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Use of Phytoplankton Culture and Multianalytical Techniques to Understand the Effects of the Organic Molecule, Siderophore , on Species Specific Bioavailability of Iron.

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Marine Coastal Development
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ABSTRACT

Organic ligands play key roles in controlling the bioavailability of trace metals in the world oceans, thus influencing major biogeochemical cycles. The chemistry of organic ligands and the iron complexes formed, that in turn affect the species–specific requirements of iron for different phytoplankton are still unknown. To study the effect of the interaction between iron and organic ligands over the growth of different phytoplankton species, a culture experiment was conducted with one species of diatom (*Skeletonema costatum)*and one of dinoflagellate (*Alexandrium catenella*), grown under two different iron concentrations and with a concentration gradient of siderophore (Des-ferroxamine B).Species were grown under EDTA-metal ion buffered seawater media Aquil (Price et al. 1988).Iron and siderophore were added every third day, to reach final concentrations for iron (1 and 10 nM) and siderophore (10,50, 100, 500, 2500 and 10000 nM).Before every addition, samples were collected for pH, quantum yield, *in vivo* fluorescence, cell abundance. Dissolved iron samples (Chelex-100 labile) were collected at the end for certain treatments. All measurements performed showed a marked effect of the high siderophore concentrations over the growth only of *S.costatum*, independent of the iron concentration for the first nine days. After this, the high siderophore treatments started to exhibit growth, reaching almost the levels exhibited in the control and lower siderophore treatments. *A. catenella* on the contrary showed slower growth pace, but with less or no evident effect of the siderophore concentration. Despite the initial inhibitory effect over the diatom growth, it can be observed that changes (e.g. release of organic ligands or changes in the siderophore chemistry) occurred in time, making *S. costatum* able to uptake the iron previously complexed. The responses exhibited show the different adaptive strategies, which under a climate change scenario may influence possible community structure shifts and changes in the biogeochemical cycles.

LIST OF ACRONYMS /ABBREVIATIONS

DFB	Des-ferrioxiamine B
DFe_{CH}	Dissolved Chelex labile iron
DFe_{DGT}	Dissolved DGT labile iron
DGT	Diffusive Gradient in Thin Films
Fe	Iron
FeExp	Iron Experiment
FSU	In vivo fluorescence
HNLC	High Nitrate Low Chlorophyll
L	Litres
Sid	Siderophore
QY	Quantum Yield

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1. INTRODUCTION

The oceanic chemistry of trace metals influences plankton production and community structure and these biological processes also strongly influences the oceanic chemistry of these trace metals. These influences that interact are particularly important for six metals of the first transition series which are required by phytoplankton for various metabolic functions: Mn, Fe, Co, Ni, Cu and Zn (Bruland et al., 1991).A deficiency of these "bioactive" trace metals may result in oceanic plankton production limitations (Brand et al.1983), and an extreme level of some of these same metals may inhibit plankton growth (Brand et al. 1986; Sunda 1988-1989). The uptake of some essential metals by planktons results in extraordinarily low concentrations in surface seawater. In order to take up these micronutrients, various microorganisms apparently release strong complexing agents and catalyze redox reactions that modify the bioavailability of trace metals and promote their rapid cycling in the upper water column (Morel and Price,2003).Trace elements are also key tracers for understanding marine biogeochemical processes (Bruland and Lohan, 2003). Understanding these biogeochemical cycles requires knowledge of their sources and sinks in addition to their transport and chemical form in the ocean .Additionally, the concentrations of bioactive trace metals are now known and that they exist at nanomolar to picomolar concentrations at oceanic waters (Bruland et al., 1991). These bioactive trace metals when dissolved have effects on phytoplankton growth and their interactions emphasize the importance of chemical speciation of trace metals in the external milieu of these organisms.

Chemical speciation is the quantitative distribution of an element in an environment and is chemically explained as different forms an ion can exhibit when in solution can either be a free ion, or in the oxidation state (Stumm and Morgan, 1996). Hence , the knowledge about the chemical forms of bioactive metals is of importance in explaining how phytoplanktons accumulate key metals despite their low concentrations in surface waters .Even though the clarification of chemical speciation of bioactive metals has created much analytical problems that has remained unsolved, some field measurements and culture studies have shown quite reasonable results which discovered that that dissolved concentrations of the bioactive metals are in their non reactive forms at the ocean surface (Huntsman and Sunda 1980; Morel and Morel – Laurens 1983; Sunda 1988-1989) through modern electrochemical method (Bruland et al., 1995; Bruland et al., 1988; Saito et al., 2002 .Hence the absence of reactivity by these bioactive metals

in biological systems has exposed that these metals are bound to some strong unknown ligands (Boyle et al., 2001; Kogut et al, 2001).). These ligands have been detected in the North Sea (Gledhill and van den Berg, 1994), the Western Mediterranean (van den Berg 1995), the North Pacific (Rue and Bruland 1995), the Northwest Atlantic (Wu and Luther 1995), and the equatorial Pacific oceans (Rue and Bruland 1997). They are present at high concentrations in seawater (0.5–15 nmol kg^{-1}) and bind practically all the dissolved iron, leaving 0.05 pmolkg^{-1} as inorganic ferric species. The exact chemical nature of the organic ligands is uncertain, but they possess stability constants similar to those measured for microbial iron chelates (Rue and Bruland 1995).Therefore there has been a general acceptance of the notion that organic compounds may play a role in changing the speciation of these bioactive trace metals by binding metal ions or by forming coordination species or complexes (Barber et al., 1971). Also, the knowledge of oceanic concentrations, distributions and cycles of trace metals have significantly advanced in recent years (Mawji et al., 2008;Bruland et al., 2001; Bergquist et al., 2007; de Baar et al.,2001; Liu et al.,2002;Boyd et al.,2001).As a result of these advances, it is now realized that the concentrations of bioactive metals are much lesser and that their chemical speciation plays a very important role in their ocean chemistry and biology than it was previously recognized (Boyle et al.,2002;Johnson et al, 2007).

Half of the photosynthetic fixation of carbon in relation to primary production on earth is determined by the phytoplankton of the oceans (Field et al,1998).As they serve the basis of the food chain, they always have to continuously produce food to keep up with the constant demand from zooplanktons. In order to continue producing food, phytoplankton have to take some micronutrients in trace concentrations in addition to nitrogen, carbon, phosphorus and silicon (in the case of diatoms) (Morel et al; 2003).These bioactive metals needed in trace concentrations are known to play biological roles such as cofactors or part of cofactors in enzymes and also structural elements in proteins (Bruland, 1995; Boyle et al., 2002; Boyd,2010; Morel and Price,2003; Johnson,2000).However, the concentrations of these bioactive trace metals are low in the sea as a result of their level of solubility and their constant uptake from the water column by phytoplankton. Hence as a result of this, their concentrations fall precipitously within short distance of the coastline leading to a difficulty in their recycling (Johnson et al; 1997).

Additionally, a continuous uptake of these elements from the photic zone to aphotic zone or depth results in their fate being controlled in the oceans making it critical for biological

processes (Wang et.al 2001).The depletion of the dissolved concentrations of these trace elements from the photic zone is so intense in the ocean, surface concentrations are only about a small fraction of those in the deep (Morel et al; 2003). The deficiency of biologically important trace elements such as iron (Fe), Zinc (Zn), and Copper (Cu) in seawater has been shown to be an important limiting factor for a phytoplankton growth and controls primary production(Takeda and Tsuda 2005).Apart from being the most abundant trace element in the Earth's crust, iron is present at subnanomolar concentrations in most near surface oceanic waters due to the insolubility of Fe(III) oxides and hydroxides, efficient scavenging by particle surfaces and uptake by phytoplankton (Martin and Gordon,1988;Martin et al;1989,1993). Additionally, it exists in it oxidized form as Fe^{3+} (ferric iron) which apparently insoluble while the bioavailable form, the reduced state Fe^{2+} (ferrous ion) is less abundant (Price et al; 1994). Additionally, it has nutrient-type characteristics that are inferred from vertical profiles (Martin and Gordon, 1988) and from rapid biological recycling (Hutchins et al., 1993) Furthermore, it exhibits scavenging characteristics with relatively short residence times both in surface waters and the oceans as a whole (Wells et al., 1994).

Iron plays specific roles in plant metabolism and here, it is required for nitrate reduction, photosynthetic and respiration electron transport, chlorophyll synthesis and detoxification of reactive oxygen species (Morel and Price, 2003). Since iron has numerous important biological roles, its role has been studied and known that it is a limiting nutrient for biological productions, especially in the areas termed as the high-nutrient-low-chlorophyll (HNLC) areas. The acronym, HNLC describes areas of the ocean where the number of phytoplankton (standing stock) are low and fairly constant in spite of high macro-nutrient concentrations (nitrate, phosphate, silicic acid). In general, some essential inorganic substances, like nitrate, may be present in oceanic waters in concentrations low enough to be limiting to plant production, but in HNLC regions the level of nitrate is never significantly depleted (Lalli and Parsons, 2004; Pitchford and Brindley, 1999). Instead, these regions are limited by low concentrations of metabolizable iron (Lalli et al., 2004).

There are two popular explanations for the existence of HNLC regions: the iron hypothesis and the 'grazing control hypotheses. HNLC regions cover 20% of the world's oceans in three major areas: equatorial Pacific Ocean, subarctic Pacific Ocean and the Southern Ocean (Lalli and Parsons, 2004; Pitchford and Brindley, 1999). In comparison with oceanic waters, coastal and

shelf waters gain high amounts of iron coming from riverine sources and bottom sediments which results in highly dissolved particulate iron concentrations. Also these dissolved particulate iron in the coastal areas are inorganic and processes that solubilize this reservoir make iron accessible to phytoplankton (Brand et al., 1983; Sunda et al., 1991).However, it is proven that even in coastal systems, iron might also be a limiting nutrient in coastal areas (Hutchins et al., 1998; Hutchins et al., 2002; Ozturk et al. 2002).

The processes that lead to Fe(III) reduction include direct and indirect photoreduction of iron (Wells and Mayer, 1991 ; Kuma et al., 1992; Waite et al., 1995; Miller et al.,1995; Maldonado and Price ,1999; 2000).Under conditions of cellular iron deprivation, many microorganisms release small (about 1,000 Da) molecules called siderophores that exhibit extraordinarily high complex formation constants for Fe(III) (Raymond et al. 1984). Siderophores solubilize Fe (III) extracellularly and facilitate its transport into the cell. They typically contain hydroxamates or catecholates that function as Fe-chelating groups; however, a number of other functional groups have also been characterized (Winkelmann, 1990). Heterotrophic bacteria isolated from various marine habitats produces siderophores in low iron media (Gonye and Carpenter 1974; Trick 1989), some of which have been isolated and chemically characterized (Takahashi et al. 1987; Jalal et al. 1989; Reid et al.1993). These siderophores chelate and solubilize iron present in minerals-like iron oxides, adsorbed on particle surfaces or bound within existing complexes (Neilands,1981, 1989) thereby minimizing its loss from the seawater medium through association with settling particles. These bacteria often possess multiple siderophore uptake systems which are capable of transporting iron bound not only to specific siderophores they themselves produce but also those that are bound to siderophores produced by other microorganisms (Sunda and Huntsman,1995).Hence some siderophores form complexes with iron making it not bioavailable to phytoplankton thereby interrupting biological activity and leading to changes in the natural structure of phytoplankton communities(Wells, 1999; Hutchins et al., 1999b)

Marine chemists have developed advanced multi analytical techniques that are extremely sensitive and selective to determine the chemical speciation of trace metals in oceanic waters. The determinations of the speciation of trace metals requires extremely sensitive analytical techniques because fractions of total concentrations are measured, and high levels of cleanliness

(Bruland et al., 1991) .This is a challenging issue because of the risk of contamination during sampling, analysis and high detection limits of the available techniques. According to Price et al., 1989, the studies of the physiology of marine phytoplankton have aid in the ability to culture them in artificial mediums. Therefore the experimentation of the cultures of phytoplankton in laboratories artificial mediums and the utilization of multianalytical techniques in determining the concentrations of bioactive metals will aid in understanding how bioavailable they can be to these biological organisms ,the various adaptations that might aid in the rapid uptake of bioactive metals by different phytoplankton species and further recommendations will create a platform for advanced research on how to facilitate the process of the trace metal-biota interactions if key growth characteristics are observed or not.

2 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

The organic molecule, siderophore will have species specific effects on iron bioavailability. Hence different concentrations of siderophore will render different effects on the biological productivity on each phytoplankton species. Additionally, the use of multianalytical techniques to determine the bioavailable forms of iron will render distinct information about the amount of iron taken up by these species.

2.2 Objectives

2.2.1 General objectives

To use multi-analytical approaches and phytoplankton culture to determine the potential bio-available fractions of certain trace elements in order to assess the characteristics of each approach and how it aids in making bioactive trace metals bio-available to biological production in aquatic systems.

2.2.2 Specific objectives

Determine how iron complexed with organic molecule, siderophore has species specific effect on phytoplankton growth.

Determine how bioavailable or non bioavailable iron expresses itself in the culture medium using different multianalytical techniques.

3. THEORY

3.1 Iron as a trace element and micronutrient

3.1.1 Definition

Trace elements are micronutrients present in trace quantities which represent essential dietary components of aquatic organisms and are known to have biological roles like cofactors or part of cofactor enzymes e.g. Copper, Zinc, Iron (Morel and Price,2003). They are passively or actively assimilated by organisms to satisfy physiological needs (Florence, 1982).

3.1.2 Categorization

Trace metals can exist in a variety of forms either physical or chemical. The basic physical distinction is the particulate versus dissolved forms. This distinction is related to an operational definition with 0.4µm or 0.2 µm pore size filters during the process of filtration. The particulate metals include those that are adsorbed onto surfaces of particles, incorporated within particles of biogenic origin and incorporated in the matrix of aluminosilicate minerals or co-precipitated in other authigenic minerals. Dissolved metals, on the other hand, are those metals which consist of the various soluble complexes of trace metals and potential colloidal forms (Bruland, 2003).Basically, this explains that the fraction that passes through the 0.45µm filter is defined as "dissolved" whiles the fraction that is collected by the filter is termed as "particulate". Generally, organisms have the tendency to uptake dissolved metals which are mostly available as compared to their fellow particulate metals which are less available (INAP, 2002).

3.1.3 Sources and sinks

Trace metals in the ocean originate from different sources which gradually end up in sinks in the ocean. The input from rivers is generally a prominent source for major ions in seawater (Jickells, 1995). Secondly, atmospheric dust input into the oceans is also a source of trace metals in seawater (Duce and Tindale, 1991).The atmospheric input of these trace metals varies spatially and temporally (Jickells, 1995).Furthermore, it has been discovered that hydrothermal vents are also major sources of trace metals like iron and manganese and a sink as well to magnesium and sulfate .The sinks of these trace metals includes biological uptake, particle scavenging and hydrothermal vents.

3.1.4 Functions of trace metals

Trace metals play key biological roles as tracers for identifying the source of element in seawater and for understanding marine biogeochemical processes (Bruland and Lohan, 2003). Some of the numerous biological roles played by key bioactive metals are oxygen transport, electron transport, redox catalysis, electron transfer, methane production and so on. Iron plays equally and extreme biological roles and these includes the table below and its numerous biological role,

Table 1. The enzymatic role of Iron (Fe) in marine organisms (Stumm and Morgan, 1996)

Enzyme & proteins	Functions
Cythochrome f	Photosynthetic electron transport
Cythochromes b and c	Electron transport in respiration and photosynthesis
Cythochrome oxidase	Mitochondrial electron transport, O2 + 4e- + 4H+ 2H2O
Fe-sulphur proteins	Photosynthetic and respiratory electron transport
Ferrodoxine	Electron transport in photosynthesis and nitrogen fixation
Nitrogenase	Nitrogen fixation
Nitrate and nitrite reductase	Nitrate and nitrite reduction to ammonia
Ribonucleotide reductase	Transforms ribose to deoxyribose (DNA repl. and cell div.)
Fe-superoxide dismutase	Disproportion of O2 - radicals to H2O2 and O2
Catalase	H2O2 breakdown to O2 and H2O
Peroxidase	H2O2 reduction to H2O
Chelatase	Porphyrin and phycobiliprotein synthesis
Succinate dehydrogenase	Fumarate synthesis
Aconitase	Isomerization of citrate
Coprporphyrinogen oxidase	Oxidative decarboxylation of Mg-protoporphrine
Lipooxygenase	Fatty acid oxidation, carotenoid degradation
Glutamate synthetase	Glutamate synthesis
Xantine oxidase	Oxidation xanthine to uric acid
Ferritin	Iron storage
Methane monooxygenase	Methane oxidation
Purple acid phosphatase	Unknown
Alkaline Phosphatase	Formation of phosphate ester

3.1.5 Speciation

The term speciation has been defined in IUPAC Recommendations as the term given to the different forms an ion can exhibit when in a solution where it can be either a free ion or be in the oxidation state. Hence the term “chemical species” is utilized as a specific form of an element

including the isotopic composition, electronic state and complex or molecular structure (Salomons, 1984).It can also be referred to as the quantitative distribution of an element in the environment (Stumm and Morgan, 1996). Here, chemical speciation analysis involves the speciation analysis which is an analytical process that is used to identify or measure quantities of one or more individual chemical species in a sample.

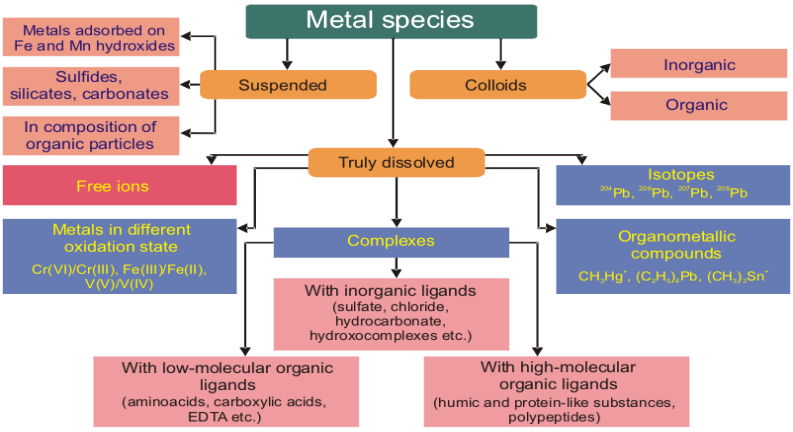


Fig3. 1 A schematic diagram showing the various pathways of speciation of trace metals (Linnik, 2003)

3.1.5.1 Variables affecting speciation

The purpose of chemical speciation is to clarify the chemical forms of trace metals in seawater and their controlling processes from both chemical and geochemical aspects (Hirose, 1985).However, according to INAP 2002, the nature of metal speciation is a function of numerous variables which includes the metal of consideration, types of concentrations of complexing agents and competing present. Additionally, it also includes some key parameters of water like pH, pE (redox potential), hardness (Ca and /or Mg concentration), alkalinity and salinity. All these parameters have an effect on the speciation of trace metals to phytoplankton or aquatic biota (INAP, 2002).

3.1.5.1.1 Complexing agents or Ligands

Trace metals exist in natural waters in a variety of chemical phases mostly as a cation complexed either by an organic or inorganic ligand (Florence,1982).The complexation of trace metals in aquatic systems may occur in reactions with soluble inorganic ligands like F^-,Cl^-, HCO_3^- , SO_4^{2-} ,HPO_4^-) or organic ligands example humic substances. Complexation with organic ligands (i.e. organic-metal complexes reduces metal bioavailability because these complexes are not readily

transported across the cell membrane whiles inorganic metal complexes (e.g. carbonates) however typically dissociate rapidly to the free metal form. For example, the bioavailability of several metals (*e.g.*, Cd, Cu, Zn) is reduced in the presence of organic chelators (Zamuda et al., 1985).Complexation with organic ligands reduces metal bioavailability because most organic-metal complexes are not readily transported across cell membranes. Examples include complexes with humic and fulvic acids which represents by-products of polymerization and condensation reactions of natural organic matter. They tend to have great importance as they serve a major component of the dissolved organic matter pool in coastal and freshwater ecosystems (Maranger and Pullin, 2003).

Even though organic ligands reduce metal bioavailability generally, some examples of organic ligands that aid in an increase of metal uptake by aquatic biota have been discovered. Siderophores, for example, are ligands with a high affinity for Fe that are released by organisms to facilitate the transport of Fe into the cell. However, further discoveries have revealed that there two opposing views about organic ligands being chelators of metals, as the one that binds metals enhancing the availability of these elements to planktonic organisms (Sunda and Huntsman, 1995) and the other stating that availability of these trace metals are reduced when chelation occurs. In this light, organic chelators kind of play the "nurse and kill" role where they have the potential to reduce the concentration of toxic metals and also reduce the bioavailable fraction of essential trace metals in biological systems of aquatic biota(Whitfield, 2001).

3.1.5.1.2 pH

Some works in relation to trace metals have demonstrated an increase in metal toxicity with decreasing pH, due to the increase in free metal-ion activity at lower pH (Hodson *et al.*, 1978). Conversely, other studies have shown a decrease in metal toxicity with decreasing pH (Bervoets and Blust, 2000; Franklin et al. 2000).The latter observations have been attributed to the increased competition of H^+ with trace metals at the cell surface.

3.1.5.1.3 Toxicity

The toxicity of metals to aquatic organisms, for example, generally decreases with increasing water hardness (Meyer et al., 1999). Two processes have been suggested to account for these observations: 1) Ca and Mg successfully compete with trace metals for membrane transport sites on cellular surfaces; and 2) the complexation of metals with carbonate (CO^{3-}) decreases the free

metal ion concentration and thus metal bioavailability (Barata et al., 1998). The effects of metal toxicity are variable, but are generally expressed as mortality, decreased growth rate, decreased fecundity and decreased metabolic activity. Metals are toxic because in sufficient concentrations they are able to compete for intracellular sites normally occupied by functional metabolites, thereby interfering with normal cell functions (INAP, 2002).

3.1.6 Bioavailability

In natural waters, the bioavailability of trace metals, including their toxicity, is thought to be related to their ability to cross biological barriers (e.g. plasma membrane) and it is most often predicted by the concentration (Franklin, 2002) or flux (Wilkinson,2004) of internalized metal. The bio uptake process depends not only on the internalization pathways and their specificity but also on the physicochemistry of the medium and the size and nature of the organism (Pinheiro, 2001). Hence, the definition of bioavailability is the fraction of total amount of an element that is available for an organism metabolism or that can be absorbed and used or stored in an organism. This bioavailable fraction of metals consists of both free metal ions and kinetically-labile metal complexes (i.e., those with rapid dissociation kinetics), the biological response is proportional to the free metal concentration only (Whitfield, 2001).As a result of this, the bioavailability and the toxicity of metals in aquatic systems is highly dependent on the nature of the species of metal present. Therefore, the determination of the chemical form of metals in the environment or aquatic systems is key to predicting impacts to aquatic biota.

3.1.7 Phytoplankton

Phytoplanktons are responsible for nearly all of the primary production that takes place in the pelagic ecosystem (Sakshaug, 2009). Phytoplankton which are also called microalgae, are likened to terrestrial plants because they contain chlorophyll and need sunlight in order to survive and grow. Most phytoplankton are buoyant and float in the upper part of the ocean, where sunlight penetrates the water. Additionally they require inorganic nutrients such as nitrates, phosphates, and sulfur which are converted into proteins, fats, and carbohydrates (NOAA, 2014).

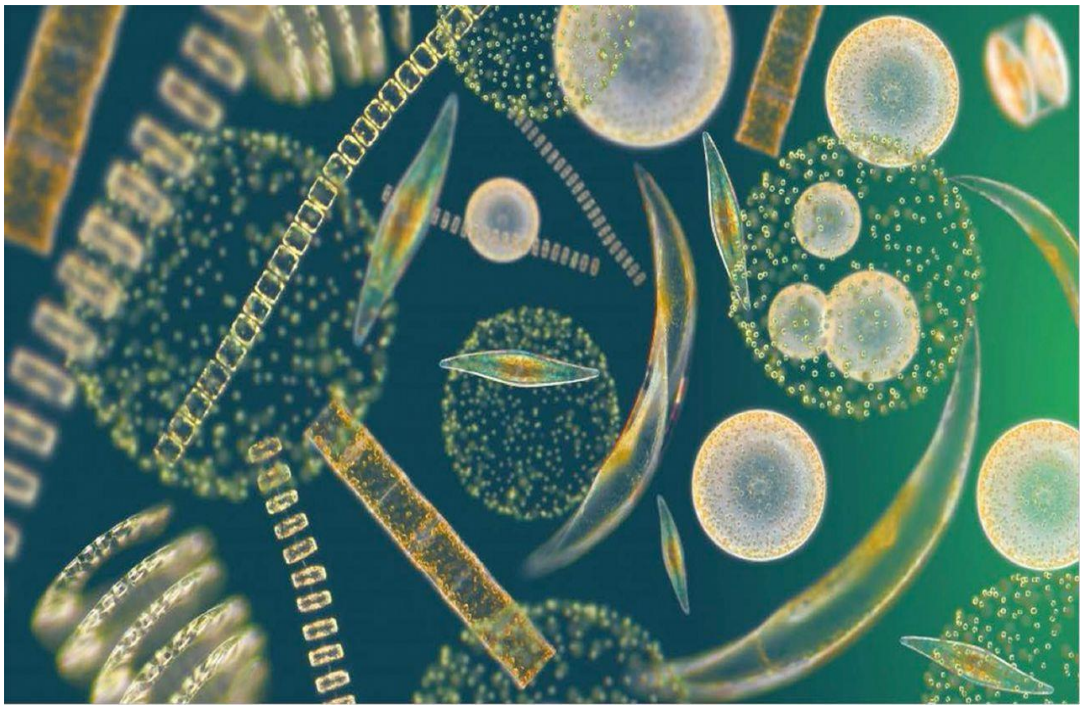


Fig 3.2 A picture showing different phytoplankton types (Ocean bites, 2015)

Phytoplanktons can be categorized into two classes which are diatoms and dinoflagellates.The two main classes of phytoplankton are dinoflagellates and diatoms .Diatoms which belong to Class Bacillariophyceae occupy a wide size range (2µm -2mm) and possess an intricately structured silicate cover and hence they require silicate for their survival (Sakshaug, 2009). This unique feature of diatom cells is called frustules .These frustules show a wide diversity in form, but are usually almost bilaterally symmetrical, hence the group name (Hasle, 1996). Additionally, diatoms possess a particular kind of division where it can create a size range of 10 fold for one and the same species because the silicate cover is unable to grow. The daughter cells inherit the top and bottom cover where the bottom cover is slightly small. However, both complete the growth process by forming new bottom halves. Diatoms form two groups namely the centric and the pennate diatom which exhibit radial and bilateral symmetry respectively. Diatoms do not rely on flagella to move through the water and instead rely on ocean currents to travel through the water.

