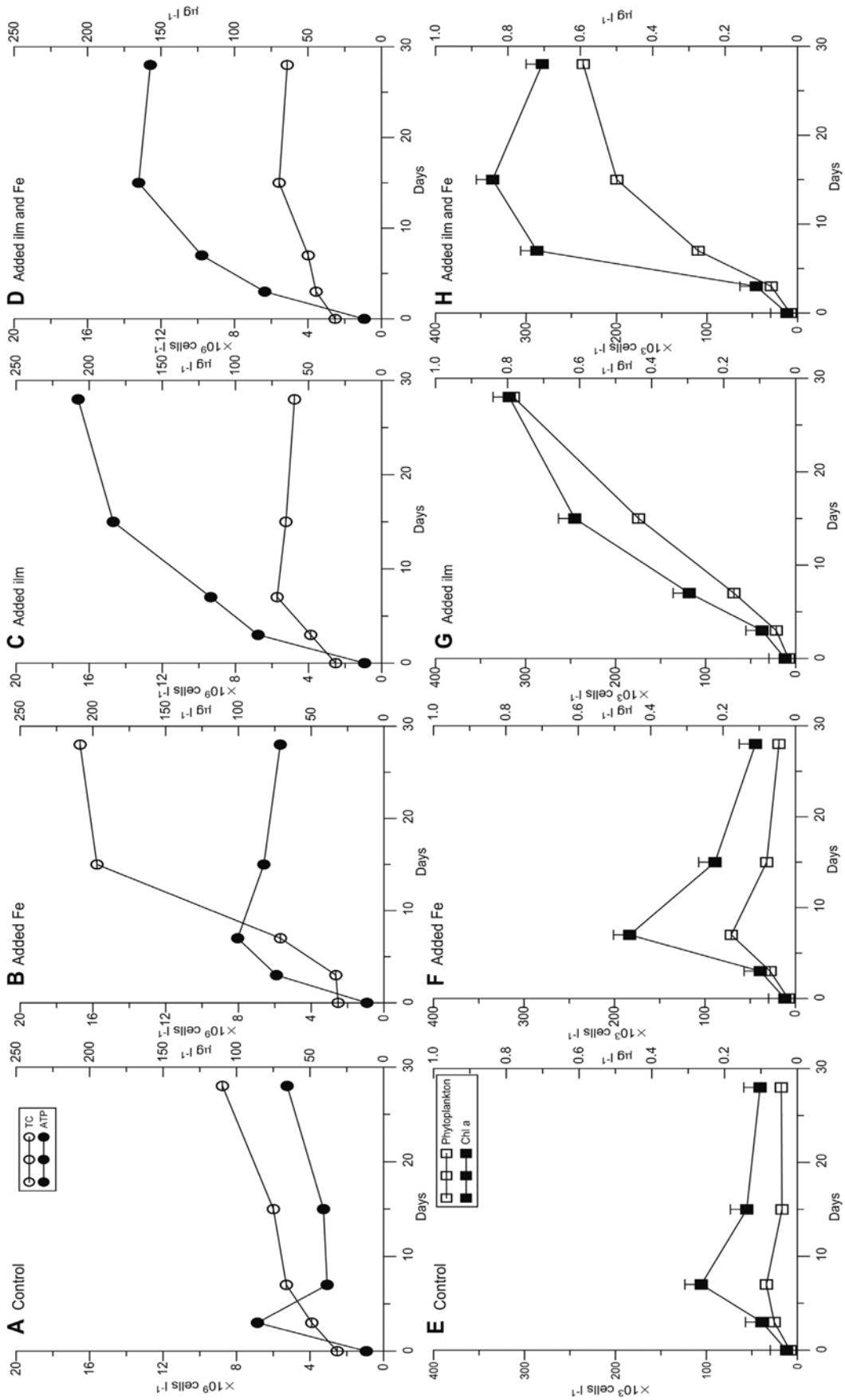
Control ("control"), added iron at a concentration of 10µM FeSO₄.7H₂O ("added Fe"), added mineral source in the form of 100mg ilmenite ("added Ilm"), and a added combination of mineral and iron (10 µM FeSO₄.7H₂O) ("added Ilm and Fe").