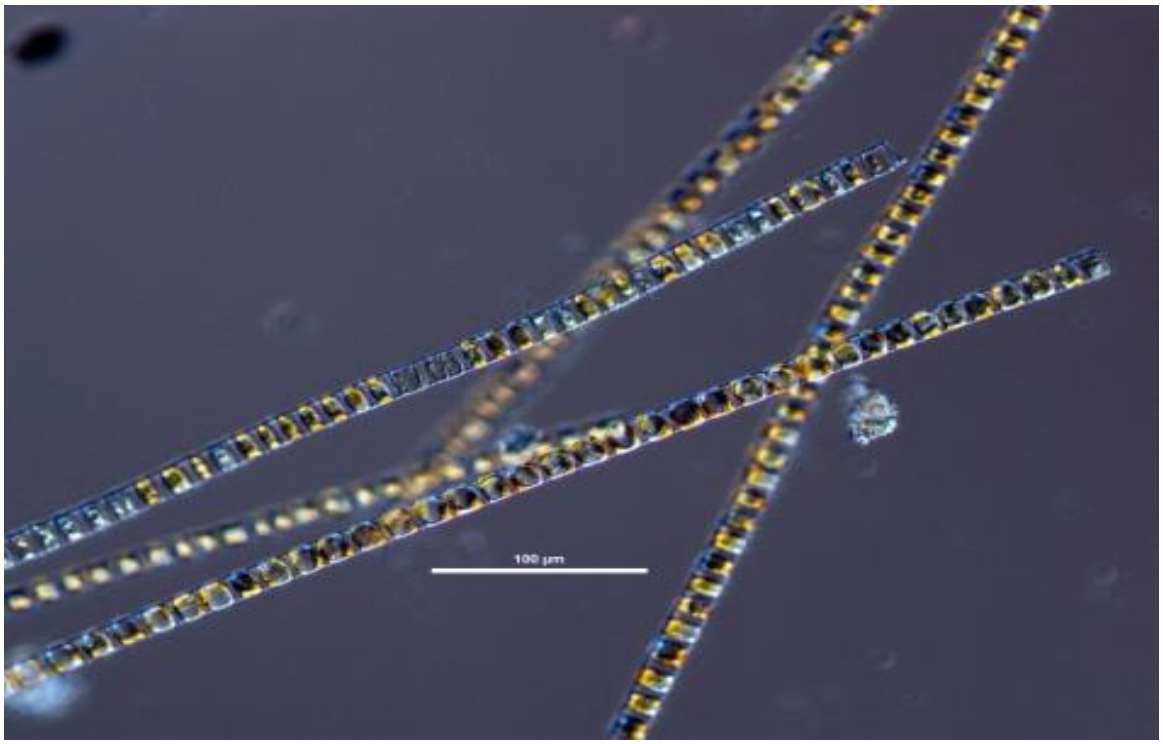


Fig 3.3. A picture of a centric diatom, Skeletonema costatum with marginal rings of spines (Guiry, 2011))

Fig 3.4 A picture of a pennate diatom , Cymbella (Lee,2008)

Dinoflagellates are a large group of flagellate protists that constitute the Phylum Dinoflagellata. They are mostly marine plankton but they are common in fresh water habitats as well. Their distributed populations depend on temperature, salinity or depth. Many of these dinoflagellates are known to be photosynthetic, but most of them mixotrophic combining photosynthesis with ingestion of prey (phagotrophy)(Stoecker,1999). Dinoflagellates use a whip-like tail, or flagella, to move through the water and their bodies are covered with complex shells (NOAA, 2014). They form one of the largest groups of marine eukaryotes, although they are smaller than the diatoms substantially (Guiry, 2012).

Dinoflagellates possess a haplontic life cycle (Fensome, 1993). The life cycle usually involves asexual reproduction by means of binary fission, either through desmoschisis or eleuteroschisis. Furthermore, complex life cycles occur especially with parasitic dinoflagellates. Also, sexual reproduction also occurs (Von Stosch, 1993) and is only known in a small percentage of dinoflagellates (Pfiester, 1973).This occurs by fusion of two individuals to form a zygote which may remain mobile in typical dinoflagellate fashion called a planozygote. This zygote may later form a resting stage (hypnozygote) which is called dinoflagellate cyst or dinocyst. After (or before) germination of the cyst, the hatchling undergoes meiosis to produce new haploid cells.

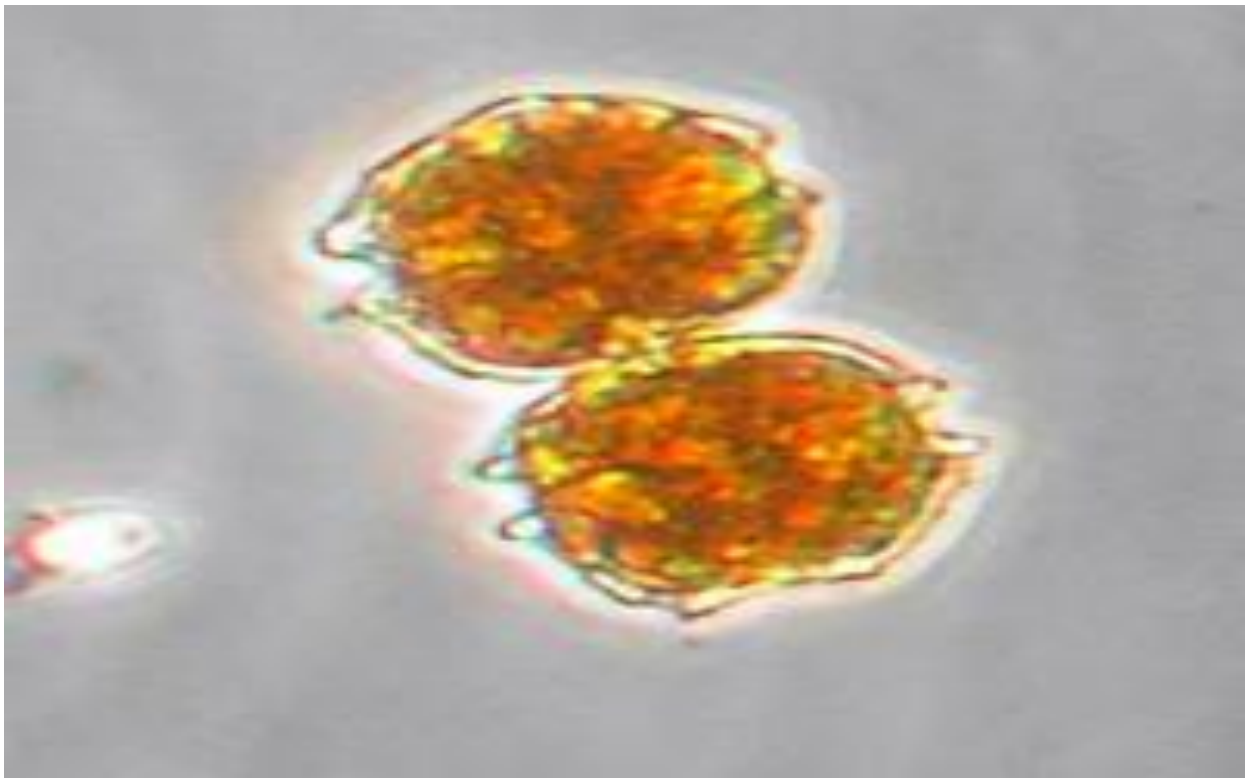


Fig 3.5 Picture of the dinoflagellate, Alexandrium catanella (Cassis, 2005)

3.1.8 Metal uptake in biological systems

Metals may also be assimilated via contact/ingestion of water and sediment pore waters. For example; waterborne metals can be biologically assimilated through binding to the gills of fish, bivalves and crustaceans (e.g., Wilkinson, et al., 1993; Hollis et al.,1996;Playle,1998; Soegianto, et al., 1999), across digestive membranes (Reinfelder et al., 1998) and through direct uptake across the cell membranes of unicellular organisms (Anderson and Morel, 1979). The regulation of materials into and out of a cell is facilitated by the cell membrane, which simply termed, is composed of phospholipids and large protein molecules.

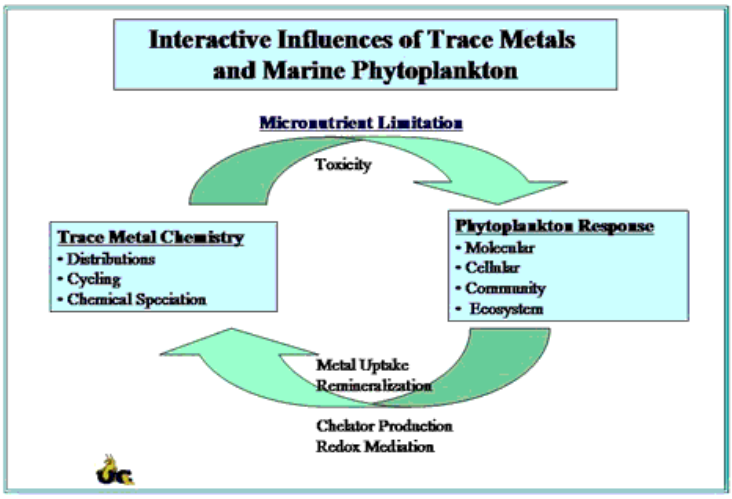


Fig. 3.6 A schematic diagram showing the interactive influences of trace metals and marine phytoplankton (Bruland, 2004).

Most metal species are extremely hydrophilic, and as a result, their passage through the hydrophobic lipid membrane is restricted. Dissolved metals are typically incorporated passively into the cells of organisms via specialized pumps, channels and carriers which operate across the membrane surface (INAP, 2002). This process is aided by the cell membrane which consists of globular proteins (integral proteins) that are dispersed throughout the lipid bilayer, and act as conduits for the transport of materials to the cell interior. Metal transport across cellular membranes may also be facilitated via extracellular chelators.

Phytoplankton cells can be considered to take up trace metal in three stages: (1) Transport of metal species to the cell surface through diffusion, (2) binding to a biologically produced ligand through sequestration or capture and (3) transfer of complex across cell membrane through internalization (Whitfield, 2001). Generally, the overall process for uptake follows the Michaelis- Menten kinetics, typical for enzyme-mediated reactions (Morel et al., 1991, Stumm and Morgan, 1996):

$$\rho = \frac{\rho_{max}\,[M']}{K\rho + [M']}$$

Where ρ is the uptake rate, p_{max} is the maximum uptake rate and K_p is the half saturation constant. M' is the available metal concentration, that is, the concentration of free metal ions and kinetically labile complexes adjacent to the cell surface. The K_p value is assumed to be fixed for

a given trace metal with a given species of phytoplankton. The value depends upon the ligand exchange rates (k_{-L}, k_L) and the rate of transport into the cell (k_{in}) (Whitfield, 2001):

$$K_\rho = \frac{k_{-L} + k_{in}}{k_L}$$

If the trace metal is limited, the surface will be under saturated. The M' value will then be smaller than the value of K_p. At steady state:

$$\rho^{55} = \frac{k_{in}[L1]^{max}}{k_\rho}[M']$$

Where $[L1]_{max}$ is the maximum concentration that the cell can produce of surface ligands (Whitfield, 2001).

3.2 Iron

Iron is a particle reactive trace metal existing at extremely low concentrations in the oceans. Its nutrient type characteristics are inferred from vertical profiles (low surface values, higher deepwater values (Martin and Gordon, 1988) and from rapid biological recycling (Hutchins et al., 1993).It is the fourth most abundant element in the Earth's crust and the second most abundant metal (Reilly, 2004).It belongs to the d-block transition element and can exist in oxidation states ranging from -2 to +6. Despite the wide range of oxidation states, iron is however limited or reduced to ferrous (+2), ferric (+3) and ferryl (+4). Three oxides are known, FeO, Fe_2O_3 and Fe_3O_4, representing the Fe (II) and the Fe(III), as well as the mixed Fe(II)–Fe(III) oxide which occurs in nature as the mineral magnetite. With non-oxidizing acids, in the absence of air, ferrous salts are formed. Many iron salts, as well as hydroxides, are insoluble in water (Reilly 2004).

Iron has a very rich redox chemistry which enables it to play a role in various oxido-reductase enzyme activities as well as in electron transfers (Reilly, 2004).It is important in plant metabolism where it is required for photosynthetic and respiratory electron transport, nitrate reduction, chlorophyll synthesis and detoxification of reactive oxygen species (Martin et al., 1989, 1991).The availability of iron to biological systems or microorganisms is very dependent on its redox state. Ferrous iron (Fe^{2+}) is highly soluble in water whiles its fellow oxidized form (Fe^{3+}) is apparently insoluble (Haese, 2005).Additionally, iron can bind to different ligands due

to its unoccupied d-orbitals. Therefore, from a biological view, this property of being able to form coordination complexes provides an important chemical property (Reilly, 2004).

3.2.1 Iron cycle in the marine environment

Iron is transported to the marine system by four major pathways: fluvial (the most important one in coastal areas), aeolian, submarine hydrothermal and glacial input (Haese, 2006). Averagely, iron concentrations show less solubility in marine water in comparison with river water((Haese, 2005).Sources of dissolved iron to the photic zone includes "new" sources from external inputs as well as "regenerated " sources recycled in-situ from various particulate phases (Wells et al.,1995). These sources include wet and dry deposition of atmospheric aerosols, vertical mixing and upwelling, inputs from rivers and bottom sediments and biogenic recycling of cellular iron in surface waters. When iron is dissolved, it is mainly present as Fe (III) oxyhydroxide, which is stabilized in colloidal dispersion by high molecular- weight humic acids (Hunter 1983).

Due to biological activity, several waters are fairly oxidized. Autotrophs produce free oxygen and on the other hand organic matter, affecting the redox state of natural waters, therefore iron speciation through photosynthetic processes (Haese, 2005). The proximity to iron sources in coastal regions resulted in the assumption that iron generally is in abundance, occurring 100 to 1000 times higher concentrations in coastal waters (Sunda and Huntsman 1995), decreasing abruptly off continental margins (Johnson et al. 1997) to the extent to become limiting in certain region in the open ocean, reaching concentrations 20 – 30 pM in the High Nutrient Low Chlorophyll (HNLC) zones (Martin 1991; Martin et al. 1991; Morel et al. 1991).

While much of the particulate iron introduced through rivers, sediment resuspension, or as mineral aerosols will be removed by settling, ascertaining the underlying basis for the removal of "dissolved" iron forms is much more difficult (Morel and Hudson, 1985).Most mechanisms for removing iron from surface waters include sorption and precipitation, biological assimilation, aggregation of inorganic or organic colloids, sinking of mineral and biogenic particles. Dissolved iron also is removed by direct assimilation by phytoplankton. The subsequent sinking of live cells or fecal matter will transport a portion of this biogenic iron from surface waters. Additionally, iron may be adsorb to colloidal organic matter which is abundant in surface waters (Wells and Goldberg, 1992).

Beneath the photic zone, where light intensity is not enough to sustain photosynthesis, biological

activity and respiration, thus oxygen consumption sustained by falling organic matter from the photic zone. Product of decomposition and mineralization, dissolve iron concentration increases resembling a nutrient type profile (Johnson et al. 1997). However, it is dictated by chemistry that iron should adopts a scavenged element profile (lead type), decreasing in concentration with depth due to particle adsorption (Whitfield 2001). Hence if the rate of respiration exceeds downward advection of oxygenated surface water, respiration depletes all available oxygen, suboxic or anoxic conditions are achieved. Under these conditions iron increase due to the mineralization of iron bearing organic matter (Haese 2005). In sediments, once oxygen is consumed, a variety of microbial activities continue utilizing other oxidants as other than oxygen. Under these suboxic and anoxic circumstances Fe occurs in its soluble form and concentrations are much higher compared to normal oxic environments. The metal then diffuse upward to the oxic-anoxic boundary in water bodies where they again are oxidized and precipitate (Haese 2005).

3.2.2 Importance of Iron in biological productivity

Iron is fundamental to the physiology of prokaryotic and eukaryotic cells (Whitfield 2001). Its key role in marine as biological production was initially discovered in the late 1980s (Martin and Gordon 1988; Martin et al. 1989; Martin 1990; Martin et al. 1990) .This has been aided by a number of extensive mass fertilization experiments through the 1990s and during the past decade (Martin et al. 1994; de Baar et al. 2005).

The properties of the oxidation-reduction of iron makes it ideally suited to catalyze electron transfer reactions. Hence in the course of evolution, microorganisms have exploited iron for photosynthetic and respiratory functions as well as for the reduction of inorganic nitrogen species, nitrate, nitrite, and nitrogen gas (Morel et al. 1991). It also acts as an acid catalyst in hydrolytic enzymes (Whitfield 2001). Iron is undeniably the most versatile and important trace element for biochemical catalysis (Morel et al. 1991)

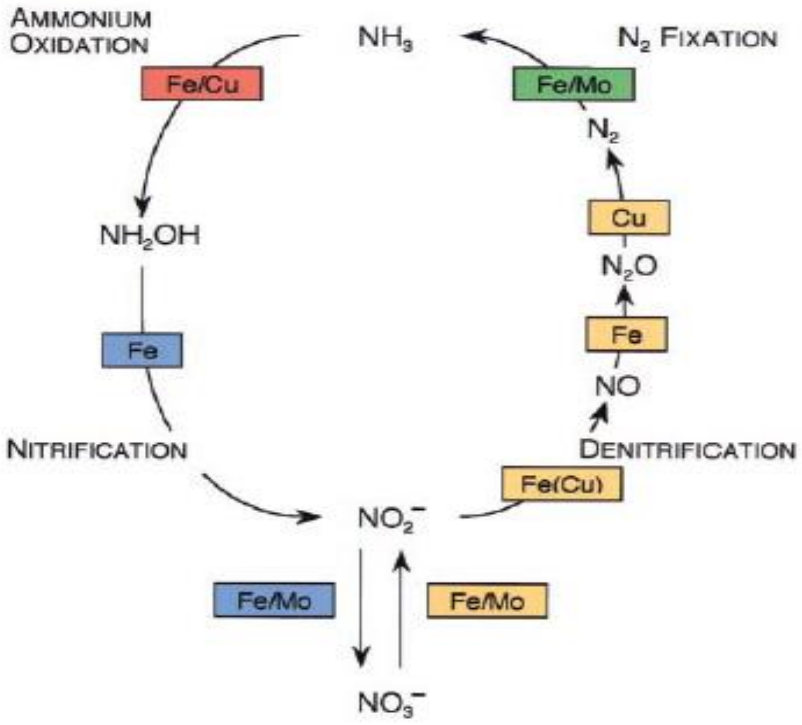


Fig 3.7 A schematic diagram showing the role of iron in Nitrogen cycle(Morel and Price, 2003)

3.2.3 Iron speciation and uptake by phytoplankton

The solution chemistry of iron is dominated (at pH 8) by extensive hydrolysis, which makes it vulnerable to rapid expulsion by oxyhydroxide colloid formation and effective scavenging onto falling particles (Whitfield 2001). Between 10 and 50% passes through 0.4 μm filter (Martin and Gordon 1988; Martin et al. 1989), and some of this may be colloidal rather than truly dissolved. The utilization of electrochemical techniques in direct seawater measurements shows that a large proportion (usually >97%) of the total dissolved fraction is been held by strong organic complexes (Rue and Bruland 1995). Hence few may be in the reduced Fe (II) form rather than the stable Fe (Ill) in oxygenated water. It appears that only the dissolved inorganic forms of iron, chiefly the dominant hydrolysis species $Fe(OH)^{2+}$, are uptaken by marine phytoplankton, therefore the need for the iron to be in dissolved inorganic form in order to be available to algae accentuates the importance of iron chemistry in surface seawater (Morel et al. 1991) according to research from culture studies.

The uptake of inorganic iron directly must involve this soluble hydroxide complexes (Whitfield 2001) .According to early researches, it has been claimed that colloidal Fe might be an available

source of iron for algae (Barbeau and Moffett 2000), while others it is not (Rich and Morel 1990).Moreover, since the uptake of iron by marine phytoplankton involves a complexation reaction between iron in the water and an uptake molecule at the cell surface, followed by internalization of the membrane-bound iron (Hudson and Morel 1990), the production of organic ligands to complex iron as bacteria (e.g., siderophore) which improves the accessibility to iron. For example, it has been noticed that phytoplankton may manufacture excess ligand in response to an influx of iron (Witter et al. 2000).

In light of this, organic complexes can play a vital role for phytoplankton by increasing the solubility of iron (Kuma et al. 1996), making it available and usable for longer periods, thereby this greatly reduce the opportunity for removal of the iron by particle scavenging. Moreover, the organic complexes also provide a potential site for the photo reduction of Fe (III) to Fe (II) that is more readily assimilated, provided that it can be accessed in a timescale that is short compared with the oxidation rate (Whitfield 2001).

3.3 Siderophores and its role in iron bioavailability

3.3.1 Definition

Siderophores can be defined as small, high-affinity iron chelating compounds which are secreted by microorganisms such as bacteria and fungi as part of a highly specific iron uptake mechanism. They are thought to be important in the bacterial acquisition of iron in seawater and to influence iron biogeochemistry in the ocean (Neilands, 1952; Neilands,1995 ; Hider and Kong, 2010; Crosa et al,2004 ; Cornelis and Andrews , 2010; Miller, 2008).These microbes release siderophores that scavenges iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Many siderophores are non ribosomal peptides (Hider and Kong, 2010; Miethke and Marahel, 2007) although several are biosynthesized independently (Challis, 2005).

Siderophores are amongst the strongest binders to Fe^{3+} known, with enterobactin being one of the strongest of these (Raymond et al., 2003). Because of this property, they have attracted interest from medical science in metal chelation therapy, with the siderophore des-ferrioxamine B gaining widespread use in treatments for iron poisoning and thalassemia (Zhou et al, 2010)

3.3.2 Classification

Siderophores are classified based on the ligands used to chelate the ferric iron. The major groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (Hider and Kong, 2010). The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores which cannot be transported by other microbes' specific active transport systems, or in the case of pathogens deactivated by the host organism (Hider and Kong, 2010; Miller,2008).

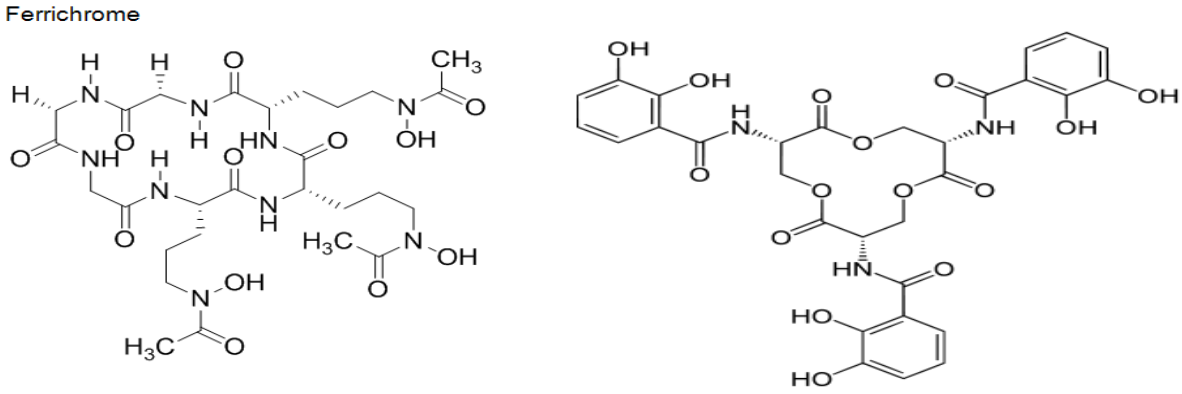
Catecholate-type (phenolate) siderophores bind Fe^{3+} using adjacent hydroxyl groups of catechol rings. Enterobactin, also known as enterochelin, is produced by a number of bacteria including E. coli and is the classic example of a catechol-type siderophore (OíBrien & Gibson, 1970, Pollack et al. 1970). It possesses the highest known affinity for Fe^{3+} with a stability constant (Kf) of 1052 (Hofte,1993). Enterobactin production has been demonstrated in some nitrogen-fixing bacteria, including Klebsiella pneumonia and K. terrigena (Hofte 1993). In hydroxamate siderophores, $Fe^{3+}$is chelated using nitrogen atoms of thiazoline and oxazoline rings (Crosa and Walsh 2002). Ferrichrome is the classic hydroxamate-type siderophore. The carboxylate group utilizes N-hydroxy amino side chains with an oxygen atom as one as one of the ligands for $Fe^{3+.}$ Anguibactin produced by Vibrio anguillarum incorporates this functional group but it also a combination of all the three siderophore types in that it is made up of all three functional groups with three different methods of binding Fe^{3+} (Crosa and Walsh 2002).

3.3.2.1 Hydroxamate siderophore (Des-ferrioxiamine B)

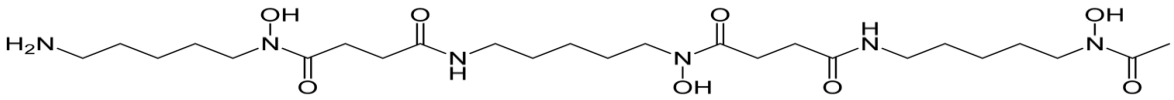
Deferoxamine (also known as desferrioxamine B, desferrioxamine B, DFO-B, DFOA, DFB or desferal) is a bacterial siderophore produced by the Actinobacteria *Streptomyces pilosus*. It has medical applications as a chelating agent used to remove excess iron from the body (Miller, 1989). Medically, desferrioxamine acts by binding free iron in the bloodstream and enhancing its elimination in the urine. The agent reduces the damage that is done to various organs and tissues, such as the liver by removing excess iron .It performs a similar function by making Fe inaccessible to the biota when it is added to high-Fe seawater .Deferoxamine may modulate expression (Lee et al., 2007) and release of inflammatory mediators by specific cell types (Choi et al., 2004).

In marine chemistry,) it has been demonstrated that additions of a trihydroxamate siderophore, desferrioxamine B (DFOB), greatly reduces community Fe uptake in the equatorial Pacific

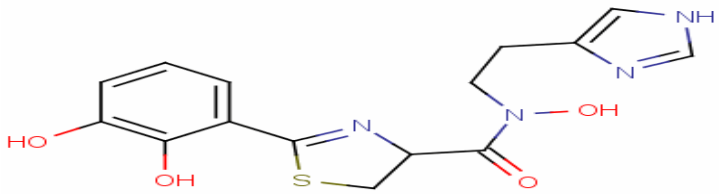
HNLC area (Wells et al., 1994).The very high conditional stability constant with respect to inorganic Fe(III) (Fe9) of the Fe-DFOB complex in seawater (;1016 M21; Rue and Bruland 1995; Witter et al. unpubl. data) allows this siderophore to effectively complex any available dissolved Fe.



A) Ferrichrome, a hydroxamate siderophore **B) Enterobactin, a catecholate siderophore.**



C) Desferrioxamine B, a hydroxamate siderophore



D) Anguibactin, a mixed siderophore

Fig 3.8 The representative structures of different types of siderophores

4. MATERIALS AND METHODS

Biological cultures and different chemical (Chelex-100 preconcentration and DGT) techniques were employed in this experiment where the former is to aid in determining the iron-siderophore effects on two different phytoplankton species whiles the latter aids in determining the bioavailable forms of iron in the culture medium.

4.1 Techniques

4.1.1 Chelex- 100

Chelex -100 is a resin that consists of styrene divinylbenzene copolymers containing paired iminodiacetate ions which act as chelating groups. It is widely applied in many analytical procedures including trace metals in natural waters. It has unusually high preference for copper, iron and other heavy metals over monovalent cations such as sodium and potassium. Its selectivity for divalent over monovalent ions is approximately 5,000 to 1, and it has a very strong attraction for transition metals, even in highly concentrated salt solution. It differs from ordinary exchangers because of its high selectivity for metal ions and its much higher bond strength (Bio-Rad Laboratories). Chelating resin is available as Analytical Grade Chelex 100 resin, Biotechnology Grade Chelex 100 resin, and Technical Grade Chelex 20 resin. The Analytical Grade Chelex 100 resin has been exhaustively sized, purified, and converted to make it suitable for accurate, reproducible analytical techniques. Biotechnology Grade Chelex 100 resin is analytical grade resin which is certified to contain less than 100 micro-organisms per gram of resin (Bio-Rad Laboratories).

The selectivity of Chelex resin for metal cations corresponds to that of iminodiacetic acid and the selectivity of this resin is dependent on the pH, ionic strength and the presence of other complex-forming species. In relation to selectivity, pH affects the quantity of cations that is exchanged, being very low below pH 2, while it reaches its maximum above pH 4. For the purposes here, pH was not variable affecting the results as the experiments were carried out in natural waters. Chelex chelating resin is efficiently regenerated in dilute acid and operates in basic, neutral, and weakly acidic solutions of pH 4 or higher. At very low pH, the resin acts as an anion exchanger (Bio-Rad Laboratories).

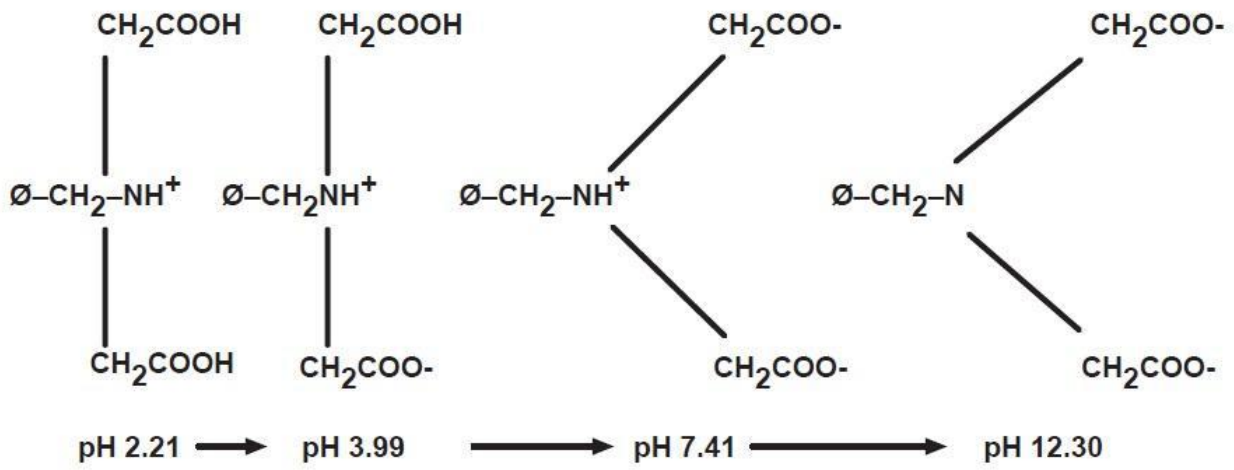


Figure 4.1 The structure of the Chelex resin at different pH (Bio-Rad Laboratories, 2000).

4.1.2 DGT

DGT comprises of a three-layer system which includes 1) a resin-impregnated hydrogel layer; 2) a hydrogel diffusion-layer; and 3) a filter membrane. The innermost two gel layers are fabricated from a polyacrylamide hydrogel. The filter membrane isolates the polyacrylamide surface from particles in the water. These layers are contained within a plastic device which makes it kind of protected from the environment. The DGT device passively accumulates labile species from solution while deployed in situ and therefore contamination problems associated with conventional water collection and filtration procedures are eliminated.

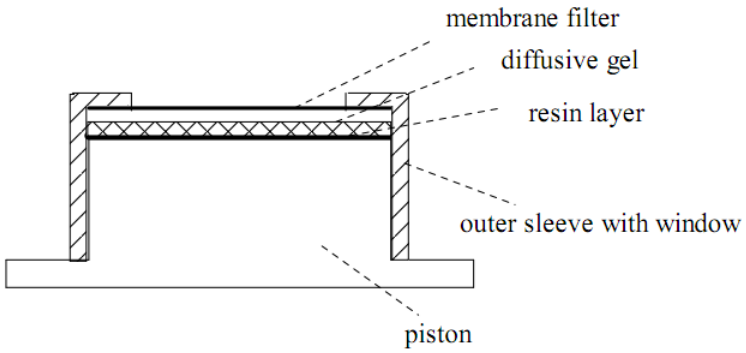


Fig 4.2 A schematic diagram of DGT (Zhang, 2003)

The theory behind DGT is based on the diffusional characteristics of metals in a hydrogel and on the ion exchange properties of a metal-binding resin. Specifically, the technique utilizes a hydrogel layer to control the diffusive transport of metals in solution to a cation-exchange resin. In addition, since the resin used in DGT (Chelex) is selective for free or weakly complexed species, it provides a proxy for the labile fraction of metals in solution. Labile metal ions in solution diffuse across the filter and gel layers and are pre-concentrated on the resin. The DGT technique principle is based on Fick's first law of diffusion. The layer of polyacrylamide hydrogel is of known thickness Δg (cm), which is backed by the layer of ion-exchange resin (Chelex-100) of thickness Δr (cm). Additionally, between the diffusive gel and the bulk solution there is the diffusive boundary layer (DBL), which is of thickness δ, where transport of ions is solely by molecular diffusion (Zhang and Davison 1995)

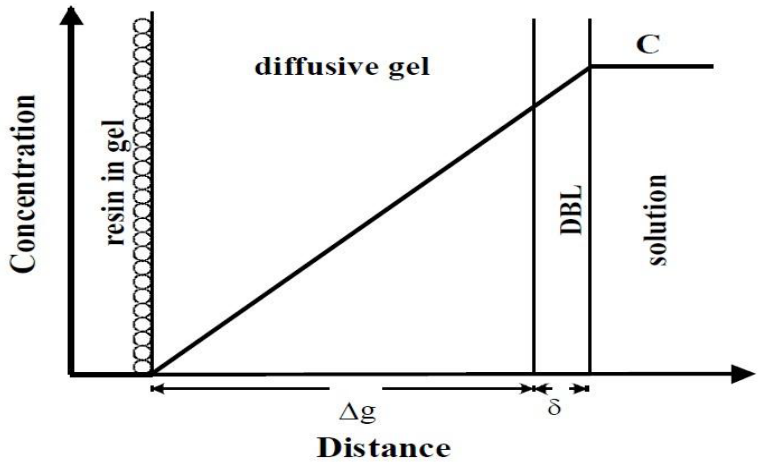


Fig 4. 3 **Schematic representation of the free concentration of ionic species in a hydrogel assembly in contact with aqueous solution, where the concentration is *Ct,* (DBL is diffusive boundary layer). The rate of diffusion is assumed to be the same in the gel and solution (Zhang and Davison 1995)**

The δ here was assumed negligibly compared to *Δg* due to effective stirring of the solution in the shaker. Then, the flux F (mol. cm^{-2} s^{-1}) of metal ions diffusing through the gel layer to the resin can be expressed by X-1

$$F = D\,(C_b - C')\,/\,\Delta g \qquad X\text{-}1$$

Where *D* is the diffusion coefficient (E-6 $cm^{-2}.s^{-1}$) of the element in the gel, C_b the free

concentration of a metal ion in bulk solution, and *C'* the free concentration of the metal ion in the resin gel layer. If the free metal ions are in rapid equilibrium with the resin, with a large binding constant, *C'* is effectively zero providing the resin is not saturated. Therefore X-1 can be simplified to X-2

$$F = D\,C_b / \Delta g \qquad \text{X-2}$$

According to the definition of flux (F = M/At), the mass diffused through an area A (cm^2), after given time t (sec) should be

$$M = D\,Cbt\,A / \Delta g \qquad \text{X-2}$$

The mass of the diffused ion *M* (ng)**,** can be obtained by The mass of the diffused ion *M* (ng)**,** can be obtained by X-3

$$M = (\,C_e\,(V_g + V_e) / f_e \qquad \text{X-3}$$

Where Ce is the concentration (μgr L^{-1}) of ions in the acid eluent obtained from the results on HR-ICP-MS analysis, *Vg* the is the volume (L) of gel in the resin gel layer, V_e the is the elution volume (L) of acid and f_e the ratio of the eluted to bound metal, known as the elution factor and here assumed 0.9 as extraction proceeded with 2M UP HNO_3 (Ardelan *pers. comm.*).

Obtaining M, the concentration of the ion in the bulk solution can be quantified by rewriting

$$Cb = M\Delta g / Dt\,Ag \qquad \text{X-2}$$

4.1.3 ICP-MS

ICP-MS is a powerful technique for analysis and quantification of trace elements, offering high precision and low interferences. It combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer. The sample is typically introduced into the ICP plasma as an aerosol, either by aspirating a liquid or dissolved solid sample into a nebulizer or using a laser to directly convert solid samples into an aerosol. Once the sample aerosol is introduced into the ICP torch, it is completely desolvated and the elements in the aerosol are converted first into gaseous atoms and then ionized towards the end of the plasma. Once the elements in the sample are converted into ions, they are then brought into the mass spectrometer via the interface cones. The interface region in the ICP-MS transmits the ions traveling in the argon sample stream at atmospheric pressure into the low pressure

region of the mass spectrometer. This is done through the intermediate vacuum region created by the two interface cones, the sampler and the skimmer. The sampler and skimmer cones are metal disks with a small hole (~1mm) in the center. The purpose of these cones is to sample the center portion of the ion beam coming from the ICP torch (Taylor, 2001, Thomas, 2004, Thomas, 2001b). Once the ions enter the mass spectrometer, they are separated by their mass-to-charge ratio. In a High Resolution mass spectrometer, both a magnetic sector and an electric sector are used to separate and focus the ions. The magnetic sector is dispersive with respect to both ion energy and mass and focuses all the ions with diverging angles of motion coming from the entrance slit of the spectrometer. The electric sector is dispersive only to ion energy and focuses the ions onto the exit slit (Hoffmann and Stroobant, 2007, Thomas, 2001a)

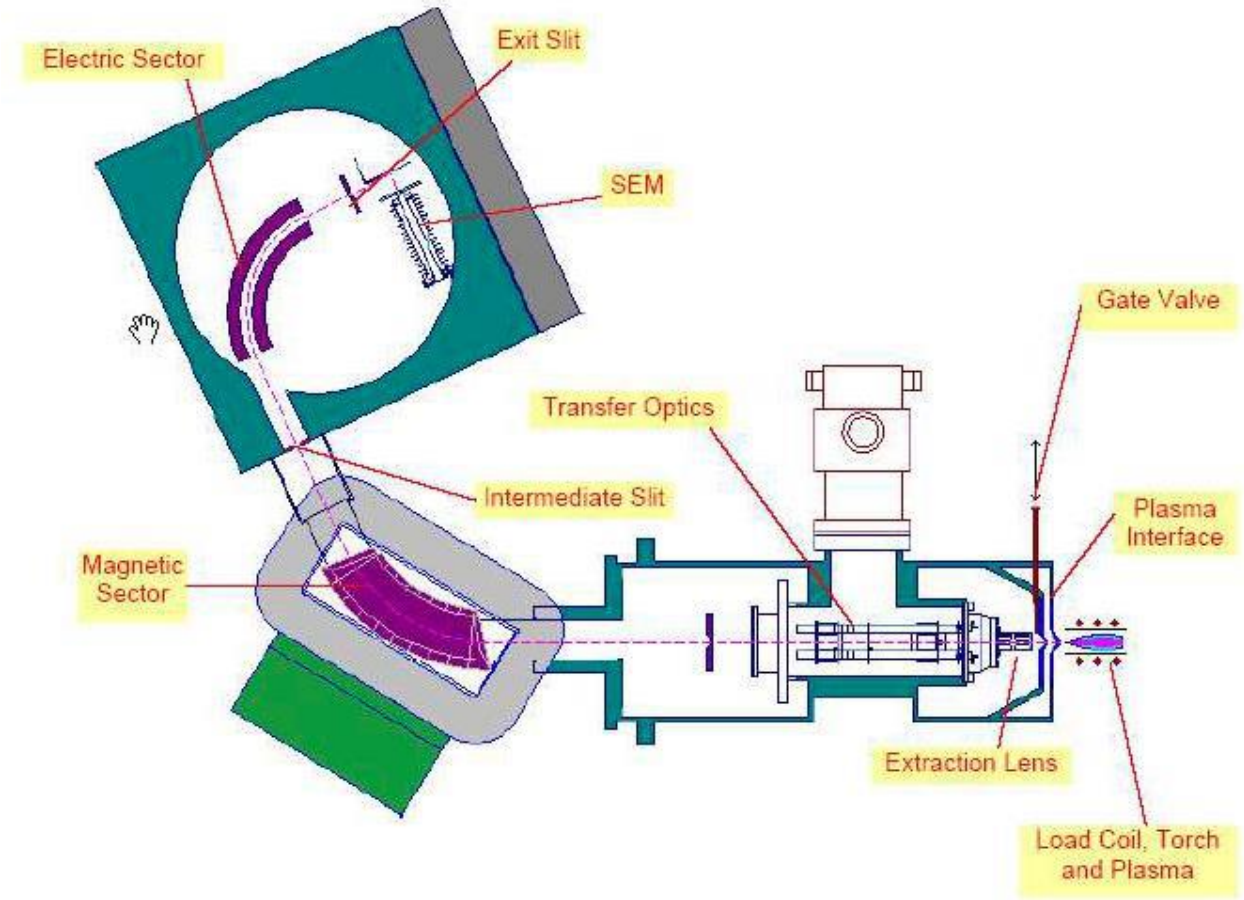


Fig 4. 4 Model of the HR-ICP-MS system showing the different components (Wolf, 2005).

4.2 Experimental work

4.2.1 Seawater sampling

Seawater collection was done with the aid of Go-Flow equipment and placed in an acid washed 10 L collapsible bottle. This were equally distributed into 4 different collapsible bottles and later brought to the laboratory and kept in the refrigerator for conservation purposes. All materials used in the collection of seawater for the experiment were acid washed in order to minimize the level of contamination during the process. Water was collected from a depth of 7 metres to 10 metres.

Seawater was filtered through a 0.4*um* + 0.25*um* Sartorius -Sartobran filters with a peristaltic pump and collected by acid washed 10L Nalgene bottles. Sartorius-Sartobran filters were replaced after every 20L filtration of seawater. The filtered seawater was sterilized by an autoclave for 30mins at 120^0C.Additionally, water was autoclaved in acid washed 10L Nalgene bottles and brought out of the Autoclave after temperature had reduced to 80^oC (to avoid burns) and poured into another acid cleaned bottle by the aid of a clean funnel. Autoclaved water was refrigerated.

4.2.2 Culture experiment

4.2.2.1 Arrangement of culture bottles

A culture consists of the growth of microorganisms in a nutritive medium under controlled conditions. For this experiment, two phytoplankton species were cultured which were Skeletonema costatum, a diatom and Alexandrium catanella, a dinoflagellate. 500ml acid washed polycarbonate bottles with pores on the cap for aeration purposes were used as the container for the growth experiment. A total of 84 bottles, with 42 bottles for each species were well aligned on a clean white paper on shelf .With the knowledge of the irradiance for each phytoplankton species, bottles were aligned accordingly from the fluorescent light tubes with 1 centimeter distance between each bottles. These bottles were clearly labeled in turns of three for replicates and these were done due to the various treatments planned for replicates and the experiment as a whole.

Fig 4.5 A picture showing clearly labelled, well aligned culture bottles with upper shelf bottles for Skeletonema costatum (diatom) and middle shelf for Alexandrium catanella (dinoflagellate).

4.2.2.2 Nutrient preparations

4.2.2.2.1 F/2 medium preparation

1L trace element solution of initially prepared individual solutions from trace elements of Fe, Cu, Mo, Zn, Co, Mn, Vn. Se, and Ni was prepared. Additionally, 4.36g of $Na_2EDTA2H_2O$ and 3.15g of $FeCl36H_2O$ were added to 950ml of Milli-Q water and manually shaken when these powdery mixtures were added in bits to ensure thorough mixing. The various volumes dispensed from each individual solution was done according to guide for f/2 medium preparation by Guillard and Ryther, 1962.

A volume of 1ml was taken from each trace element solution and added to 990ml of Milli-Q water. Additionally, 1ml each of $NaNo_3$, $Na_2H_2PO_4H_2O$ and $Na_2SiO_39H_2O$ were also prepared separately. Before these solutions were prepared, they were first cleaned with Chelex resin in order to trap every metal in the macronutrients solution. Here, 0.8ml of Chelex slurry was dispensed into each macronutrient solution and placed on shaker for 48 hours and as mentioned below, these solutions were cleaned the same way as the Chelex samples for this experiment.

Vitamin solutions were also prepared which include 200mg of thiamine (vitamin B_1), 1ml of biotin (vitamin H) and 1ml of cyanocobalamin (vitamin B_{12}).Hence, with 950ml of filtered natural seawater and addition of trace element solution plus vitamin solution, the f/2 medium for the growth of the phytoplankton species were prepared. Each culture bottle was filled with the nutrient solution up to the 400ml mark on the 500ml polycarbonate bottles under a Class-100 Air clean Chamber with a laminar flow to reduce levels of iron contamination.

4.2.2.2.2 Preparation of Iron and Siderophore Solutions

Different concentrations and volumes of iron was prepared from $Fe(SO_4)_2$ with aluminium around bottle due its ability to easily oxidize to Fe^{3+} in the presence of light .Here 0.07907g of $Fe(SO_4)_2$ (iron sulphate) was added to 500ml of Milli-Q water in a bottle and was placed on a shaker for proper mixing for about 2 hours. This bottle was labeled **FeExpZero stock**. Furthermore, 1ml of FeExpZero solution was added to 999ml of Milli-Q water and the bottle was labeled **FeExp 1st Stock.** Then 20ml of the **FeExp 1st Stock** was added to 80ml of Milli-Q water in a bottle labeled **FeExp 2nd stock.**

Des-ferrioxiamine B (DFB) siderophore solutions were prepared from 0.0786g of its powdery state which was added to 100ml of Milli-Q water in a bottle and placed and shaker for hours for thorough mixing. The light in the clean chamber was switched off since siderophores are biosensitive. This siderophore solution was labeled **Stock A** and **Stock B** siderophore solution was prepared from 99ml of Milli-Q water and 1ml of **Stock A** siderophore solution.

4.3 Culture sampling and parametric measurements

All 84 culture bottles were washed with seawater. In total, about 36 litres (4*10L) Nalgene bottles) of filtered, autoclaved and seawater cleaned with Chelex on different days were well shaken. 10 litres of seawater was taken in turns and poured in an acid washed 10L Nalgene bottle with an outlet where a tube was connected plus a regulator. Afterwards, 10ml each of the micronutrients i.e. Nitrates, Silicate, Phosphate and Vitamins) and trace element solution was added by the aid of an Eppendorf pipette. These processes were done in the absence of light and under a Clean Chamber with Laminar flow. The solution was well shaken manually for homogeneity purposes. With the aid of the tubing and the regulator connected to the outlet, 400ml of nutritive culture medium was dispensed into each culture bottle. Each of the culture bottles for Skeletonema costatum were inoculated with 4ml of the stock solution whiles 2ml of

Alexandrium catanella stock solution was dispensed each into for bottles meant for Alexandrium. Afterwards, each bottle for both species were well shaken manually .Spilling was avoided. However, since it was the first day, random bottles were chosen for parametric measurements. After sampling for each phytoplankton species, volumes of different concentrations of iron and siderophore were respectively added to replicates of bottles according to the design of the experiment. The table showing the various volumes of iron and siderophore treatments that were added to each bottle including replicates and the control(See Appendix 1). Culture bottles for each species were well shaken after treatments and well aligned on the shelf to ensure equal irradiance from light tubes in the culture room.

According to the design for the experiment above, culturing of Skeletonema costatum and Alexandrium catanella were planned for 15 days where sampling was done every second day i.e. sampling was done on day 1, 3, 5,7,9,11,13 and 15.On these sampling days, samples of the culture medium were taken for parametric measurements which included pH, quantum yield, In vivo fluorescence, bacteria abundance and cell abundance for each phytoplankton species. Samples were poured in 50ml vials from each culture bottle and pH measurement was first done, later to in vivo fluorescence, quantum yield measurements, cell abundance and lastly bacteria abundance measurement.

4.3.1 pH

The pH meter was always calibrated to a 2 point calibration system before measurements began. Vials containing sample were well shaken before the probe of the pH meter was inserted and values were recorded after pointer displayed on pH meter stops flashing.

4.3.2 In vivo fluorescence

2ml cuvette was washed with Milli-Q and thoroughly dried with paper. Sample was poured from the 50ml vials up to ¾ (three quarters) of the cuvette. The outside of the cuvette was well cleaned and later placed in the fluorometer .Raw fluorescence values were measured and recorded.

4.3.3 Quantum Yield

2ml cuvette was washed with Milli-Q and thoroughly dried with paper. Sample was poured from the 50ml vials up to half of the cuvette. The outside of the cuvette was well cleaned and later placed in the aqua pen. Quantum yield value was measured and recorded.

4.3.4 Cell Abundance and Bacteria Abundance

0.5ml of sample for each species' culture medium was dispensed into 1ml vials by the aid of an Eppendorf pipette after sample was well shaken. For bacteria abundance, 1.5ml of sample was dispensed into sterilized 2ml vials. Under a Class-100 Air Clean Chamber with laminar flow, 30µl of gluteraldehyde (which was very toxic) was added and later vials were thrown into a small tank containing Nitrogen gas.