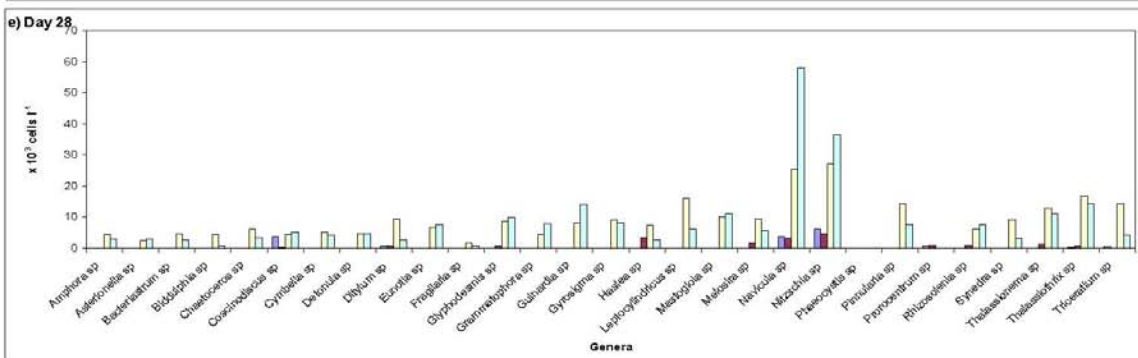
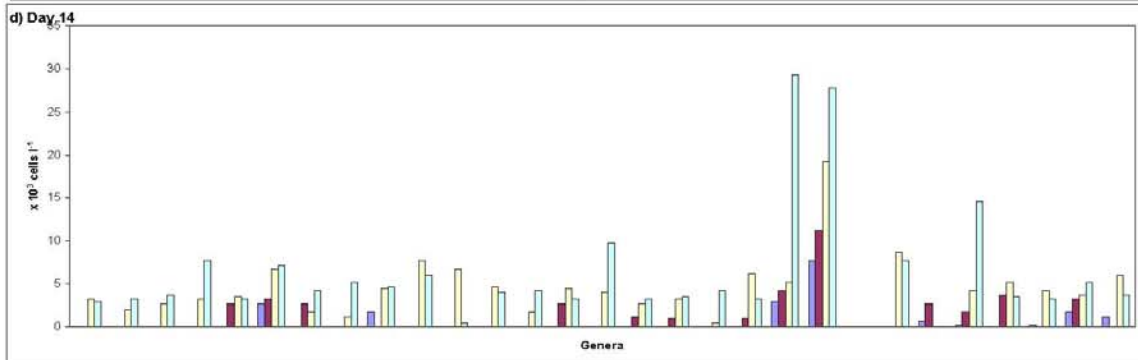
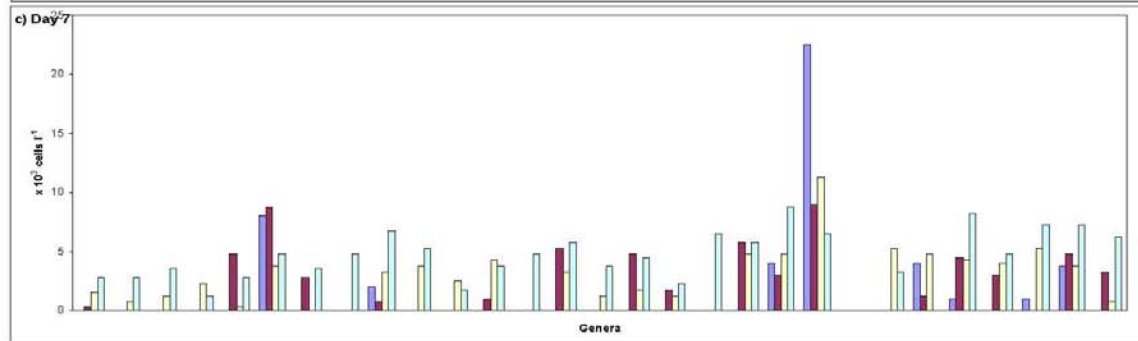
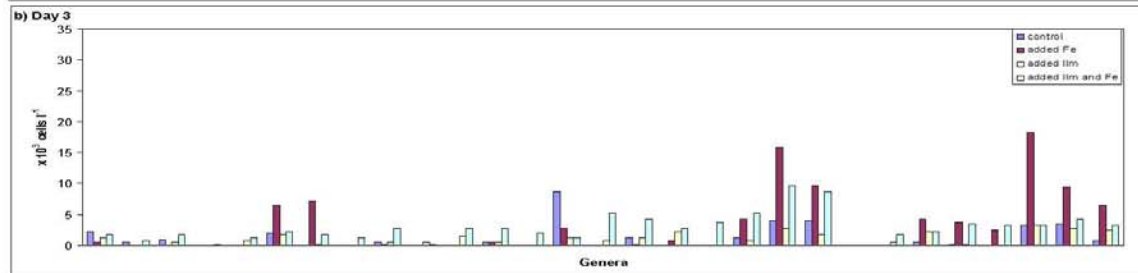
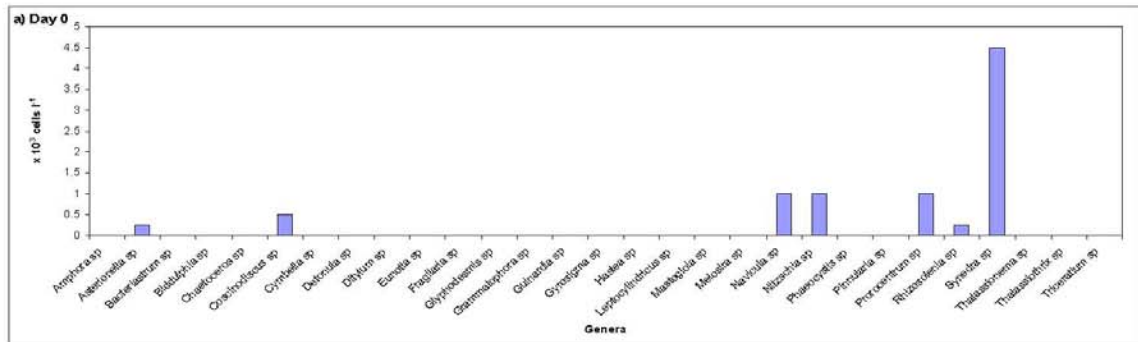