4.4 Chelex samples

Water samples were collected in acid washed plastic bottles for Chelex labile iron (Fe_{Ch}).A volume of 0.8ml of Chelex slurry (well shaken before used to ensure uniform mixture) was dispensed by an Eppendorf multiplette automatic pipette to a volume (120 – 150 ml) of water sample. Each sampling bottle was placed in clean plastic bags and put on a shaker (65 – 8 rpm) for 48 hours. Sampling bottles after this period were taken and poured into acid washed plastic Chelex Columns (Bio-Rad Laboratories).The water was transferred into the column by the aid of a funnel and a mesh at the end of the column collects the Chelex-100 resin in the water sample when the water is being drained through tubings and regulators. The Chelex resin in the emptied sampling bottle were washed with 15 ml of Milli-Q water, secondly with 10 ml of Ammonium Acetate buffer ($C_2H_4O_2.NH_3$) and 5 ml of Milli-Q water to remove residues of Mg^{2+} and Ca^{2+} ions present in seawater. All columns for the extraction of trace metals were placed in a well aligned grid system under a clean fume hood with laminar flow to minimize levels of contamination during extraction process. A two step acidifying process was employed for the trace metal extraction process. Firstly, 1 ml of 2M UP HNO_3 was dispensed by an automatic pipette into the column closed with a stopper. It waited for 5 minutes, then gently shaken for re-suspension of Chelex-100. After an additional 15 minutes, the content of the column was drained into acid washed Polyethylene tubes. Secondly, 4ml of 0.25 M UP HNO_3 was again added to the columns with stoppers. After 10 minutes, the content was poured into the Polyethylene tubes gaining a total volume of 5ml sample (Ozturk et al. 2002; Ardelan et al., 2010)

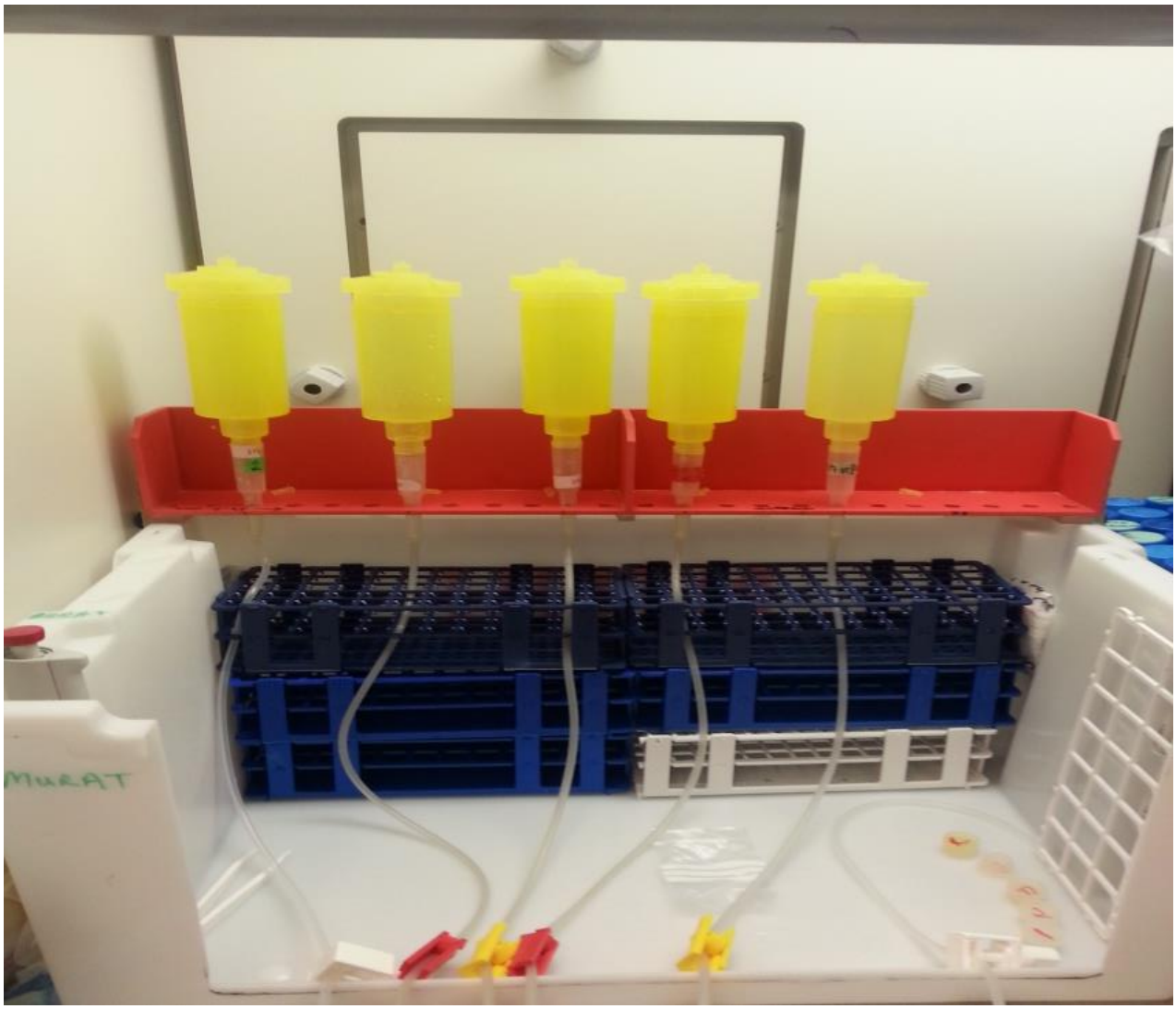


Figure 4.6 The set up for Chelex-100 pre-concentration columns for iron and other trace metals.

4.4.1 Initial iron concentration for Chelex samples before experiment.

The initial iron concentration of iron after seawater was cleaned with Chelex resin was 0.76nM.The table below displays the average iron concentration from liters of Chelex cleaned seawater.

TESTS performed	µgr.L (raw)	RSD (%)	Wt (gr)	Vol (ml)	µgr.L^{-1}	nmol.kg	nM
Smpl-15	0.96	1.1	132.2	154.98	**0.03**	0.65	0.55
Smpl-16	0.66	0.7	132.7	153.85	**0.02**	0.45	0.38
Smpl-17	1.46	1.1	126.5	154.57	**0.05**	1.03	0.84
Smpl-18	2.18	3.3	127.7	154.78	**0.07**	1.52	1.26
							0.76

Table 4.1 The average of initial iron concentration of Chelex samples before culture experiment

4.5 DGT Samples

Approximately 1.5 L of filtered, autoclaved and Chelex cleaned seawater was poured into acid washed 2L plastic bottles. 6 DGT units were placed in it. Afterwards, the plastic container was put in clean plastic bags to reduce level of contamination and later on a shaker (70-90 rpm) for 72 hours. After this time period, DGT units were taken out of the water sample and stored in the freezer (Ardelan et al. 2009).

All DGT units were processed on Teflon sheets under a clean fume hood with laminar flow. All units were opened carefully where the first two layers (membrane filter and diffusive gel) were removed. The resin gel (third layer) was transferred into polyethylene tubes and 1 ml of 3M UP HNO_3 was added. These tubes were placed on a shaker (70-90 rpm) for 12 hours. After this period, the acid in the Polyethylene tubes was transferred into new acid washed tubes that were clearly labelled keeping the resin was in the old tube. Furthermore, ensuring that all extracted metals had been transferred, the resin was washed twice. Firstly with 1ml of Milli-Q water and later 3ml and then poured into the new tubes resulting in a total volume of 5ml sample.

4.5.1 Initial iron concentrations of DGT samples before experiment

The initial concentration of iron for DGT samples was 1.60nM and the table below shows an average of the iron concentration from DGT units in seawater before the experiment.

Samples	Technique	Initial Fe conc.
INITIAL	DGT	1.85
INITIAL	DGT	3.42
INITIAL	DGT	0.00
INITIAL	DGT	0.00
INITIAL	DGT	2.07
INITIAL	DGT	2.25
	Average	1.60

Table 4.2 The average initial iron concentration for DGT samples before the experiment.

Figure 4.7 A DGT unit sampler taken out of seawater sample and ready for the separation of the three layers.

4.6 Filtration

In order to determine the cellular concentration and the distribution of total particulate iron content present within the culture medium for the phytoplankton culture, filtration of the culture medium (seawater) was done. By the aid of a peristaltic pump, carefully selected culture bottles with the same nutrient, iron and siderophore treatments were filtered through acid washed

0.4µm+0.2µm Sartorius-Sartobran filters cartridges and drained through acid washed polycarbonate tubes into clean 2L plastic bottles. A volume of between 150-170 ml was filtered for Chelex samples and replicates with 10000nm of siderophore treatments and Control (without siderophore treatments) were filtered for DGT samples. Sartorius-Sartobran filters were replaced after remnants of phytoplankton clogged the pores of the filter.

4.7 Blank and detection limits

Detection limits are defined as the minimum concentration that is different statistically from the instrumental blank value and this is a part of the quality control of analytical method (Grasshoff et al. 1999) .For Chelex blanks, 800uL of Chelex slurry was dispensed with an automatic pipette into each polyethylene columns and run through tubes. 15 ml of Milli-Q water was used to wash excess Chelex resin on the walls of the column. Afterwards, 10ml of 0.1M UP Ammonium Acetate buffer and 10 ml of Milli-Q were added to the columns. Using two acidifying process explained above, a total volume of 5 ml was obtained. The acid clean and conditioned Chelex-100 gel in the DGT units used as blank. These Gels were treated similarly as sample-DGT units.

4.8 Statistical analyses

Microsoft Excel 2010 was the main spread sheet used for statistical analyses. The mean and standard deviation of all data was generated in this spreadsheet. No other statistical analyses were done.

5. RESULTS

5.1 pH levels in phytoplankton culture medium

In order to measure the effect of CO_2 removal by photosynthesis and the implication of net CO_2 removal(photosynthesis and respiration) from the culture medium by the different phytoplankton species, the pH values of Skeletonema costatum and Alexandrium catanella cultured with different iron concentrations (specifically 1nm and 10nm) and siderophore treatments together with their standard deviation respectively are being presented in a graph form to show the various levels each species exhibited and its relation to growth activities during these days.

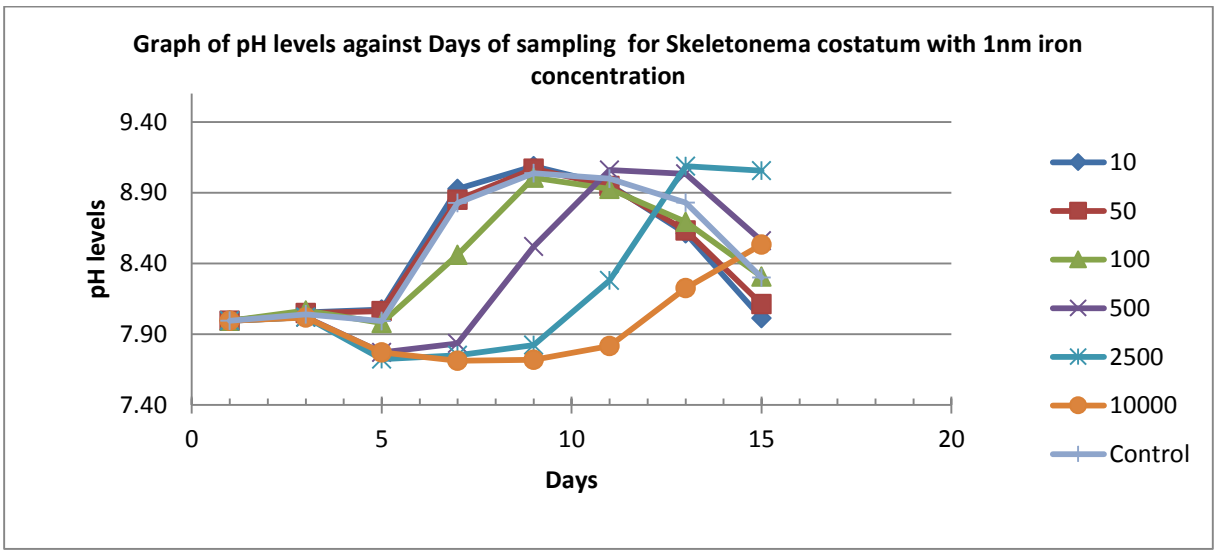


Fig 5.1 The levels of pH recording over 15 days of culture experiment for Skeletonema costatum with 1nm iron concentration and different siderophore concentrations as shown in the chart legend (10nM,50nM,100nM,500nM,2500nM, 10000nM and Control)

The response of pH to siderophore increase was very clear or distinct in Skeletonema costatum than in Alexandrium catanella. DFB siderophore did apparently have an effect on the pH response (effective CO_2 removal by photosynthetic activity) in Alexandrium catanella but not as prominent and explicit as in Skeletonema costatum.

The initial pH recorded before the experiment was 8.00 for cultures treated with 1nM Fe concentration and 7.87 for cultures with 10nM Fe concentration treatment. For 1nM Fe concentration cultures, the pH was observed increasing after a level of 8.90 in cultures with

treatment 10nM, 50nM, the control and 8.40 in 100nM DFB siderophores quick as 7 days. Additionally, the maximum pH in cultures with treatment 10nM, 50nM, 100nM DFB siderophore concentrations and the control were been reached on the 9th day with values recorded as (9.09±0.01),(9.07±0.02),(9.01±0.01) and (9.04±0.05) respectively. However, cultures with treatment 500nM, 2500nM and 10000nM DFB siderophore concentration delayed in reaching their maximum pH. Treatment 500nM, 2500nM and 10000nM reached their maximum pH at (9.06±0.12), (9.09±0.02), (8.53±0.03) on days 11, 13 and 15 respectively. These shows a very clear response treatment between the DFB siderophore concentrations and pH levels especially the low DFB siderophore concentration group (10nM, 50nM and 100nM) and in the control despite a little delay in the DFB 100nM treatments. In contrast, the response of pH to the higher DFB siderophore group was clearly different from the rest of DFB siderophore concentrations group. Hence whiles siderophore concentrations increased, levels of pH delayed in reaching its maximum peak. See Figure 5.1

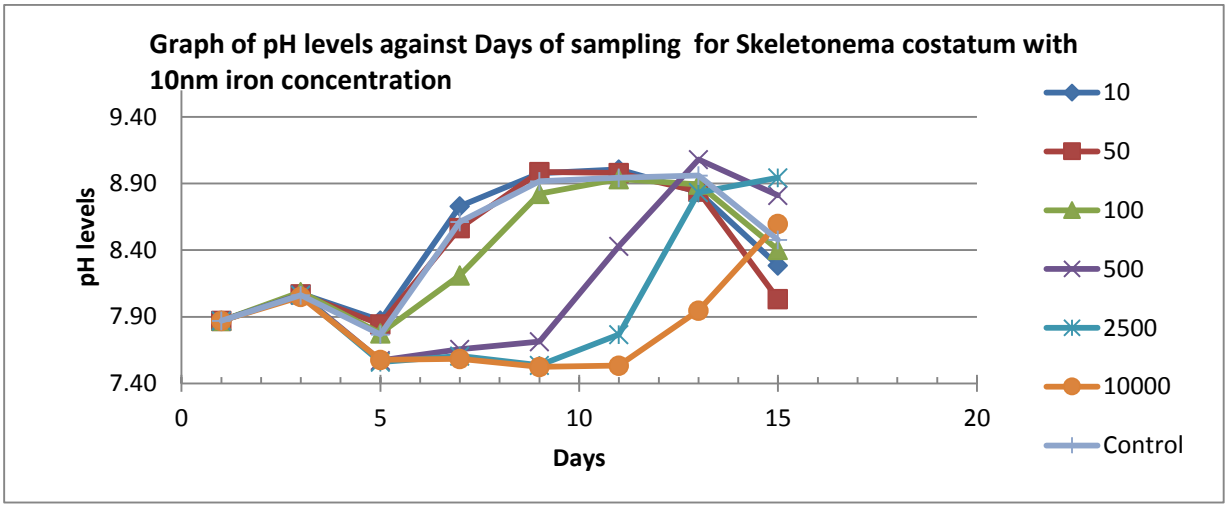


Fig 5.2 The levels of pH recording over 15 days of culture experiment for Skeletonema costatum with 10nm iron concentration and different siderophore concentrations .

A similar pattern can be seen with cultures of Skeletonema costatum treated with 10nM Fe concentration. The pH began to increase after a level of 8.73 for the lower DFB siderophore groups and the control as well. It could be noticed that CO_2 was effectively removed during photosynthetic activities as early as the 7th day with most changes in pH occurring between day 5

and 9 for the lower DFB siderophore treatments and Control cultures .The maximum pH values recorded were (9.01±0.02), (8.99±0.03), (8.93±0.02) an (8.96±0.03) in treatments 10nM, 50nM, 100nM and control cultures. Increase in pH in both 500nM and 2500Nm DFB siderophore cultures started on day 9 and day 11 for 10000 nM DFB cultures. A similar clarity of an increase in siderophore and a delay in pH increase was established here however in comparison with 1nM Fe treated cultures, 10nM Fe treated cultures experienced an increase in pH 2 days (lag) after the 9th day prominent pH changes that occurred in the high DFB siderophore concentration group (500nM, 2500nM and 10000nM) for 1nm Fe treated cultures. See Fig 5.2.

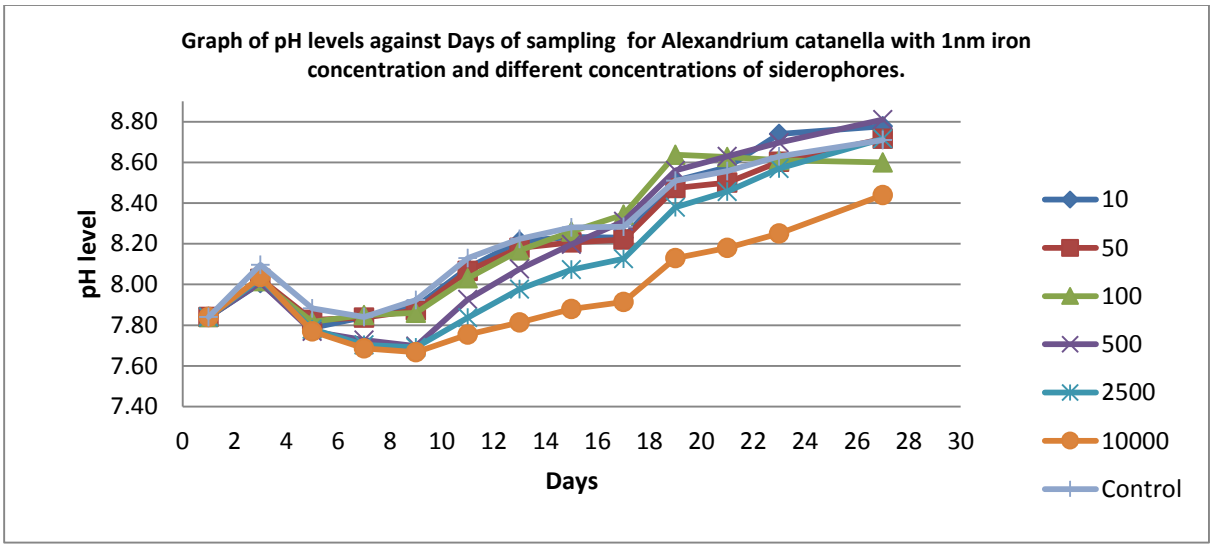


Fig 5.3 The levels of pH recorded over 27 days of culture experiment for Alexandrium catanella with 1nm iron concentration and different siderophore concentrations.

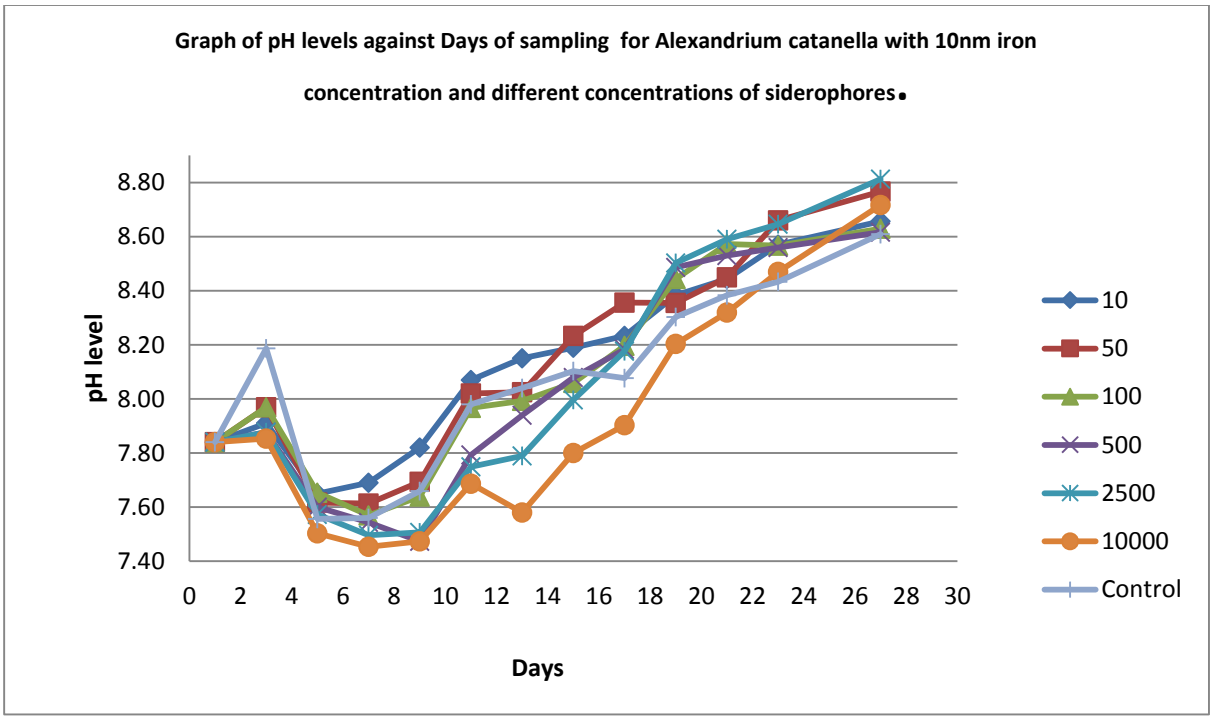


Fig 5.4 The levels of pH recorded over 27 days of culture experiment for Alexandrium catanella with 10nm iron concentration and different siderophore concentrations.

Alexandrium catanella was cultured for 27 days due its slow response to growth and pH was generally around 7.80 before the beginning of the experiment. Comparing the two different iron treatments in Alexandrium catanella cultures, it could be clearly observed that the impact of siderophore on pH levels were much visible in 1nM Fe than in 10nM Fe In 1nM Fe treatments, pH began to increase after day 5 in the lower DFB siderophore treatments and control cultures whiles pH began to increase after day 9 for 500nM, 2500nM and 10000nM cultures. However, as the days for the growth experiment increased, DFB 10000 siderophore treatments began to show it effects on iron bioavailability especially after day 11 where it became so distinct (reaching a pH of around 8.4) whiles the other treatment groups rose closely in gaining their highest pH levels ranging between 8.60 and 8.80 .Additionally, there were fluctuations that were shown in each treatment graph(line) until day 17 graphs for 500nM and 2500nM joined in till day 27 reducing the clarity in their effects of iron bioavailability towards the end of the culture experiment. pH was therefore increasing all way through, till the 27th day in all treatments. See figure 5.3

An indistinguishable trend was repeated in the 10nM Fe treated cultures of Alexandrium. Here, pH began to increase after the 5th day till the 27th day in the lower DFB siderophore treatments (10nM and 50nM with a bit of delay in 100nM which started to increase after day 7) and control cultures .on the 19th day. The higher DFB siderophore treatments did also delay until after day 9 where pH began to increase. However on day 13, DFB 10000 did show a distinct separation by showing clarity of its impact on Alexandrium cells till day 25 where it became obscure. See figure 5.4.

5.2 Quantum yield of phytoplankton species in culture medium

In order to determine the amount (moles) of CO_2 fixed per mole of quanta (photons) absorbed or the efficiency with which light is converted into fixed carbon by Skeletonema costatum and Alexandrium catanella, the quantum yield for each species was measured and presented in the form of graphs.

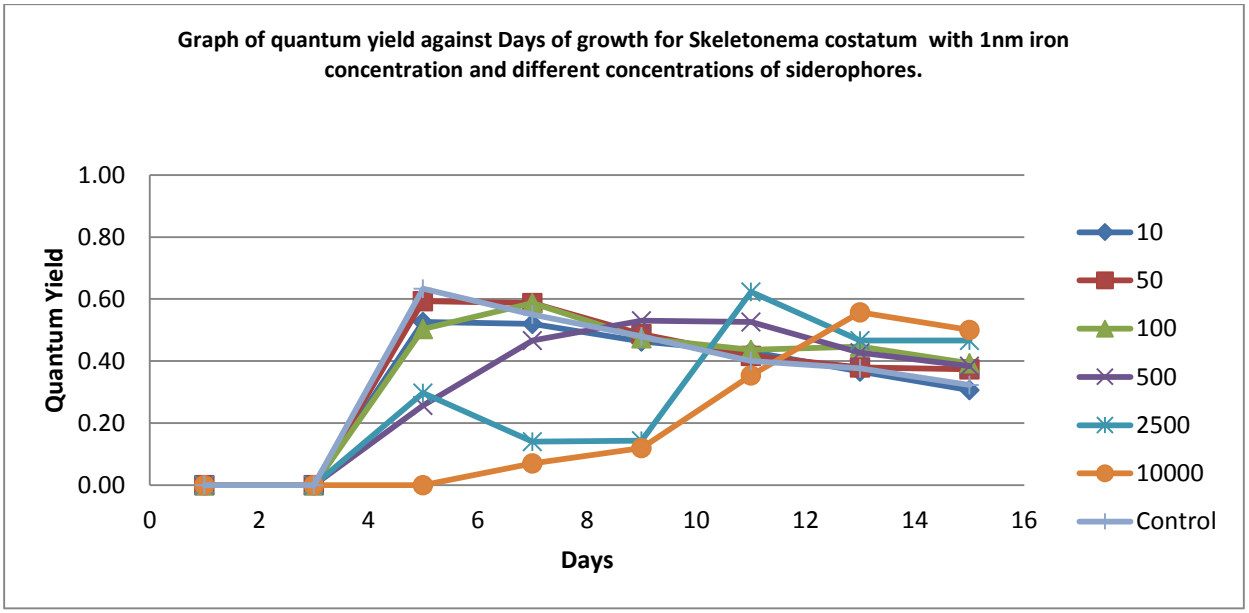


Fig 5.5 Quantum yield recorded over 15 days of culture experiment for Skeletonema costatum with 1nm iron concentration and different siderophore concentrations.

With reference to quantum yield measurements , Skeletonema costatum presented a distinctive and transparent levels of QY as a result of more carbon that were fixed over a short period of time despite the presence of high concentrations of siderophores whiles Alexandrium catanella still not showing eminent effects of different DFB siderophore concentrations in its growth medium. In 1nM Fe treatments for Skeletonema costatum, QY began to increase from day 3 in

the lower DFB siderophore treatment (10nM, 50nM and 100nM), control, 500nM and 2500nM siderophore cultures whiles those 10000nM DFB additions did delay after day 5 when QY began to accelerate. The initial QY was around zero for al treatments till the 3rd day. Additionally, most carbon was fixed on the 5th day and 7th day in the 10nM, control cultures and 50nM, 100nM DFB siderophore treated cultures respectively. The maximum QY were (0.53± 0.06) , (0.59± 0.09),(0.59±0.01) and (0.63± 0.05) for 10nM, 50nM, 100nM and the control cultures. The higher siderophore group exhibited highest QY values mostly on day 11 for 500nM and 2500nM whiles that for 10000nM was on the 13th day .The values were (0.53±0.03) , (0.62± 0.01) and (0.56± 0.05) for 500nM, 2500nM and 10000nm respectively. See figure 5.5

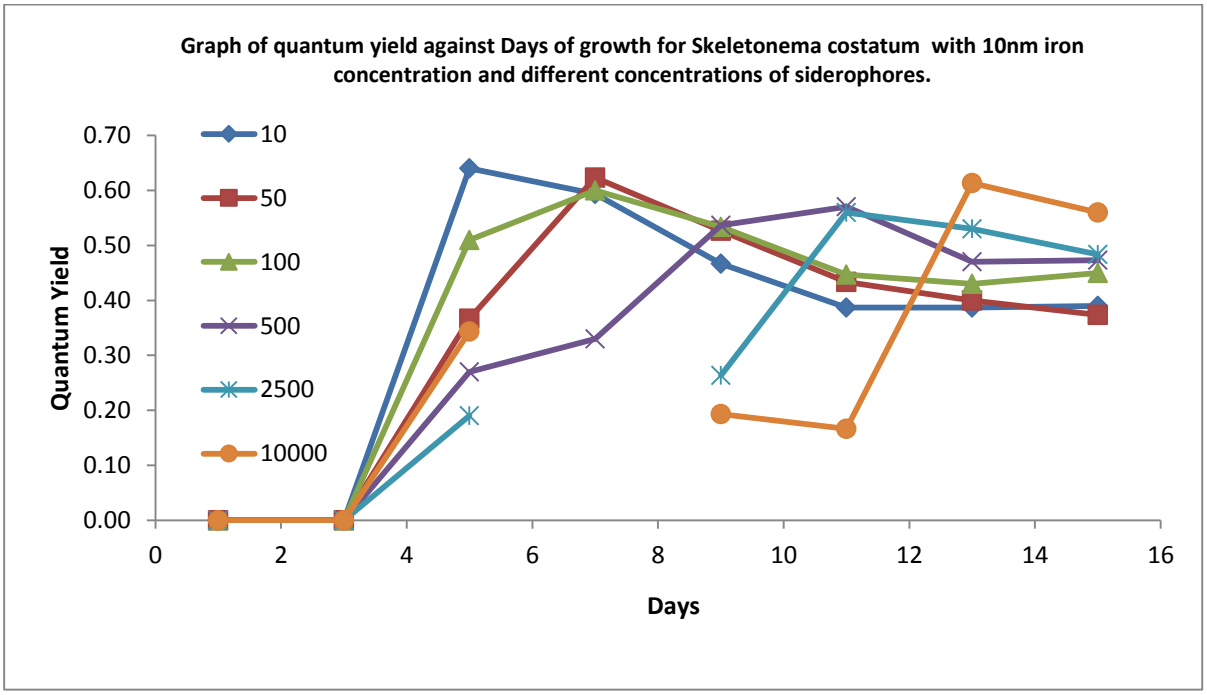


Fig 5.6 Quantum yield recorded over 15 days of culture experiment for Skeletonema costatum with 10nm iron concentration and different siderophore concentrations.

It could be plainly stated that the response of Quantum Yield to additions of various concentrations of DFB siderophore was quite clear and not very rapid because in both 1nM Fe and 10nM Fe treated Skeletonema cultures ,the lower DFB siderophore and the control cultures displayed levels of carbon fixation between day 3 and 5 but slight change in the higher siderophore group with almost a negative QY value for 2500nM and 10000nM on days 7 for

10nM Fe treated cultures unlike in 1nM Fe where they appeared increasing gradually between day 5 and 7.QY increased after day 3 till day 11 where a decrease was exhibited in the lower DFB siderophore treatments and control cultures with the range of highest QY levels being between (0.56±0.06) and (0.64 ± 0.02) . Here again, QY was initially zero until the 3rd day.. In the higher DFB siderophore groups, QY began to increase from day 5 till day 13 especially in 500nM and 2500nM cultures whiles in 10000, QY distinctively began to increase after day 13. See figure 5.6

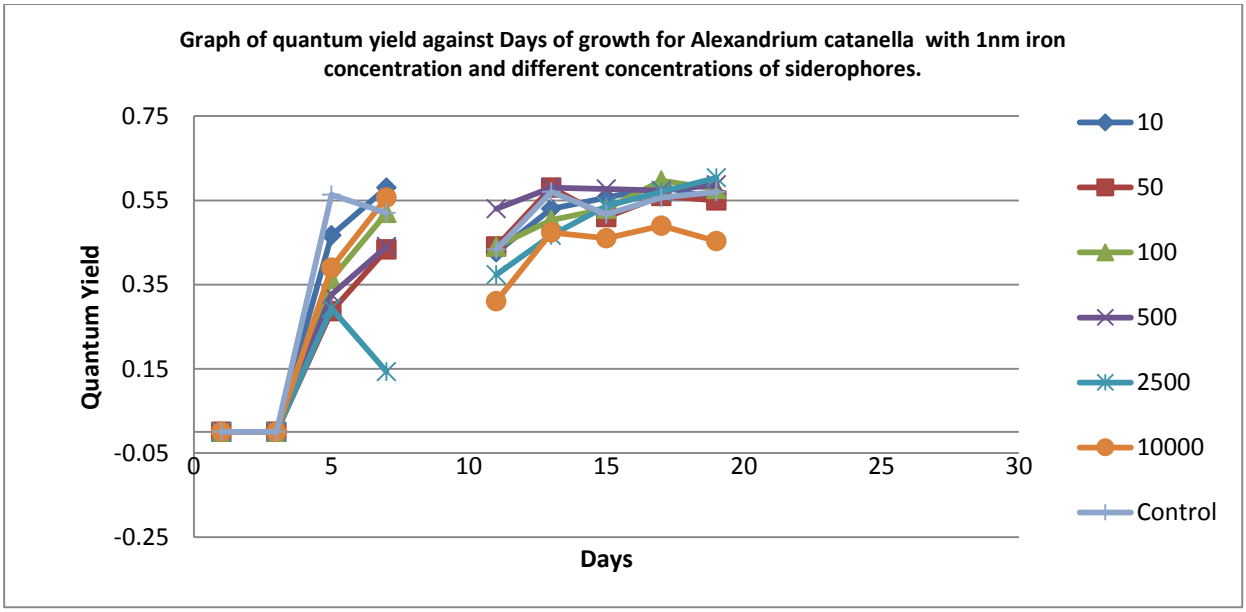


Fig 5.7 Quantum yield recorded over 27 days of culture experiment for Alexandrium catanella with 1nm iron concentration and different siderophore concentrations.

Obviously, a very similar trend of QY response to effects of addition of DFB siderophore concentrations to Alexandrium catanella treated with 1nM Fe concentration was obtained in the 10nM Fe cultures. Basically the same trend or graph pattern was repeated but with different QY values recorded on different days.

QY in Alexandrium cultures was quite unclear especially in the 1nM Fe treatments .All the treatments were show obscure effects initially on QY levels Here, QY started to increase after day 3 in all the treatments but after day 11, 10000nM DFB siderophore treatments began to show a conspicuous effect on the interference of iron bioavailability to these cells. That of the lower

DFB siderophore groups, control, 500nM and 2500nM was indistinct and did show minimal effects of siderophore concentration on QY levels in their cultures. Initial QY values were still zero until after day 3 where a change occurred. The highest QY values for the low siderophore group and control ranged between (0.58 ± 0.01) and (0.60 ± 0.05). That of the high siderophore treatments ranged between (0.49 ± 0.10) and (0.60 ± 0.02). See fig 5.7

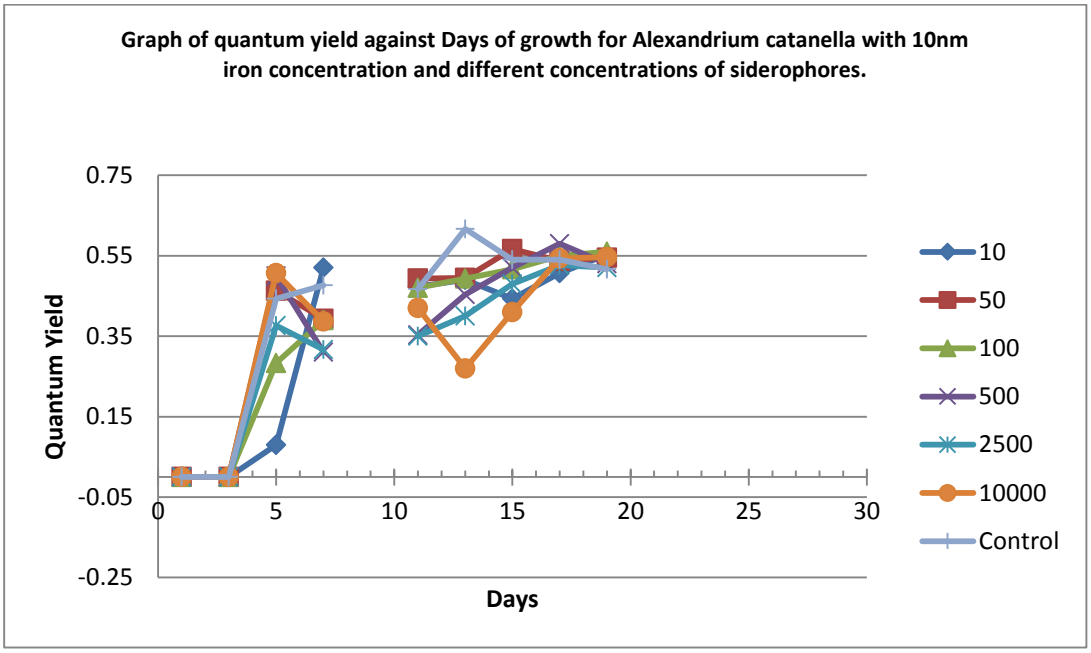


Fig 5.8 Quantum yield recorded over 27 days of culture experiment for Alexandrium catanella with 10nm iron concentration and different siderophore concentrations.

In 10nM Fe additions of Alexandrium catanella, QY levels started to increase again after day 3 in the lower siderophore treatments, 500nM and 10000 DFB siderophore treatments, but on the 5th day for 2500nM after an initial level of zero. However an increase in the days of the growth experiment resulted in a clear trend of the effects of 10000 DFB siderophore treatments on QY levels on day 11 precisely where a decrease occurred on day 13 but a quick rise after day 13 till day 17.On day 17, it became less clear. The range for maximum QY values measured in the lower DFB siderophore treatments and cultures were between (0.55 ± 0.02) and (0.62 ± 0.02) whiles for the higher DFB siderophore group it was between(0.53 ± 0.02) and (0.58 ± 0.04).See figure 5.8

5.3 In vivo fluorescence

In the process of monitoring the levels of photosynthetic activities due to the uptake of CO_2 supplied to the growth medium of the cultures, it was also necessary to measure the efficiency with which light impinging the phytoplankton algae is being used for photosynthetic processes and hence this was done by measuring the lost energy from the phytoplankton algae in the form of fluorescence.

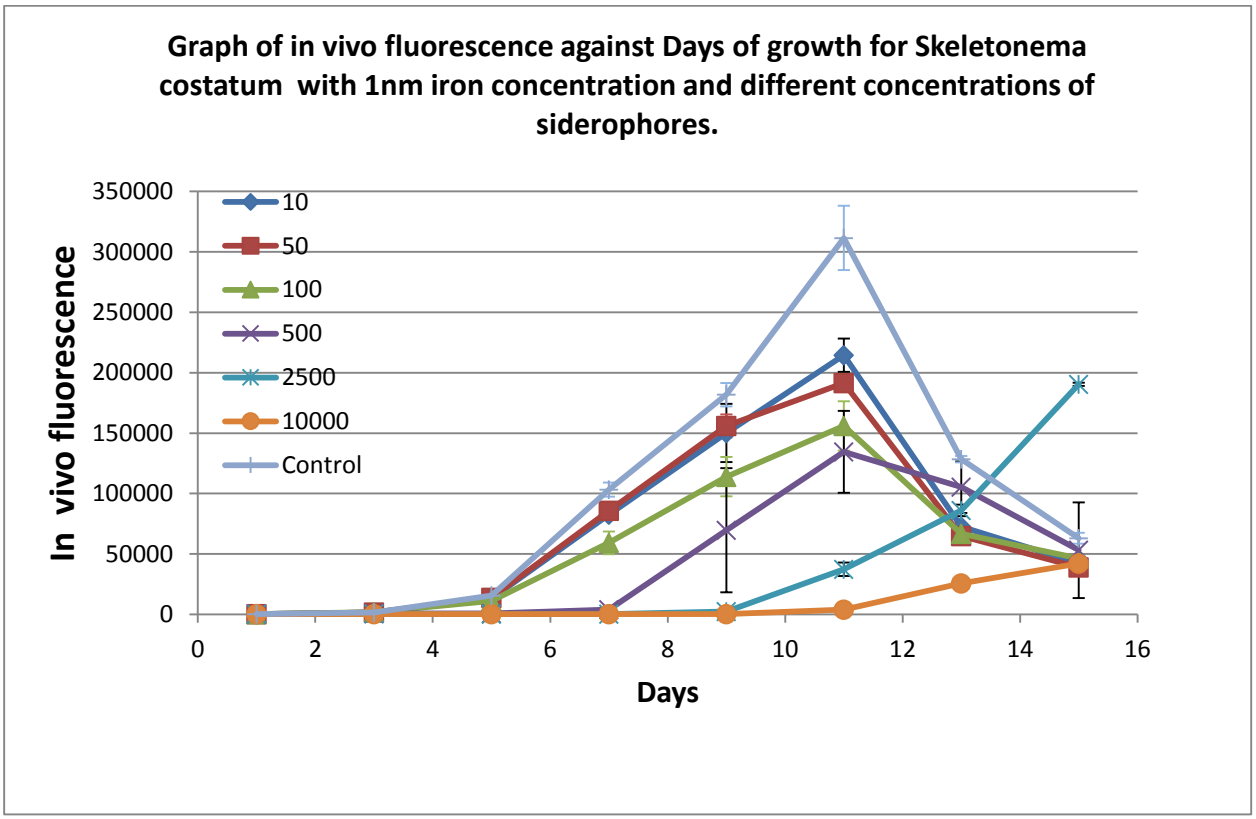


Figure 5.9 In vivo fluorescence recorded over 15 days of culture experiment for Skeletonema costatum with 1nm iron concentration and different siderophore concentrations

A close comparison of FSU measurements in Skeletonema costatum and Alexandrium catanella reveals a marked and conspicuous effect of DFB siderophore additions on FSU in the former phytoplankton cells since that of Alexandrium was minimally shown as siderophore additions increased .There was no clear difference in attaining higher FSU values in all treatments since it was very slow at growth.

There was an obvious response of In vivo fluorescence to the effect of additions of DFB siderophore to the culture medium of 1nM Fe treated cultures .Initial values of FSU (in vivo fluorescence) were recorded on the first day of experiment which was (382.69 ± 62.52). There was clarity in the FSU graph for 1nM Fe treated Skeletonema cultures but all treatments delayed from day 1 to day 3. In the lower siderophore group and control cultures, FSU began to increase after day 5. There was a delay in the higher siderophore concentration treatments where FSU levels began to increase after day 7 for 500nM, day 9 for 2500nM and after day 11 for 10000.The maximum FSU measured for (10nM, 50nM and 100nM, 500nM) and control cultures were (214443 ± 13740.18) , (191380.67± 1981.43) , (155956.67 ± 20479.8) ,(134494.33 ± 33938.60) and (311542.67± 26684.59) respectively. Higher siderophore (2500nM and 10000nM) had maximum FSU values as (190486 ± 1330.79) and (42005.33 ± 1629.67). See figure 5.9

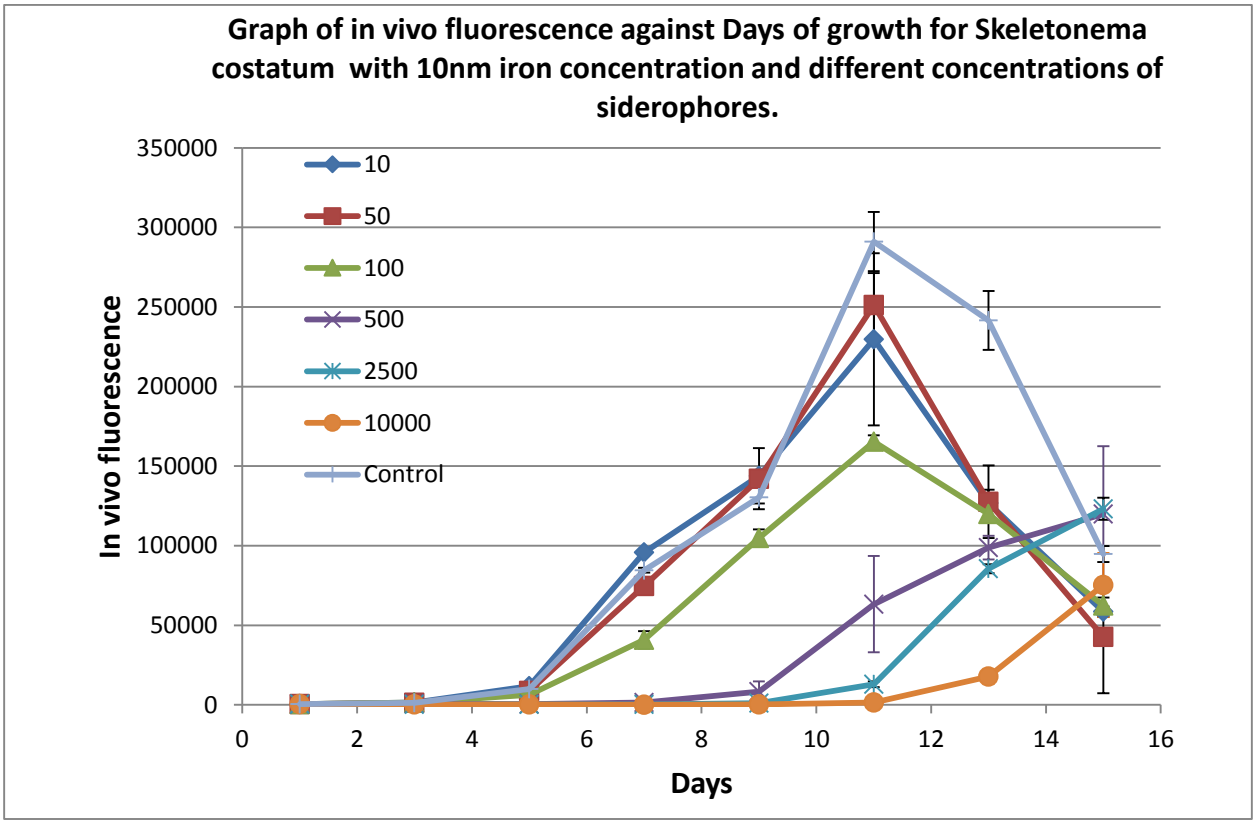


Figure 5.10 In vivo fluorescence recorded over 15 days of culture experiment for Skeletonema costatum with 10nm iron concentration and different siderophore concentrations.

A similar trend was shown for Skeletonema cultures with 10nM iron content as compared to with 1nM iron content with control exhibiting higher FSU. This occurred after day 5 for the

lower DFB siderophore treatments and control cultures. In the higher DFB siderophore group, FSU values began to increase after day 9 in 500nM, day 11 for 2500nM and 10000nM .Their maximum FSU measured were (229634 ± 54126.30) for 10nM, (251288.00 ± 19960.21) for 50nM, (165363.33 ± 4074.94) for 100nM and (291040 ± 18617.81 for control cultures. respectively.2500nM and 10000nM siderophore containing cultures. showed lower trends from the beginning of the experiment until the 13th day where an increase in FSU was noticed. Their maximum FSU values were (123186.00 ± 6890.85) and (75272.67 ± 20025.25) respectively. See figure 5.10

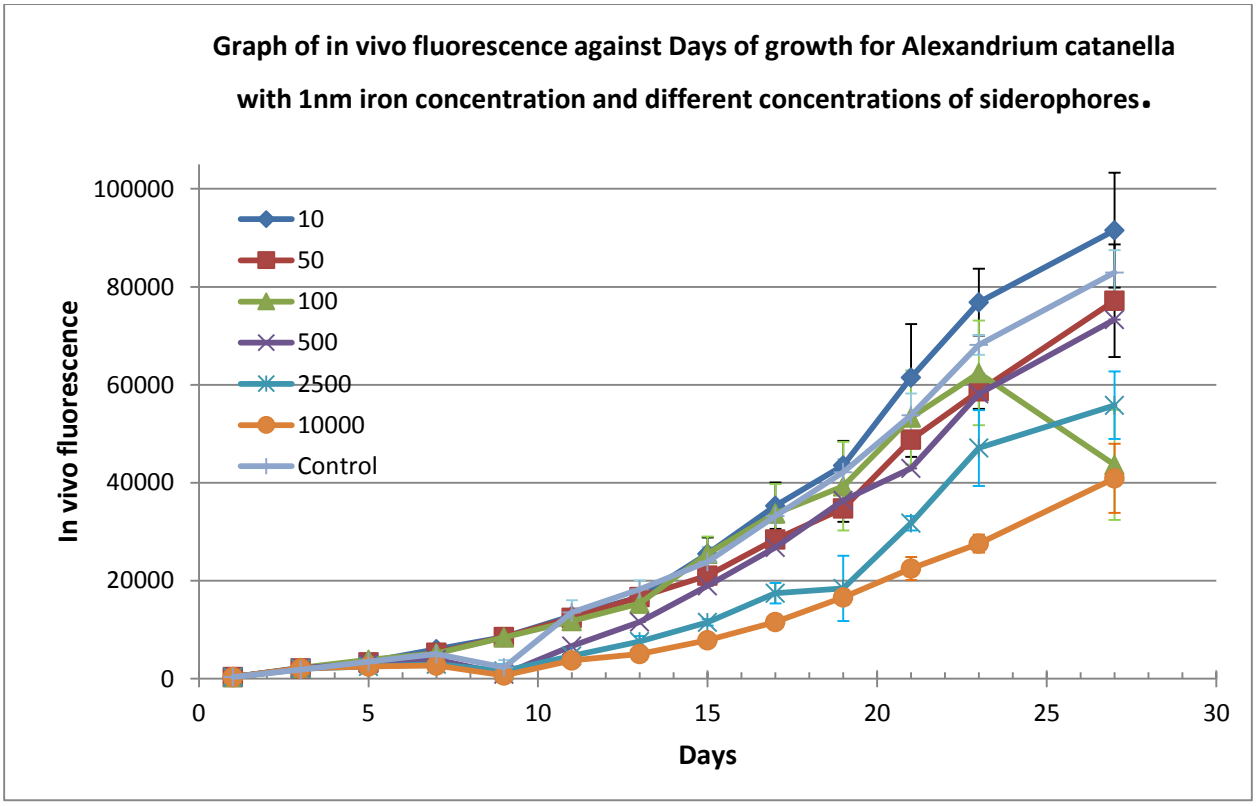


Figure 5.11 In vivo fluorescence recorded over 27 days of culture experiment for Alexandrium catanella with 1nm iron concentration and different siderophore concentrations.