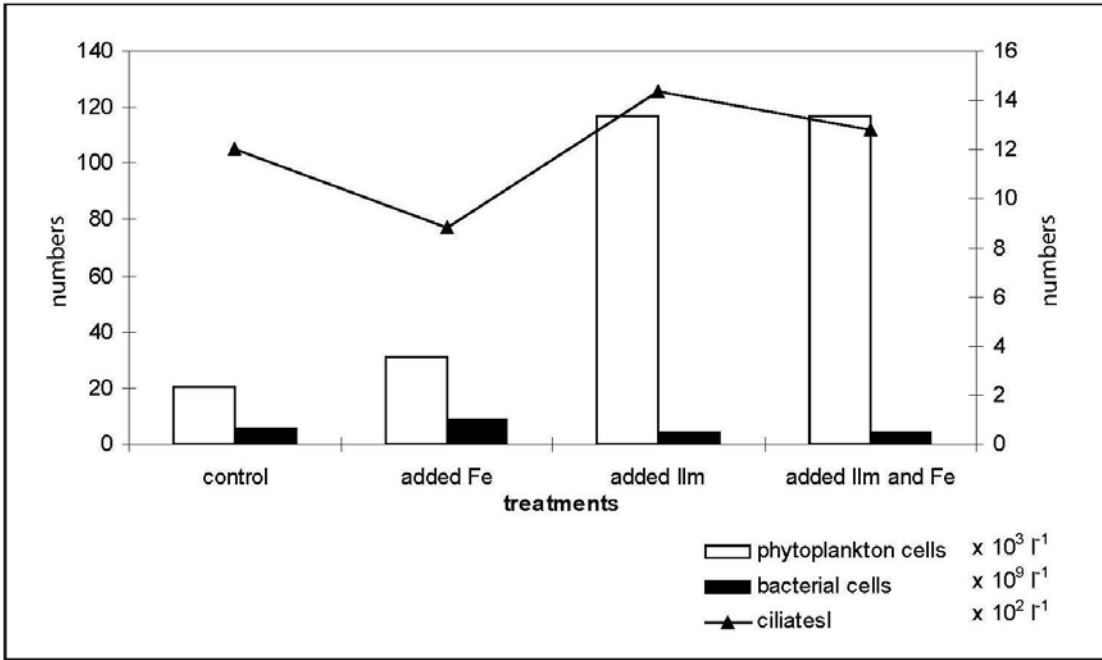




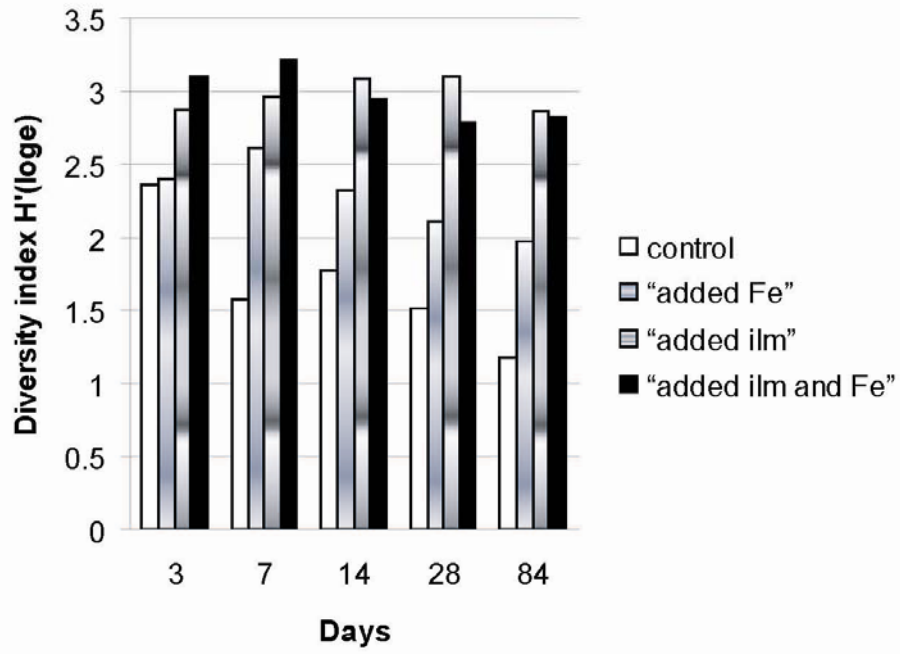








a.



b.

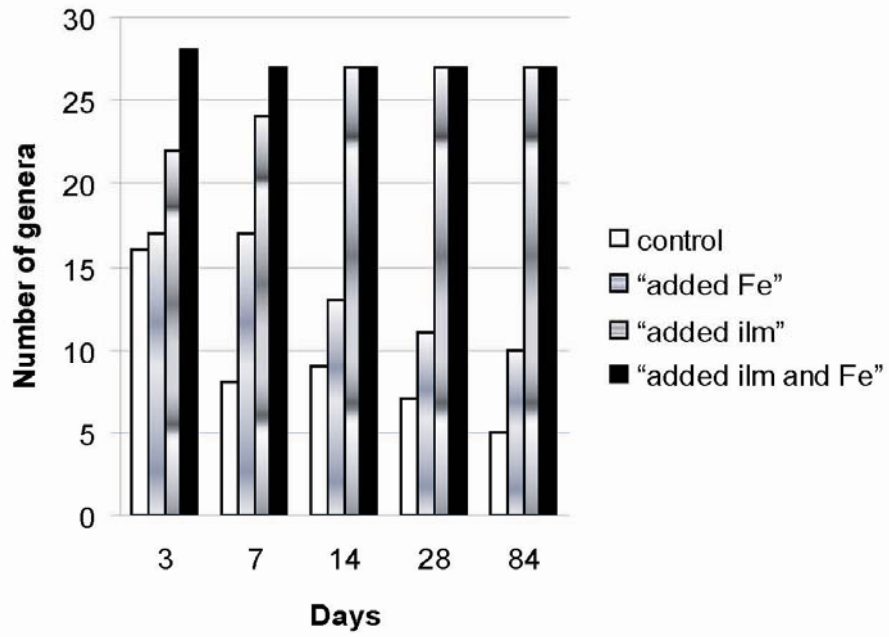


Fig 8