As represented above, it can be seen very clear trend that only highest siderephore treatments with 2500 nM and 10000 nM made considerable impact with the rest of the treatments including control considered to be very close to each other. Here FSU increased after day 5 for the lower DFB siderophore group and control cultures and after day 9 in the higher DFB siderophore

cultures. After day 9, the higher siderophore started to become obvious with relation to their effects on iron in the growth medium especially 2500nM and 10000nM. The maximum FSU presented were (91549.33 ± 11731.51) , (77189.00 ± 11502.19) (73321.67 ± 8930.43) , (55813.67 ± 6885.62), (40893.67 ± 7061.87) , (82900.67 ± 4595.28) for 10nM, 50nM, 500nM, 2500nM, 10000nM and control cultures respectively as whiles for 100Nm ,it was (62422.67 ± 10667.87) but on the 23rd day of the experiment. See figure 5.11

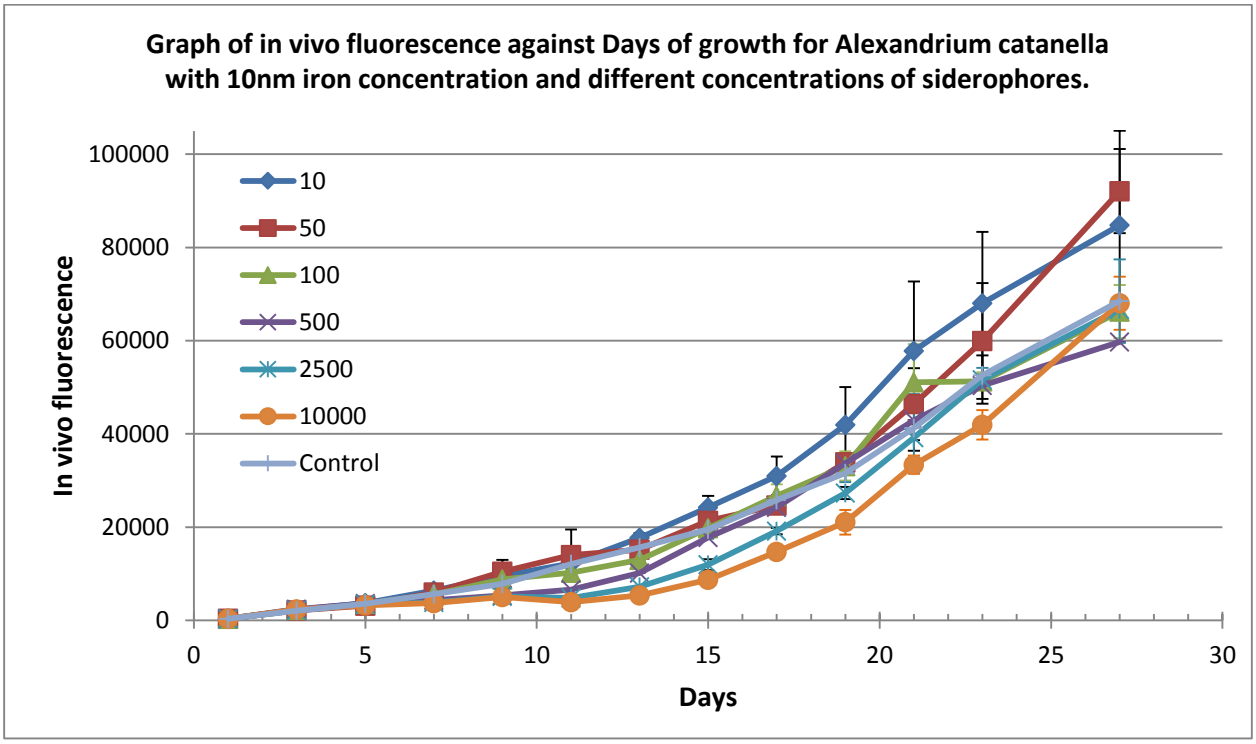


Figure 5.12 In vivo fluorescence recorded over 27 days of culture experiment for Alexandrium catanella with 10nm iron concentration and different siderophore concentrations

Here, differences of the response of in Alexandrium catanella to siderephore between high and low siderephore treatments are not very clear. An increase in FSU began to show after 9 in all treatments except 10000 where the FSU began to increase after day 11. This therefore shows how slow these phytoplankton cells performed in relation to light efficiency used to photosynthetic activities. The initial values of in vivo fluorescence level was recorded as (354.99 ± 168.4) for every culture treatment. The values were (84757.67 ±20269.2) for 10nM, (92091.50 ±9038.95) for 50nM, (66236.33 ±5710.28) for 100nM, (59739.33 ±7414.40) for 500nM,

(66707.67 ±67 ±82.78) for 2500nM, (68018.00 ±5690.46) for 10000nM and that for the control replicates was (68488.00 ±8961.95). See figure 5.12

5.4 Cell abundance

The abundance of cells produced as a result of rate of photosynthetic activities performed in the growth media for the cultures with different siderophore concentrations were counted and recorded. The charts with different graphs for siderophore concentrations are presented below:

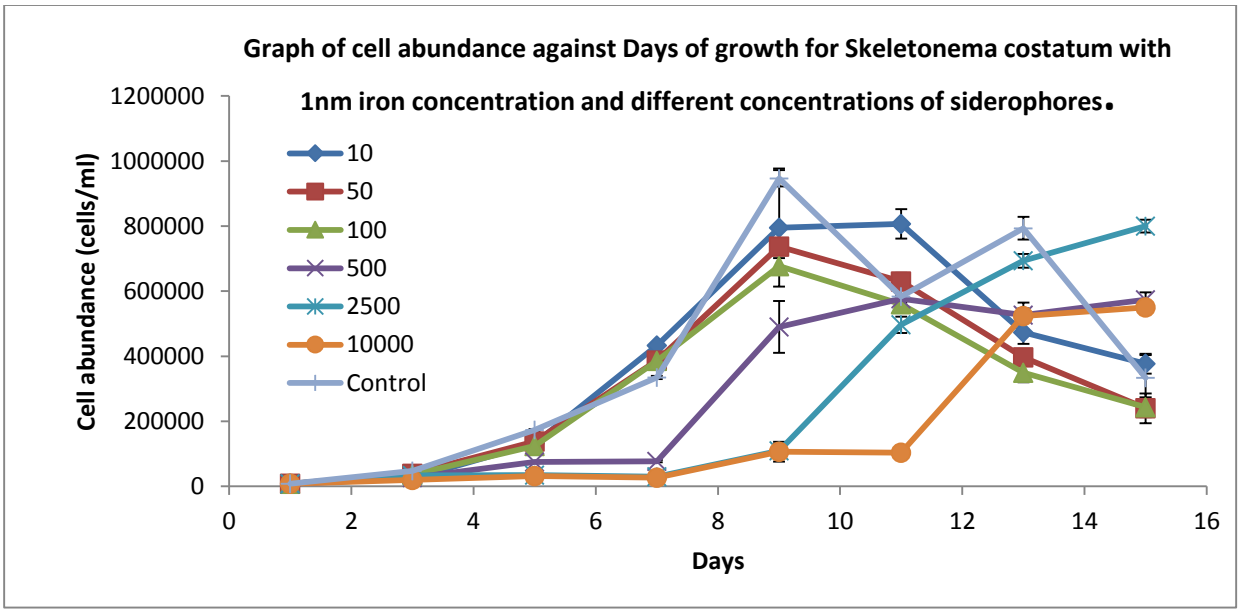


Figure 5.13 Cell abundance recorded over 15 days of culture experiment for Skeletonema costatum with 1nm iron concentration and different siderophore concentrations

It was noticed that while the cell abundance was decreasing in low siderephore treatments after approximately day 9, the cell abundance is increasing in the high siderephore treatments. As shown in the chart, all graphs for various siderophore concentrations rose gradually from day 3 to day 10 with the exception of the higher siderophore concentrations (2500nM and 10000nM) but in all cases a sigmoidal growth curve was presented. Most cell densities were maximally reached on the 9th in the lower siderophore treatments and control with the exception of the 10nM DFB siderophore treatments. The chain forming cells of Skeletonema costatum was observed mainly in the first week and in the early part of the second week of the 15-day culture experiment. Between the 1st day and the 3rd day culture replicates with lower siderophore contents (10nM 50nM and 100Nm) and control cultures experienced an initial lag phase where a

slow increase in cell density was observed. After the 3rd day an exponential growth phase occurred as days increased and from day 5 to day 11 .The highest values and the respective days on which various culture treatments were presented as follows. (806666.67± 45092.50) cells/ml, (736666.67± 25166.11), (676666.67 ± 26457.51) and (946666.67 ± 25166.11) for 10nM, 50nM,100nM and control culture treatments respectively. (576666.67±11547.01)cells/ml, (800000 ± 20000.00) cells/ml and (580000 ± 20000) cells/ml were the maximum cell density values recorded for 500nM, 2500nM and 10000nM respectively. See figure 5.13.

A more similar pattern of growth was also observed in Skeletonema costatum cultures with 10nM iron treatments. Most maximum cell densities were reached in treatments of siderophores with lower concentration and in the control on day 11 which was 2 days after the 9th day in the 1nM Fe treated cultures. Here an initial lag phase was established from the 1st day to the 5th day for lower siderophore containing replicates (10nM, 50nM and 100nM) and control culture followed by an exponential phase from day 7 to 9. However, there was a decreased stationary phase with a rapid transformation into the terminal phase (diminishing growth phase) which occurred from the 13th day towards the 15th day. For cultures with higher siderophore concentrations (500nM, 2500nM and 10000nM) established the same pattern of growth phases as described in cultures with 1nM iron concentration. Hence, the highest values presented were (776666.6± 25166.11) cells/ml for 10nM, (716666.67± 25166.11) cells/ml for 50nM, (763333.33± 20816.66) cells/ml for 100nM and (783333.33± 20816.660 cells /ml for Control replicate cultures. The other siderophore concentration replicates (500nM, 2500nM and 10000nM) presented values for highest cell density over the 15-day experiment were (776666.67±35118.85)cells/ml on day 11, (723333.33±40414.52) cells/ml on day 15 and (783333.33 ± 40414.52) cells/ml on day 15 respectively. See figure 5.14.

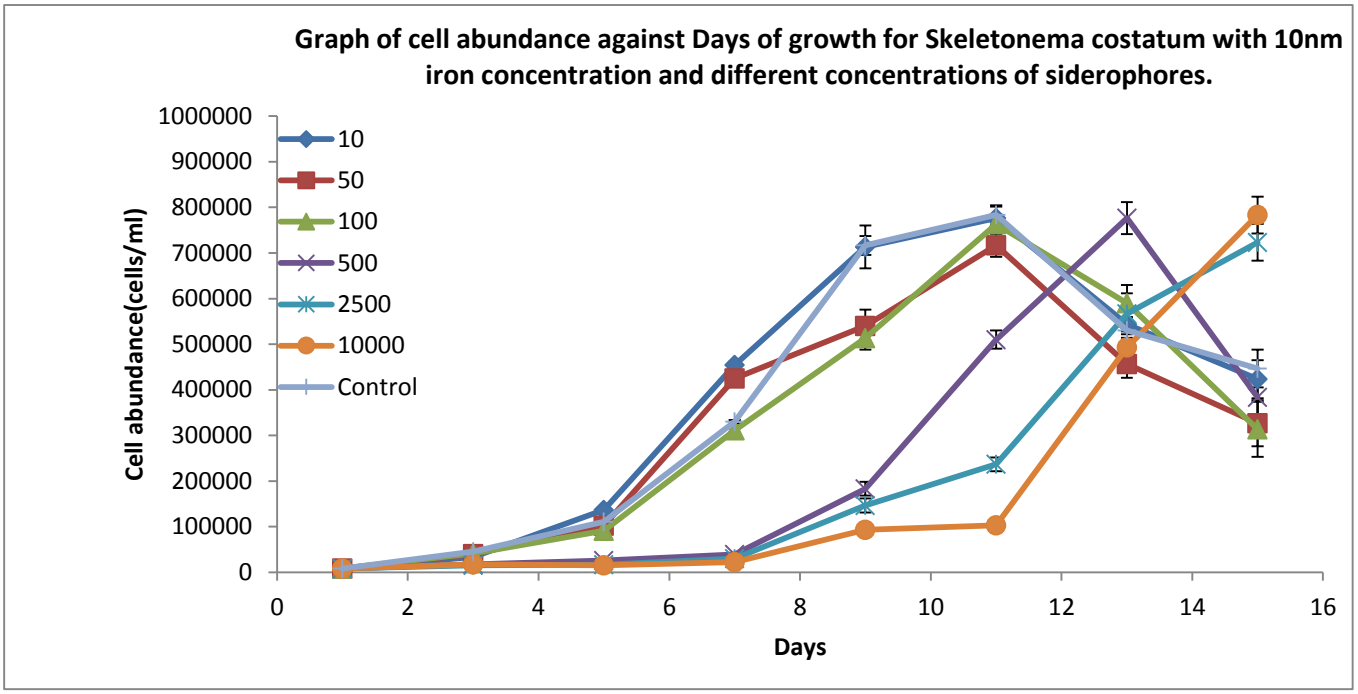


Figure 5.15 **Cell abundance recorded over 15 days of culture experiment for Skeletonema costatum with 10nm iron concentration and different siderophore concentrations**

A unclear graph was presented by Alexandrium catanella with 1nM Fe concentration as graphs of the treatments were fluctuating .Here it could be observed that after the gradual rise o the 1st day to the 5th day , changes did set in relation to the different phases of growth. The different phases of growth where partially exhibited here showing that cell densities of Alexandrium catanella was not stable. The lag phase, exponential phase, stationary phase and terminal phase were all shown in culture treatments with lower siderophore concentrations and the Control whiles the higher siderophore concentrations hardly reached their peaked values or approached the stationary phase. The maximum cell densities reached by the various culture treatments were (4520 ± 111.36) cells/ml for 10nM , (6360.00±60.00) cells/ml for 50nM , (6360.00±72.11) for 500nM , (5446.6±110.15) for 2500nM and (4573.33± 50.33 for 10000, all recorded on the 27th day with the exception of 100nM and Control culture replicates that presented their highest values earlier before the 27th day with values (3913.00± 64.29) cells/ml on day 19 and (5233.33 ± 100.66) cells/ml on day 21 respectively. See figure 5.15

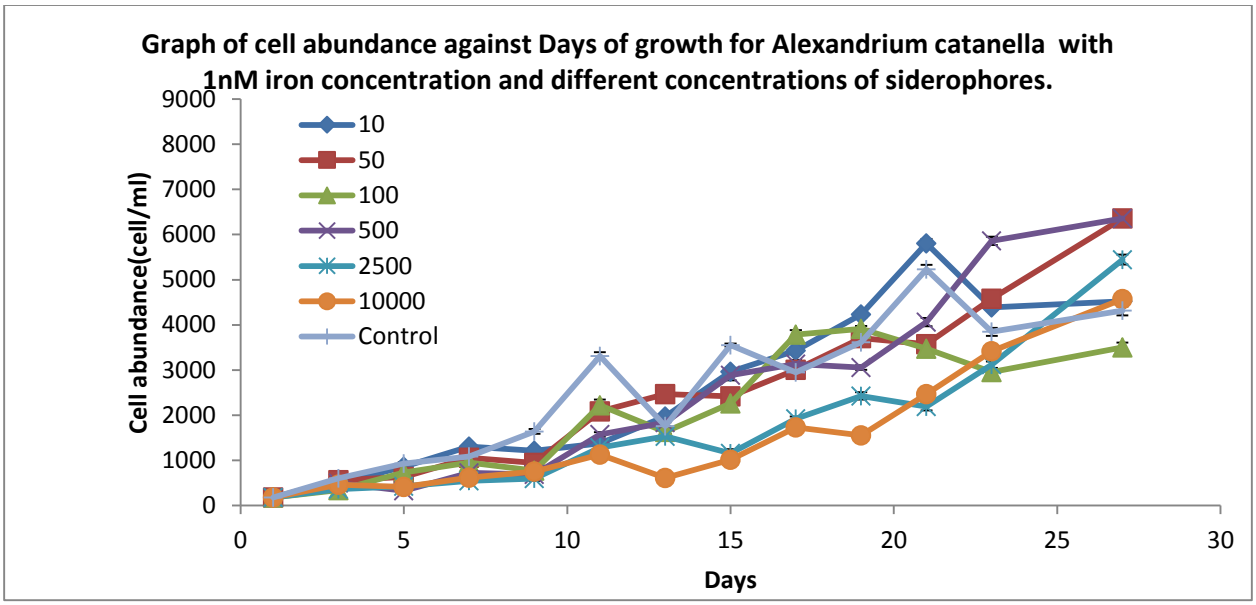


Figure 5.16 Cell abundance recorded over 27 days of culture experiment for Alexandrium catanella with 1nm iron concentration and different siderophore concentrations

Despite difference in iron concentration for Alexandrium catanella, 10nM iron treated cultures of Alexandrium showed a very similar pattern of growth over the span of the experiment. Here also, levels of fluctuations in cell density were high resulting in a less distinct description of the various phases of a growth curve. All graphs observed on the chart showed initial lag phases and exponential phases which kept fluctuating over the experiment period. However, the highest values were mostly presented on the 27th day with the exception 500nM which gained its highest value on the 21st day. These values were (7260.00± 91.65) cells/ml for 10nM, (6220±56.571) cells/ml for 50nM, (4406.67±70.24)cells/ml for 100nM, (4960.00±87.18) cells/ml for 500nM (4353.33 ±83.27) for 2500nM, (4066.67±120.55) for 10000nM and (6540±131.15) cells/ml for control culture replicates. Hence there was not much a distinctive difference registered between Alexandrium cultures with 1nM and 10nM iron concentrations or treatments except for the maximum cell density values gained on day 19 and 21 by 1nM Fe treatments in 100nM and control cultures whiles in the 10nM it occurred on the 27th day. See fig.5.16

Comparing the various biological parameters that were measured, pH presented a much clearer parameter because it was easy to measure as compared to Quantum Yield in this culture experiment. In vivo fluorescence was also quite conspicuous in both phytoplankton species as well as cell abundance.

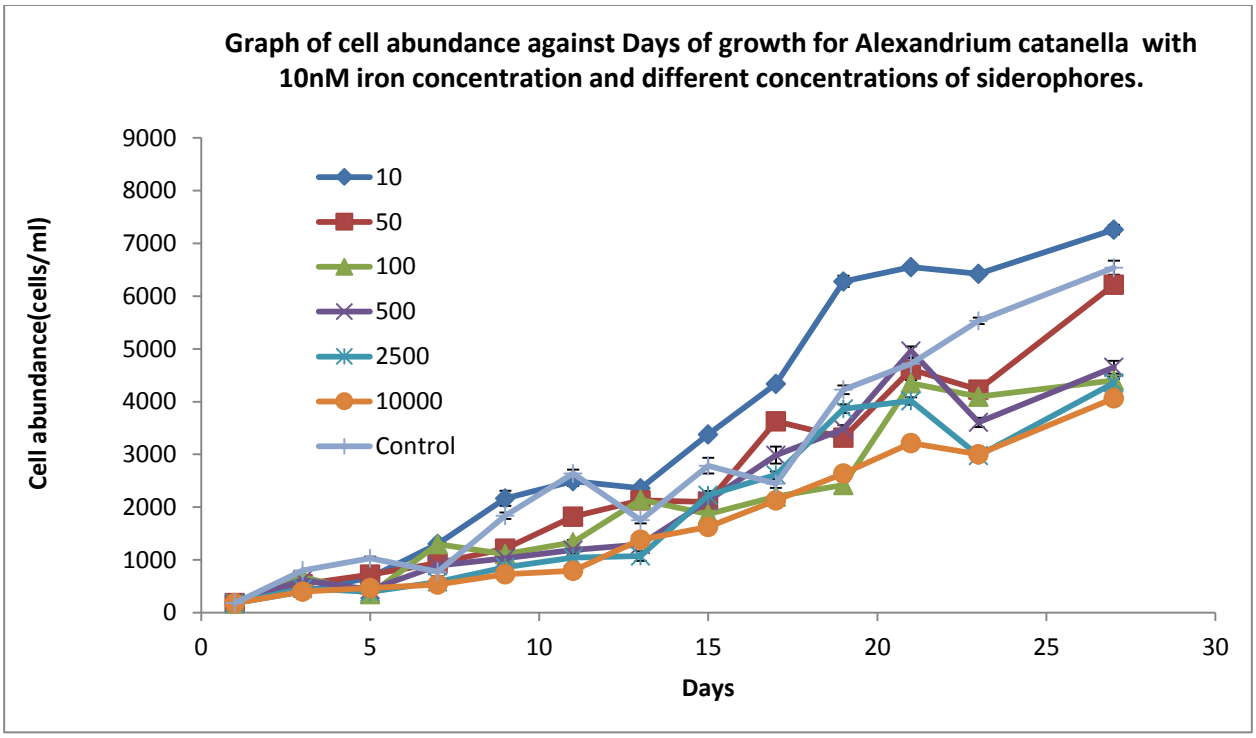


Figure 5.17 **Cell abundance recorded over 27 days of culture experiment for Alexandrium catanella with 10nm iron concentration and different siderophore concentrations**

5.5 Final iron concentrations in samples of multianalytical techniques (Chelex and DGT)

5.5.1 Chelex samples

For Chelex samples, an initial Iron concentration of 0.76nM was present in the Chelex cleaned seawater medium before the experiment begun. In order to know the final concentrations of iron present in the culture of the phytoplankton species, 16 final culture samples after the experiment were chosen for Skeletonema costatum and 16 samples for Alexandrium catanella. Here 8 out of the 16 for each species represented 1nM iron treated cultures and the other half representing 10nM iron treated cultures. Hence,2 replicates each belonging to cultures treated with DFB 50nM , 500nM ,10000nM concentrations and Control were randomly picked and the necessary procedures for metals extraction by the Chelex technique was conducted. Generally, it was observed that Skeletonema culture replicates treated with DFB 50Nm AND DFB 500nM possessed higher amounts of iron with final iron concentration of 3.30nM and 2.78nM respectively after experiment as compared to DFB1000nM and the control with 1nM iron treatments whose values were 2.93nM and 1.31nM respectively. See figure 5.17

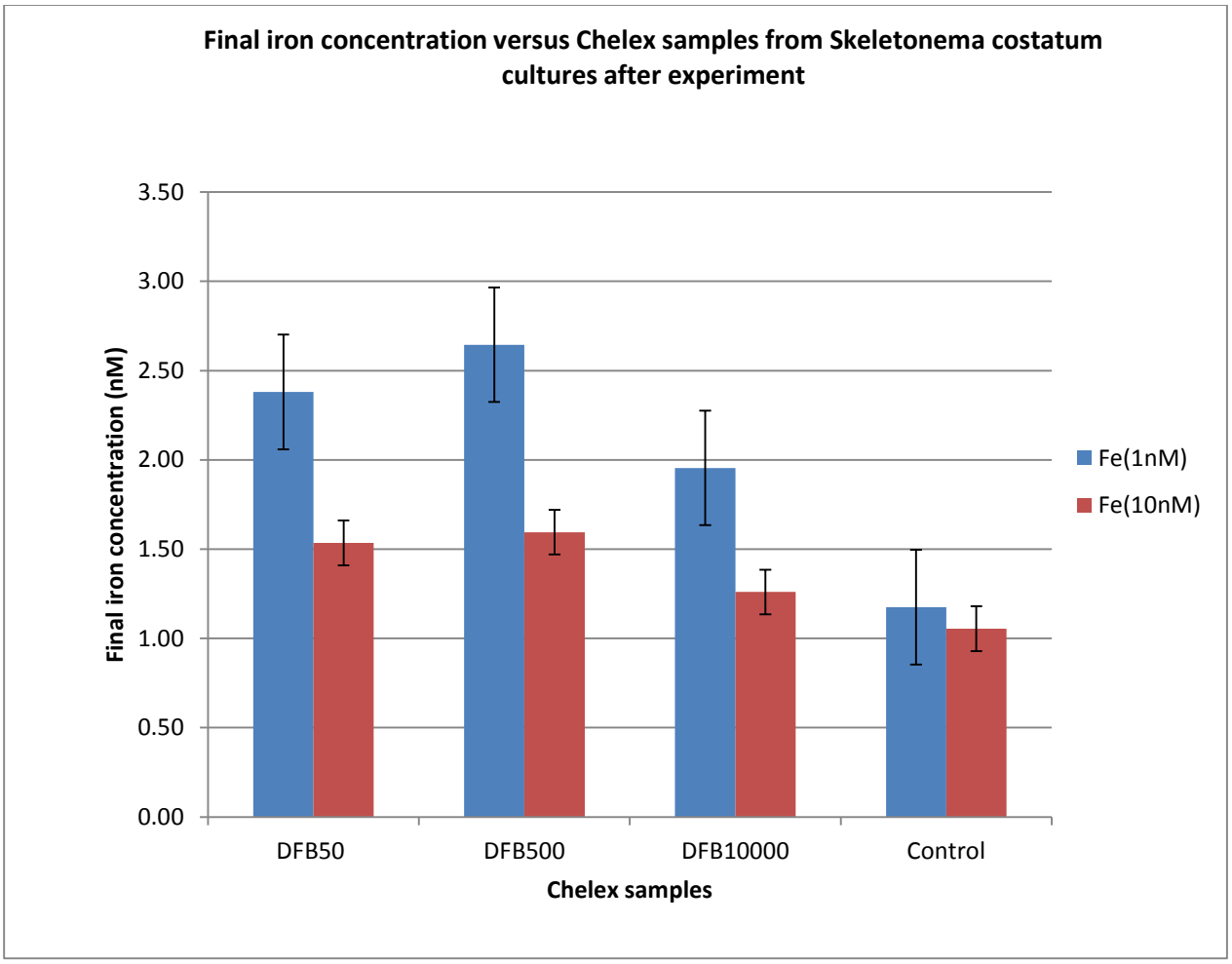


Figure 5.18 **Final iron concentrations retained in different iron and DFB concentration treatments for Skeletonema costatum cultures with Chelex technique.**

Higher amounts of iron was retained also in DFB 50nM and DFB 500nM cultures of Skeletonema costatum with 10nM iron treatments which were followed by DFB 10000nM and the control respectively .The final iron concentrations were 2.20 nM and 1.65 nM iron concentrations in DFB 50nM and 500nM respectively whiles DFB 10000 and the control showed minimal values when compared to the above siderophore concentrations with values of 1.76nM and 1.46nM respectively.

Alexandrium catanella exhibited rather an extremely high iron content in growth media after the duration of the experiment with cultures treated with 1nM iron concentration showing maximum iron concentration in growth medium than their fellow counterparts in 10nM iron treated cultures. Repeatedly, high iron levels were possessed by DFB 50nM and DFB 500 Nm

siderophore treated cultures with values 6.73nM and 4.87 nM respectively. DFB 10000 recorded its maximum final iron concentration as 4.7 nM and that of control culture replicates were between 3.28nM. For 10nM iron treated Alexandrium cultures, DFB50nM again recorded the highest dissolved Chelex labile iron with a value of 5.44nM, 3.36nM for DFB500, 2.59nM for control and to 2.53nM for DFB 10000 in decreasing order. See figure 5.18

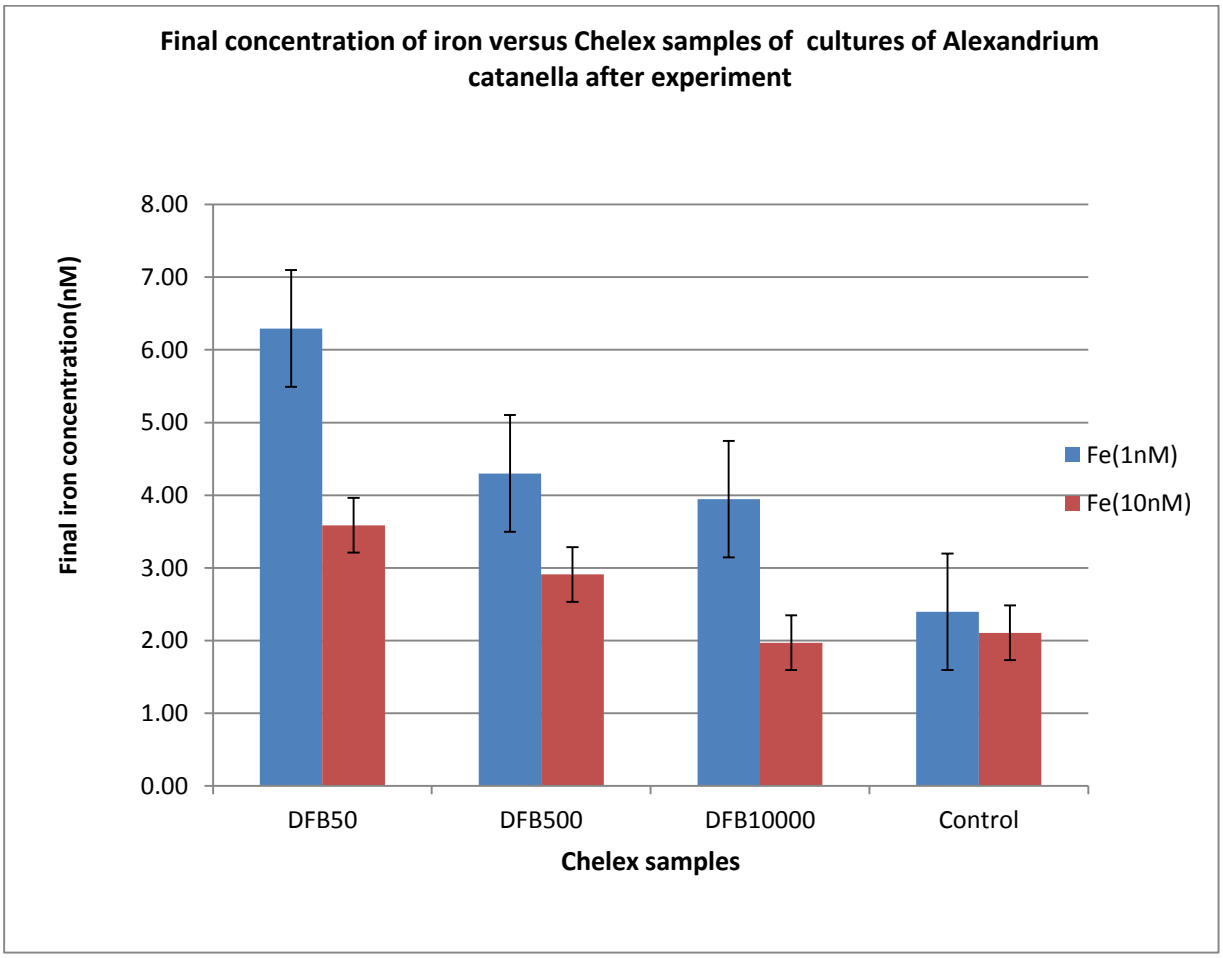


Figure 5.19 **Final iron concentrations retained in different iron and DFB concentration treatments for Alexandrium catanella cultures with Chelex technique.**

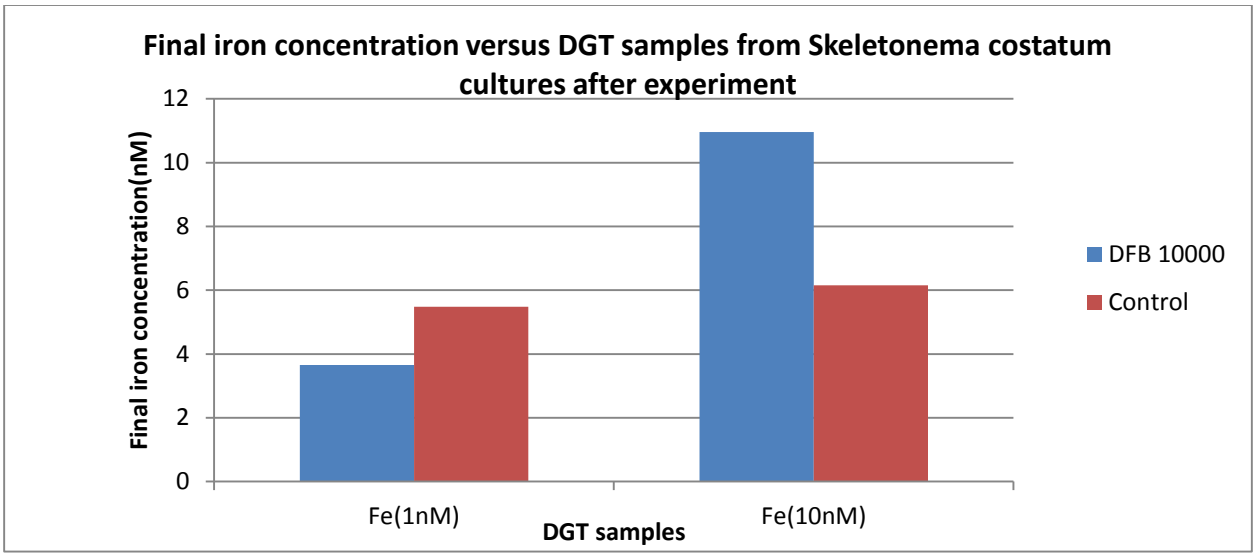


Figure 5.20 Final iron concentrations retained in different iron and DFB concentration treatments for Alexandrium catanella cultures with DGT technique.

5.5.2 DGT samples

For DGT, the dissolved DGT labile iron concentration was apparently high in the control cultures than in DFB 10000 siderophore culture replicates that were all treated with 1nM iron concentration with values 5.48nM and 3.66nM iron concentrations respectively for Skeletonema costatum. With 10nM iron treated Skeletonema costatum cultures a repetitive pattern was established. Here also DFB 10000 siderophore cultures showed highest dissolved DGT labile iron with value 10.96nM whiles that of the control was 6.15nM.See figure 5.19

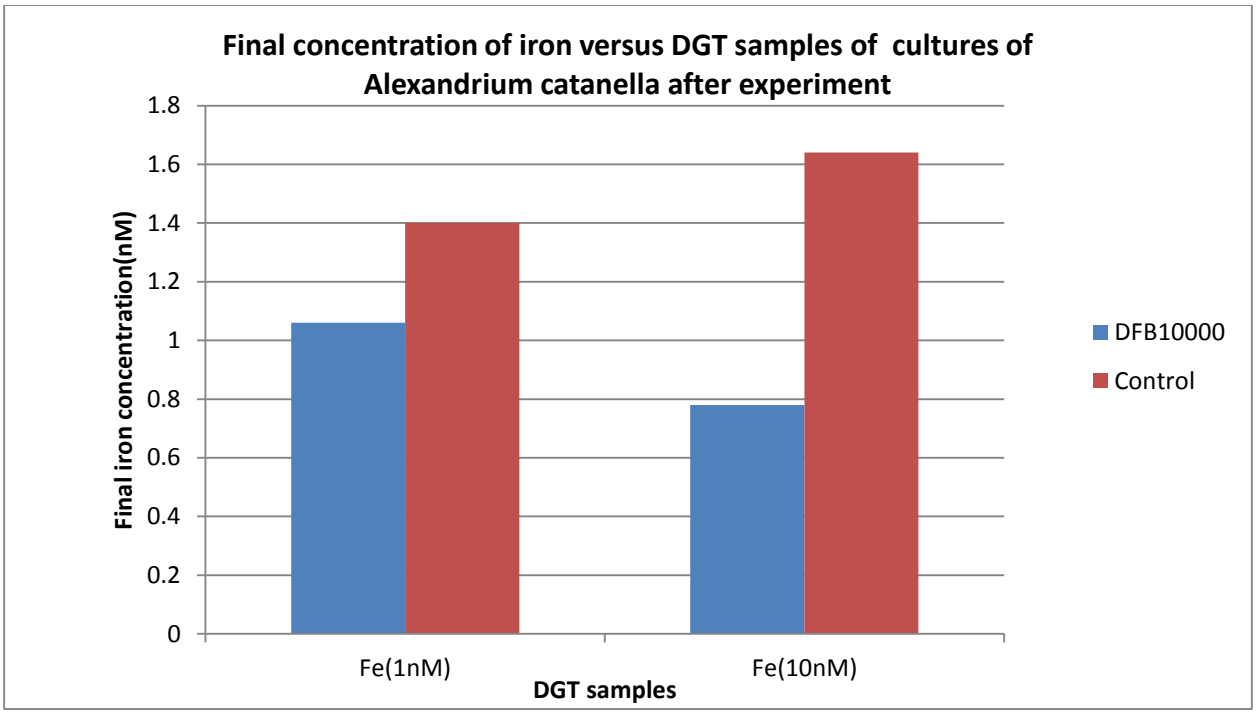


Figure 5.20 Final iron concentrations retained in different iron and DFB concentration treatments for Alexandrium catanella cultures with DGT technique.

Alexandrium catanella registered low values for dissolved DGT labile iron with a value of 1.06nM for DFB 10000nM cultures and 1.4nM for control cultures that had 1nM iron additions. That of the 10nM iron treated Alexandrium cultures did show a similar trend presenting a difference in the concentration of dissolved DGT labile iron. The values that were recorded were 0.78nM and 1.64nM for DFB 10000 and Control culture replicates respectively. See 5.20

6. DISCUSSION

6.1 Environmental factors in different phytoplankton environments

The response of pH to additions of DFB siderophore concentrations was very clear in both 1nM and 10nM Fe treated Skeletonema cultures than in 1nM Fe treated Alexandrium catanella cultures which exhibited minimal effect of siderophore on its pH levels.

Alexandrium catanella response of pH to additions of different concentrations was very minimal. Both the 1nM and 10nM Fe treated cultures showed the same pattern of pH response and as it was noted, it was not as conspicuous as in Skeletonema costatum. This difference might be as a cause of the inability of Alexandrium catanella to effectively remove the iron that was captured by DFB siderophore due to a probable physiological adaptation they possess where the amount of iron removed from the growth medium at a particular time does not exceed the threshold of the cells necessity. Hence, even in the presence of DFB siderophores or not (as in the case of the control, this threshold of iron uptake by the cells is not exceeded as well as the levels of iron that might be present. Therefore in comparing Skeletonema costatum to Alexandrium catanella, it could be deduced that Skeletonema had high iron requirements than Alexandrium catanella where iron that is highly needed by Skeletonema , when captured , does affect its effective removal of CO_2 in the growth medium for photosynthesis whiles Alexandrium catanella barely required iron after its maximum level of bioavailable iron had been reached. Also, a physiological adaptation possessed by Skeletonema might have aided in its ability to remove iron complexed by siderophore when required excessively whiles Alexandrium on the other hand is unbothered about the iron complexed by DFB siderophore unless needed in very important cell activities.

The 10nM Fe treated Skeletonema cultures created a difference in the days of attaining maximum pH levels in the higher siderophore group (500nM, 2500nM and 10000nM) as compared to their 1nM Fe treated cultures and this can be attributed to a delay in the acquisition of iron taken up by the vast amount of DFB siderophore in the growth medium. Additionally, more CO_2 was removed in 1nM Fe treated cultures than in 10nM Fe treated cultures. This may be the effective uptake of iron by Skeletonema cells in 1nM Fe treated cultures where they uptake readily available iron at a particular period thereby resulting in less amount of iron left to be complexed by the DFB siderophore. On the other hand, in 10nM Fe treated cultures,

Skeletonema cells might have taken the amount of iron needed for photosynthesis at a particular time and the rest becomes quickly complexed by DFB siderophore hence after total utilization of previously taken iron, the cells tends to perform an extra activity that will aid in taking up the bioavailable iron complexed by DFB siderophore thereby creating some sort of delay in this activity hence making Skeletonema cells unable to remove CO_2 in the higher DFB siderophore cultures as they performed In their 1nM Fe treated cultures.

The quantum yield as a biological parameter measured in this culture experiment was very insensitive to the additions of DFB siderophores when compared to pH response .It was quite difficult in measuring the quantum yield Control culture replicates of Skeletonema costatum with 1nM iron additions fixed the highest amount of carbon dioxide based on the light that was absorbed during the culture experiment earlier than the other culture treatments even though the highest QY values were recorded on the same day as lower DFB siderophore culture replicates .This mirrors the result obtained by culture experiment conducted by Wells (Wells,1999) on manipulation of iron availability in near shore waters where control cultures in a DFB added culture experiments showed most peaked level of carbon fixation. It was quite expected for this trend to occur because a culture treatment without the presence of DFB siderophores is expected to fix more carbon since there will less suppression effect of siderophores on iron but the difference in the 10nM culture treatment having the least carbon fixed among the lower FB siderophore group might be due to the remaining bioavailable iron in the growth medium been complexed by DFB siderophore at the time culture was sampled since DFB siderophores and iron additions are done after biological parametric measurements. It was also anticipated that the higher group of DFB siderophore treatment will fix less carbon in phytoplankton culture due to constant repressive effect of siderophore on iron in the growth medium and this was also similar to similar to (Wells, 1999) short term culture experiment were the net carbon assimilated by the cultures with DFB treatments were identical and was 20% of their control cultures.

A difference created here in 10nM Fe treated of Skeletonema cultures with 10nM DFB treatments fixing more carbon than the control might be due to a source of an organic ligand which might be of no concern but complexing iron in the system. Also since bacteria is ubiquitous and also known for siderophore production in marine systems, there is a possibility of this activity occurring resulting in a slight effect of iron present in the control cultures. There

is clear evidence here that DFB siderophore treatments affected levels of carbon fixation since an increase in DFB siderophore resulted in a decrease in quantum yield levels.

The numerous fluctuations encountered in the dinoflagellate, Alexandrium catanella for QY measurements with 1nM Fe treatments is related to a change in chemistry of the siderophore during the growth experiment or an interruption in cell activities for biological production since this was much seen in 10nM DFB treated and the control cultures .Despite, the lower siderophore group and control fixing more carbon about 6 days earlier than the higher DFB siderophore group, it wasn't expected for repetition to occur in QY with 10nM DFB and control cultures. Due to these feature exhibited by Alexandrium, it was very decisive not to have continued the measurements since they were growing at a slow pace and initial effects of siderophore on QY in the various treatments was quite minimal and inconspicuous. This was in contrast to what was recorded for Skeletonema costatum. The 10nM iron treated Alexandrium cultures showed a different pattern as their 1nM iron treated counter parts .However, the difference established here was the Control cultures showed the highest QY value and then followed by 50nM, 100nM, 500nM, 2500nM 10nM, 10000nM and DFB siderophore concentration which quite expected for control but not for the rest being out of anticipation especially 10nM and this can again be as a result of an interference in the interactions between siderophore and the available iron needed by the cells. There might be a possibility that the available iron in the 10 DFB siderophore cultures were highly complexed for some period of time as compared to the others before the parametric measurements were made.

FSU levels was very high in control culture replicates of Skeletonema costatum with 1nM iron additions with the lower DFB siderophore concentrations continuing to the higher DFB siderophore concentrations. This was also repeated in the 10nm iron treated Skeletonema costatum cultures. Generally, it could be explained that higher efficiency of light reached control cultures better than the other culture replicates .This efficiency made light for photosynthesis very available and with the easy uptake of iron needed in biological cycles by the cells in the absence of siderophore, a high rate of biological activity was expected to occur in the Skeletonema cells. Additionally, lower DFB siderophore culture replicates showing higher light efficiencies reaching Skeletonema cells establishes an inverse relationship. Therefore, it could be deduced that the higher the concentration of DFB siderophore in a phytoplankton (diatom)

community, the lower the levels of FSU (in vivo fluorescence) regardless of the concentration of bio available iron present.

In Alexandrium cultures, 100nM DFB siderophore replicates registered the highest value of FSU four days earlier, to the end of the experiment. All others showed their highest on the last day of experiment. However, the highest value reached was by the 10nM cultures and then to the control, the 50nM, 100nM, 500nM, 2500nM and 10000 cultures. This was also same for Alexandrium cultures with 10nM iron treatments but the 50nM DFB culture replicates showed the highest FSU values. Here, it was observed that light reaching cells in the lower DFB siderophore cultures were more efficient in the uptake of carbon dioxide for photosynthesis. Hence cells were able to trap more to produce more but surprisingly the control without siderophore exhibited a slower pace in utilizing the efficient light reaching its cells and this might be due to cells shading other cells hence preventing them from acquiring the needed amount of light of photosynthesis. Additionally, high chlorophyll a concentrations in a medium might suggest possible light limitations to metabolic processes due to self shading of cells (Thoresen et al., 1984).

The physical environment in which cultures are maintained in the laboratory doesn't necessarily correspond to that in which phytoplankton grows naturally .Environmental factors are widely different since these factors are comparably stable. Since laboratory cultures have controlled conditions simulated to the environment of phytoplanktons, they might encounter lower levels of some environmental factors (Berman et al., 2012). The spectral composition of light penetrating the water changes with depth is different for different kinds of water and varies according to the weather (Kirk, 1983). Except at the immediate surfaces, it is very different from ordinary sunlight and it is difficult to duplicate even approximately with artificial sources (Jitts et al., 1964)

As anticipated, the cell densities of Skeletonema costatum were clearly numerous in the control culture. Cell densities were initially very low from the start of the experiment until the 9^{th} day where most changes occurred .On this day, the lower DFB siderophore concentrations recorded their highest cell densities (50nM, 100nM) with the exception of 10nM, whose was on the 11^{th} day. The higher groups of DFB siderophore presented their highest cell densities at the end of the experiment.10nM iron treated Skeletonema cultures presented similar cell density trend through

the experiment. Control gained highest cell abundance on the same day as the lower DFB siderophore group but the difference between these and the 1nM iron treated cultures were the days the former cultures acquired its highest cell density levels where the lower iron concentration cultures cell density values were registered 2-day earlier than the 10nM iron concentration cultures

It could be obviously explained that the Control cultures with the absence of siderophores experienced less iron complex formation resulting in the increased bioavailability of iron that was necessary for productivity biologically. Here as well, the amount of siderophore in the culture replicates did have effects on the bioavailability of iron to the Skeletonema cells initially. Hence the levels of DFB siderophore did affect the rapid uptake of iron by these diatom cells. However, it was noticed that the suppressive effect of DFB wasn't exhibited after a long period of time because higher DFB siderophore concentrations reached cell densities that were almost the same as the control and the lower siderophore concentration group at the end of the culture experiment. Hence it could be deduced that siderophore does interrupt in cells biological activity but to a shorter extent or time period for Skeletonema costatum communities. The release of captured iron in higher DFB siderophore groups to these phytoplankton cell culture towards the end of the experiment might be attributed to an interference in the chemistry of DFB siderophore and a possible adaptation of these phytoplankton cells to be able to produce organic matter where some of them may start to compete with siderophore in taking up the iron which might be possible to utilize for biological activity.

Alexandrium catanella cultures expressed a lot of fluctuations in cell densities and due to the slower pace of growth presented by these dinoflagellates, the effect of siderophore concentration was minimally observed. Almost all the maximum cell densities recorded for the culture replicates with 1nM iron treatments was on the last day of the culture experiment. Different DFB siderophore concentrations obtained higher cell densities faster as in 100nM DFB cultures followed by the control cultures .In similarity with this, 10nM iron concentration cultures of Alexandrium catanella had increased levels of fluctuations throughout the experiment.

Despite the additions of iron and siderophore on each sampling day, the effect was expressed on the low. It revealed that the amount of iron needed by these groups of phytoplanktons at a particular time was independent of the levels of siderophore. This can also be that no matter the

levels of DFB siderophores that might be present in this group of phytoplankton species, the amount of iron necessary for biological production per time will be used and never above the threshold hence the slower rate of growth. Hutchins et al., 1999 also recorded the effects of DFB siderophore additions on phytoplankton growth where the diatom genera were numerically dominant in all experimental bottles with at least an order of magnitude higher in its control replicates. Additionally, he also registered that dinoflagellates were scarce but in contrast were significantly abundant in DFB treated culture bottles as observed in this experiment as well.

The high iron uptake and storage capacity of some diatoms (coastal) allows these species to accumulate excess iron during periods of high availability (Sunda and Huntsman, 1995) explaining why Skeletonema costatum grew rapidly despite the levels of iron concentration that was present in the growth medium .Hence this high capability of diatoms to store iron may thus be an important factor in making them outcompete other species during episodic blooms (Sunda et al., 1995). Furthermore, diatoms support largely the productive food webs in coastal upwelling areas and fuel their high export production fluxes and are also vulnerable to Fe limitation (Hutchins et al., 1999) and this dependency of diatoms on high Fe concentrations has now been well documented in both oceanic and coastal HNLC regions (Martin et al., 1991; Coale et al., 1996; Hutchins and Bruland 1998). A look at the maximum cell densities produced by Skeletonema costatum within a short time frame as compared to Alexandrium explains their high Fe cellular requirements which has been demonstrated in laboratory cultures using coastal diatoms (Sunda et al., 1991; Sunda and Huntsman, 1995).

From the above, it could be noticed that diatoms exhibited that they have a different strategy of taking bioavailable iron because even if the iron is complexed by DFB siderophores or not , it has no problems because it is only making photosynthesis and their cells uptake of elements is by diffusion whiles with dinoflagellates presented a more interesting strategy in the sense that not all but most of the species are mesotrophic meaning that they engulf particles , deform and transport them and the medium supplies the cells food that even in the absence of light they cannot photosynthesize but rather swallow other smaller flagellates or literally eat them when captured. Additionally, they engulf the whole particle with iron and siderophore.

For diatoms, they may be releasing the siderophore attached to the surface of the cell surface and in the process of reduction, the diatom takes up the siderophore and when it reaches the

membrane, they may reduce the iron complexed by the siderophore and by this way they manage to release the iron and use it but dinoflagellates just literally engulf and prey on what it encounters and sense as food. Generally, it is very obvious that low DFB siderophore concentration in a diatom species community does have minimal effect in the uptake of iron by cells as compared to unrealistic higher DFB siderophore concentrations in the marine system. Interestingly, from the results registered it could obviously explained that the chemistry of the DFB siderophore changed because the trend of its effects shows that it ends up releasing the complexed iron after some period of time and hence this released iron is been used by the phytoplankton species. For instance, it was clearly observed that after long periods of time, culture replicates treated with DFB 500nM, 2500nM and 10000nM gained cell densities when the experiment was approaching its end , that were almost that of the control and the lower DFB siderophore concentrations recorded in the initial stages of the experiment.

This an overall indication that explains that with the less siderophore group, siderophore decomposes faster a short period time whiles in higher siderophore group, there are extra amounts of iron that is complexed and extra siderophore present hence a delay or decrease in decomposition time or process for iron to be made bioavailable again to the phytoplankton cells .This is because cells might have or develop some sort of response which may lead to them producing an organic matter that might out compete siderophore in taking up iron making it more bioavailable.

In addition to the above, the most clearly sensitive biological parameter measured in this culture experiment is pH as compared to quantum yield and in vivo fluorescence since its response was very distinct despite additions of DFB siderophore especially in Skeletonema costatum. Quantum yield was unable to provide a much clearer picture for both phytoplankton species and was difficult to measure making it very insensitive for this experiment. In vivo fluorescence on the other hand was better than QY but also displayed many fluctuations but a bit conspicuous graph of its response to DFB siderophore additions to growth cultures. Cell abundance was also very sensitive in its response to DFB siderophore additions where it displayed maximum cell densities mostly in lower DFB siderophore cultures and in control in a few number of days than in the higher DFB siderophore group .Therefore an evidence of these biological parametric measurements and their individual responses, pH, Cell abundance and In vivo fluorescence

provide the best sensitive biological parameters necessary to measure in culture experiments with siderophore additions.

6.1.4 Final Iron levels in growth medium of cultures for the different phytoplankton species

The different multi analytical techniques employed in describing species specific bioavailability of iron showed separate trends of iron uptake and the levels of iron concentration that remained. The dissolved Chelex labile iron present in 1nm cultures of both Skeletonema costatum and Alexandrium catanella exhibited the same trend which explains that these phytoplankton cultures did utilize most of the bioavailable iron in their control cultures which had no DFB siderophores. Bioavailable iron present in these siderophore treated cultures did show higher iron content in 50nM, 500nM and 10000nM (decreasing order). As the above trend was repeated in 10nM, concentrations of iron present in Alexandrium catanella did have a slight difference. Here dissolved Chelex labile iron present in DFB 10000 was extremely low as compared to the Control, DFB 50nM and DFB 500nM siderophore.

With dissolved DGT labile iron, Skeletonema costatum cultures with 1nM iron treatments gained low values in cultures with DFB 10000 but was high in the control. The opposite was exhibited in the 10nM cultures of Skeletonema costatum where iron remains were high in the DFB 10000 treated cultures and low in the control. In the case of Alexandrium, dissolved DGT labile iron was very high in the control than in the DFB 10000 siderophore cultures. In comparing dissolved Chelex labile iron concentrations to the dissolved DGT labile iron concentrations, it could be noticed that DGT values measurements were very high for Skeletonema cultures with 1nM and 10nM iron treatments that it was in dissolved Chelex labile iron concentration of Skeletonema costatum. For Alexandrium catanella, dissolved Chelex labile iron concentrations were higher than dissolved DGT labile iron concentrations. This trend is related to the inability of the chelex resin in the DGT sampler to trap much free iron in the culture medium meaning that even dissolved iron concentrations, a whole lot of iron can be complexed and these complexed irons might not reach or diffuse properly on the gel as free ion or as an iron oxide.

The above techniques and their individual results suggests that DGT technique might be better than Chelex -100 preconcentration technique in that the former clearly show that much iron was barely used by these phytoplankton species as in contrast with the Chelex technique. This could

be due to drops of acid extracted metals that were lost due to the leakage of the yellow stopper during waiting times for different concentrations of HNO_3 to fully extract iron hence resulting in a decrease in volume obtained for analysis in the Chelex preconcentration technique metals extraction.

Furthermore, the two multi analytical technique employed in this culture experiment encountered some problems. This included an obvious contamination in the inoculation culture where there might be a possible amount of iron introduced in the new growth medium for the experiment hence cells using this(until it is finished) despite iron and siderophore additions . Also, these techniques were also prone to possible contamination during process or procedures (background noise and human errors in activity) and hence not very suitable for iron mixed siderophore differentiation in a growth medium since in reality it is known that iron has been complexed with siderophore and hence without siderophore a possible difference could be seen .This therefore has a link with the probable interference in the chemistry of the DFB siderophore in its complexation ability on iron.

7. CONCLUSIONS

The focus in this thesis work has been the effect of the organic molecule, Des-ferrioxiamine siderophore on species specific bioavailability of iron using phytoplankton culture and multi analytical techniques. The hypothesis for this work was that the organic molecule, DFB siderophore will have species specific effects on iron bioavailability. Hence different concentrations of siderophore will render iron bioavailability differently on each phytoplankton species.

From the work conducted, it can be concluded that DFB siderophore did have effects on iron bioavailability in different phytoplankton species growth media. The evidence of measurements of biological parameters and their sensitive responses to the effects of this organic molecule did provide a baseline data on which one is best and necessary during cell culture experiments with siderophore additions where in almost all, the control and low concentration DFB siderophore treated cultures did exhibit not much of an effect from siderophores as compared to the higher DFB siderophore group indicating less threat by DFB siderophores with iron complexation in a marine phytoplankton community unless an encounter with these unrealistic high DFB siderophore group which might cause a huge shift in cell's biological production but just for a short period of time. Moreover, it can be said that, the interference in the chemistry of DFB siderophore in this culture experiment surprisingly, reveals a less residence time of DFB siderophore effect on iron complexation within marine phytoplankton communities and the ability of the cells to produce organic matter that might outcompete these organic molecules is an excellent physiological adaptation towards cells biological production.

Furthermore, the use of Chelex-100 preconcentration and DGT technique also has provided a baseline information about which one can display most dissolved labile iron concentrations during measurements of which DGT was a better technique than Chelex. However, due to contamination problems, both techniques were also concluded not to be very distinct in their various dissolved iron labile measurements and hence a better and more reliable analytical technique should be employed with less contamination process.

7.1 Further recommendations

I recommend that future studies into iron measurements in a culture experiment with siderophore additions should make sure that the measurements of iron are done in different ways because for DGT and Chelex techniques, a lot of water was needed for effective display of dissolved labile iron concentrations especially DGT but in this work, we were restricted to a small volume of growth medium that was analyzed. Therefore the measurements were not completely precise since these techniques needed a lot of water to function properly.

Due to the above mishaps, it is noticed that these techniques are not very efficient for dissolved labile iron concentrations in small volumes of samples. However, a recent technique called ICPMS SeaFast is promising since it only needs very little volume of sample about 10- 15mL as compared to 350-400mL of samples that were analyzed.

In addition to the above I recommend that, more studies should be done about the residence times of DFB siderophores in a culture medium or in marine systems to reveal what interferes in its chemistry of complexing iron just for short periods of time and then releasing it back into the phytoplankton nutrient pool.

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APPENDIX 1. CULTURE EXPERIMENT AND VARIOUS ADDITIONS.

Figure A1. **The set up for the culture experiment showing the respective additions of Iron (Fe) and different DFB siderophore concentrations in two phytoplankton species.**

Fe experiment TBS

Experiment Setup

Setup 1	1 nM Fe																	
Sdph (nM)	10			50			100			500			2500			10000			CONTROL		
Skeletonema costatum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

Setup 2	10 nM Fe																	
Sdph (nM)	10			50			100			500			2500			10000			CONTROL		
Skeletonema costatum	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42

Setup 3	1 nM Fe																	
Sdph (nM)	10			50			100			500			2500			10000			CONTROL		
Alexandrium catenella	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63

Setup 4	10 nM Fe																	
Sdph (nM)	10			50			100			500			2500			10000			CONTROL		
Alexandrium catenella	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84

APPENDIX 2 – pH, Quantum Yield, In vivo Fluorescence, Cell abundance.

A.2.1 pH

Table A2. The pH levels measured from samples of Skeletonema costatum and Alexandrium catanella in a culture experiment.

SKELETONEMA

pH

Skeletonema

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	8.00	8.00	8.00	8.00	8.00	8.00	8.00
3	8.05	8.05	8.07	8.02	8.02	8.02	8.04
5	8.07	8.06	7.98	7.77	7.72	7.77	7.99
7	8.93	8.85	8.46	7.83	7.75	7.71	8.83
9	9.09	9.07	9.01	8.52	7.82	7.72	9.04
11	8.96	8.95	8.93	9.06	8.28	7.82	9.00
13	8.61	8.63	8.69	9.03	9.09	8.23	8.83
15	8.01	8.11	8.31	8.56	9.06	8.53	8.30

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.03	0.03	0.03	0.03	0.03	0.03	0.03
3	0.02	0.02	0.02	0.02	0.01	0.03	0.00
5	0.02	0.02	0.03	0.04	0.02	0.02	0.04
7	0.01	0.00	0.10	0.05	0.01	0.01	0.03
9	0.06	0.03	0.03	0.48	0.04	0.00	0.05
11	0.02	0.01	0.02	0.12	0.05	0.01	0.01
13	0.01	0.03	0.03	0.09	0.03	0.03	0.01
15	0.04	0.02	0.05	0.15	0.02	0.03	0.06

10 nM Fe

10	50	100	500	2500	10000	Control
7.87	7.87	7.87	7.87	7.87	7.87	7.87
8.07	8.07	8.09	8.07	8.06	8.05	8.06
7.87	7.84	7.78	7.57	7.56	7.58	7.77
8.73	8.57	8.21	7.66	7.61	7.58	8.61
8.98	8.99	8.82	7.71	7.54	7.52	8.92
9.01	8.98	8.93	8.43	7.77	7.53	8.94

8.84	8.84	8.89	9.08	8.83	7.95	8.96
8.29	8.03	8.40	8.81	8.94	8.60	8.48

Standard deviation

10	50	100	500	2500	10000	Control
0.03	0.03	0.03	0.03	0.03	0.03	0.03
0.01	0.01	0.01	0.01	0.03	0.05	0.04
0.01	0.08	0.01	0.01	0.04	0.01	0.01
0.04	0.08	0.06	0.08	0.01	0.02	0.03
0.03	0.03	0.03	0.10	0.02	0.06	0.02
0.02	0.02	0.02	0.39	0.02	0.02	0.02
0.02	0.04	0.01	0.02	0.04	0.09	0.03
0.05	0.50	0.01	0.10	0.02	0.16	0.04

ALEXANDRIUM

Alexandrium

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	7.84	7.84	7.84	7.84	7.84	7.84	7.84
3	8.01	8.03	8.02	8.01	8.03	8.04	8.10
5	7.79	7.83	7.82	7.77	7.78	7.77	7.88
7	7.84	7.84	7.85	7.73	7.70	7.69	7.84
9	7.89	7.87	7.86	7.70	7.69	7.67	7.92
11	8.08	8.07	8.03	7.93	7.84	7.75	8.13
13	8.21	8.18	8.17	8.08	7.98	7.81	8.22
15	8.23	8.21	8.26	8.20	8.07	7.88	8.28
17	8.23	8.22	8.34	8.31	8.13	7.91	8.28
19	8.51	8.47	8.64	8.56	8.38	8.13	8.51
21	8.58	8.50	8.63	8.63	8.46	8.18	8.56
23	8.74	8.60	8.61	8.70	8.57	8.25	8.63
27	8.78	8.72	8.60	8.81	8.72	8.44	8.71

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.03	0.03	0.03	0.03	0.03	0.03	0.03
3	0.02	0.02	0.03	0.03	0.01	0.01	0.01
5	0.02	0.01	0.02	0.01	0.00	0.02	0.03
7	0.00	0.03	0.01	0.01	0.01	0.02	0.01
9	0.05	0.01	0.01	0.01	0.01	0.02	0.01

11	0.01	0.01	0.00	0.05	0.01	0.03	0.02
13	0.02	0.01	0.04	0.02	0.01	0.02	0.02
15	0.03	0.01	0.10	0.02	0.02	0.03	0.01
17	0.03	0.05	0.14	0.08	0.03	0.05	0.02
19	0.03	0.05	0.12	0.10	0.03	0.09	0.01
21	0.04	0.06	0.03	0.09	0.03	0.10	0.02
23	0.08	0.06	0.08	0.08	0.04	0.11	0.01
23	0.08	0.06	0.08	0.08	0.04	0.11	0.01
27	0.02	0.05	0.11	0.09	0.06	0.15	0.01

10 nM Fe

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
7.87	7.87	7.87	7.87	7.87	7.87	7.87
8.07	8.07	8.09	8.07	8.06	8.05	8.06
7.87	7.84	7.78	7.57	7.56	7.58	7.77
8.73	8.57	8.21	7.66	7.61	7.58	8.61
8.98	8.99	8.82	7.71	7.54	7.52	8.92
9.01	8.98	8.93	8.43	7.77	7.53	8.94
8.84	8.84	8.89	9.08	8.83	7.95	8.96
8.29	8.03	8.40	8.81	8.94	8.60	8.48

Standard deviation

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
0.03	0.03	0.03	0.03	0.03	0.03	0.03
0.01	0.01	0.01	0.01	0.03	0.05	0.04
0.01	0.08	0.01	0.01	0.04	0.01	0.01
0.04	0.08	0.06	0.08	0.01	0.02	0.03
0.03	0.03	0.03	0.10	0.02	0.06	0.02
0.02	0.02	0.02	0.39	0.02	0.02	0.02
0.02	0.04	0.01	0.02	0.04	0.09	0.03
0.05	0.50	0.01	0.10	0.02	0.16	0.04

A2.2 Quantum Yield (QY)

Table A 3. Quantum Yield measurements for Skeletonema costatum and Alexandrium catanella respectively in the culture experiment.

QY

Skeletonema

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.53	0.59	0.50	0.26	0.30	0.00	0.63
7	0.52	0.59	0.59	0.47	0.14	0.07	0.55
9	0.46	0.49	0.47	0.53	0.14	0.12	0.48
11	0.43	0.42	0.44	0.53	0.62	0.35	0.40
13	0.37	0.38	0.45	0.43	0.47	0.56	0.38
15	0.31	0.37	0.39	0.38	0.47	0.50	0.32

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.06	0.09	0.15	0.22	0.29	0.00	0.05
7	0.01	0.01	0.01	0.11	0.02	0.07	0.04
9	0.01	0.03	0.02	0.11	0.30	0.21	0.02
11	0.01	0.02	0.02	0.03	0.01	0.15	0.03
13	0.03	0.02	0.03	0.03	0.01	0.05	0.03
15	0.04	0.02	0.01	0.08	0.01	0.01	0.07

10 nM Fe

10	50	100	500	2500	10000	Control
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.64	0.37	0.51	0.27	0.19	0.34	0.62
0.59	0.62	0.60	0.33	-0.29	-0.03	0.61
0.47	0.53	0.53	0.54	0.26	0.19	0.50
0.39	0.43	0.45	0.57	0.56	0.17	0.42
0.39	0.40	0.43	0.47	0.53	0.61	0.35
0.39	0.37	0.45	0.47	0.48	0.56	0.37

Standard deviation

10	50	100	500	2500	10000	Control
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	0.11	0.08	0.41	0.21	0.43	0.02
0.01	0.02	0.01	0.33	0.56	0.40	0.03
0.04	0.01	0.02	0.15	0.14	0.32	0.01
0.02	0.01	0.02	0.06	0.05	0.16	0.01
0.03	0.03	0.04	0.03	0.02	0.03	0.01
0.03	0.05	0.04	0.01	0.01	0.01	0.03

ALEXANDRIUM

Alexandrium

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.47	0.29	0.36	0.33	0.29	0.39	0.56
7	0.58	0.43	0.52	0.44	0.14	0.56	0.52
9							
11	0.43	0.44	0.44	0.53	0.37	0.31	0.43
13	0.53	0.58	0.50	0.58	0.47	0.47	0.57
15	0.56	0.51	0.53	0.58	0.54	0.46	0.52
17	0.58	0.56	0.60	0.57	0.57	0.49	0.56
19	0.55	0.55	0.58	0.59	0.60	0.45	0.57
21							
23							
27							

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.17	0.15	0.13	0.08	0.16	0.29	0.09
5	0.22	0.26	0.07	0.16	0.36	0.37	0.21
7	0.09	0.12	0.17	0.14	0.25	0.17	0.18
9							
11	0.15	0.02	0.07	0.08	0.06	0.23	0.08
13	0.04	0.03	0.02	0.11	0.04	0.06	0.02

15	0.05	0.02	0.04	0.03	0.07	0.10	0.01
17	0.01	0.05	0.05	0.05	0.04	0.07	0.04
19	0.03	0.03	0.04	0.02	0.02	0.10	0.03
21							
23							
27							

10 nM Fe						
10	**50**	**100**	**500**	**2500**	**10000**	**Control**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.08	0.46	0.28	0.50	0.38	0.51	0.44
0.52	0.39	0.39	0.31	0.32	0.39	0.48
0.47	0.49	0.47	0.35	0.35	0.42	0.47
0.49	0.50	0.49	0.45	0.40	0.27	0.62
0.44	0.57	0.52	0.52	0.48	0.41	0.54
0.51	0.54	0.55	0.58	0.53	0.54	0.54
0.55	0.55	0.56	0.53	0.52	0.55	0.52

Standard deviation

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.15	0.17	0.12	0.14	0.11	0.10	0.11
0.09	0.04	0.22	0.14	0.26	0.21	0.11
0.05	0.14	0.11	0.10	0.18	0.21	0.06
0.09	0.08	0.09	0.13	0.11	0.06	0.04
0.07	0.04	0.10	0.14	0.14	0.22	0.02
0.06	0.05	0.05	0.05	0.07	0.07	0.07
0.03	0.05	0.01	0.04	0.02	0.06	0.04
0.02	0.02	0.01	0.02	0.03	0.06	0.04

A.2.3 In Vivo Fluorescence (FSU)

Table A3. The FSU levels measured from samples of Skeletonema costatum and Alexandrium catanella in a culture experiment.

FSU

Skeletonema

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	382.69	382.69	382.69	382.69	382.69	382.69	382.69
3	1530.28	1549.20	1873.80	693.67	455.83	296.87	1449.90
5	13189.13	13807.33	11002.33	837.23	274.53	236.13	15724.00
7	82552.33	85863.67	59242.00	3838.40	238.13	148.37	103292.33
9	150180.67	155984.33	113904.67	69677.00	2410.67	384.77	181837.67
11	214443.00	191380.67	155956.67	134494.33	37411.33	4030.33	311542.67
13	72427.67	64933.00	66848.67	105312.00	86061.00	25658.67	128423.33
15	42496.67	38896.00	46298.67	53160.67	190486.00	42005.33	62980.33

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	62.52	62.52	62.52	62.52	62.52	62.52	62.52
3	162.10	76.96	280.03	121.16	67.47	35.91	93.47
5	974.51	552.65	1309.94	321.35	12.80	17.15	902.77
7	3985.99	675.59	9374.11	2758.08	7.84	11.55	5737.54
9	24139.25	9558.56	16293.85	51452.06	236.28	43.59	9654.70
11	13740.18	1981.43	20479.82	33938.60	5578.60	510.08	26684.59
13	1244.97	590.62	4315.71	21330.25	4813.24	2308.77	2683.00
15	1882.93	1877.88	1229.56	39630.13	1330.79	1629.67	4584.13

10 nM Fe

10	50	100	500	2500	10000	Control
382.69	382.69	382.69	382.69	382.69	382.69	382.69
1527.20	1311.43	1467.10	612.60	346.96	308.65	1353.90
11813.67	8741.50	6322.80	538.53	235.97	206.69	9927.90
95795.00	74702.00	40876.67	1386.93	212.27	118.73	84571.67
143912.00	142043.00	104770.33	8410.00	1117.33	262.27	130444.67
229634.00	251288.00	165363.33	63268.00	12937.00	1461.33	291040.00
126785.67	127593.33	119953.00	98788.00	85516.00	17796.67	241507.00
58665.00	42676.33	62315.33	119919.67	123186.00	75272.67	94758.00

Standard deviation

10	50	100	500	2500	10000	Control
31.36	31.36	31.36	31.36	31.36	31.36	31.36
257.60	127.47	159.15	40.27	26.91	20.92	83.83
1583.06	1353.94	128.91	166.89	8.86	21.93	518.68
1528.11	3169.73	5483.55	710.11	19.16	16.71	1534.65
17329.92	2866.49	5560.87	6429.02	112.52	21.07	7625.16
54126.30	19960.21	4074.94	30289.29	1773.20	255.04	18617.81
8444.15	22855.79	3896.63	7420.17	2835.97	4377.39	18455.65
3222.12	35292.08	5090.28	42518.43	6890.85	20025.25	5085.11

ALEXANDRIUM

Alexandrium

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	354.99	354.99	354.99	354.99	354.99	354.99	354.99
3	1973.00	2084.13	2253.77	2206.80	2033.77	2136.03	1882.30
5	3490.00	3363.17	3852.87	3095.60	2483.53	2533.40	3470.70
7	6013.33	5299.00	5157.67	3948.00	2932.67	2687.33	5065.33
9	8501.20	8480.27	8433.77	905.67	1575.10	655.80	2297.77
11	12799.00	12421.33	11808.33	6692.33	4632.00	3743.00	13572.67
13	16660.00	16677.67	15358.33	11614.33	7598.67	5064.33	18225.67
15	25490.67	21046.33	25474.00	18985.33	11518.33	7869.00	23892.00
17	35321.00	28415.67	33707.33	26833.67	17472.00	11610.00	33229.33
19	43504.33	34757.67	39297.00	36408.67	18445.33	16601.67	42161.67
21	61503.33	48828.00	53315.00	42923.33	31751.00	22487.67	53795.00
23	76861.00	58678.00	62422.67	58187.33	47089.33	27603.67	68149.33
27	91549.33	77189.00	43647.67	73321.67	55813.67	40893.67	82900.67
Standard deviaton							
Dia / Sid	**10**	**50**	**100**	**500**	**2500**	**10000**	**Control**
1	155.75	155.75	155.75	155.75	155.75	155.75	155.75
3	198.16	205.84	238.76	300.20	364.19	461.57	65.29
5	336.68	397.97	440.02	249.18	289.68	186.64	670.54
7	466.33	692.47	629.94	464.75	197.70	369.95	133.81
9	747.45	1031.04	1324.95	131.00	1392.51	310.00	1485.58
11	273.37	732.00	934.07	144.53	262.67	220.17	2472.73
13	1651.83	469.23	1957.83	937.42	1135.20	377.84	1871.94
15	3352.72	579.45	3538.11	1071.00	646.16	874.63	2995.47

17	4718.83	1320.40	6021.24	2146.60	2101.92	178.60	1481.11
19	5080.31	2724.50	9030.64	3069.94	6667.88	1573.08	2695.66
21	10911.82	3533.88	9599.26	11418.25	1460.10	2337.08	4446.12
23	6826.31	3594.17	10667.87	9337.25	7737.98	1830.51	1992.25
27	11731.51	11502.19	11234.91	8930.43	6885.62	7061.87	4595.28

10 nM Fe

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
354.99	354.99	354.99	354.99	354.99	354.99	354.99
2269.43	2106.63	2246.23	2358.50	2084.10	2309.20	2038.20
3704.37	3126.80	3584.43	3626.50	3433.93	3216.40	3589.50
6395.33	5990.67	5542.67	4329.00	3846.33	3688.67	5597.33
9512.07	10424.97	8795.03	5365.33	5114.20	5031.33	7830.33
12200.00	14008.33	10212.33	6576.33	4791.67	3884.00	12065.33
17691.00	15135.00	12977.67	10165.33	7190.33	5364.00	15658.67
24215.00	21352.50	19787.33	17802.33	11928.00	8698.33	19483.00
30965.33	24641.00	26718.33	24232.67	19135.33	14686.33	25836.67
41947.67	33918.50	33111.67	33686.00	27287.67	21034.67	31560.67
57813.33	46385.00	51103.00	42933.67	39215.67	33371.67	41239.00
68049.67	59945.00	51310.33	50411.33	51638.00	41929.67	52568.33
84757.67	92091.50	66236.33	59739.33	66707.67	68018.00	68488.00

Standard deviation

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
168.44	168.44	168.44	168.44	168.44	168.44	168.44
171.94	350.48	220.63	74.07	198.46	424.99	96.36
389.31	598.09	342.48	114.99	412.75	225.28	680.16
817.15	418.25	232.78	300.79	262.00	669.15	366.09
309.24	2561.27	469.37	104.63	635.33	1057.83	848.46
235.65	5489.38	591.62	540.62	692.08	36.37	427.62
1064.98	2110.01	622.80	885.36	101.51	213.44	1869.08
2513.39	1163.19	1302.93	755.59	1190.67	308.93	2003.39
4163.54	1183.70	2460.68	1135.84	688.14	1225.90	854.78
8080.40	1819.39	3130.51	5735.74	1304.02	2659.59	1918.65
14865.01	7728.68	8168.29	2102.32	2835.29	1958.00	7259.99
15304.97	12443.67	1909.35	2742.91	5188.61	3151.47	1590.04
20269.23	9038.95	5710.28	7414.46	82.78	5690.46	8961.95

A.4 Cell Abundance

Table A2. The cell abundance recorded from counted samples of Skeletonema costatum and Alexandrium catanella in a culture experiment.

SKELETONEMA

CELL ABUN.

Skeletonema

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	8000.00	8000.00	8000.00	8000.00	8000.00	8000.00	8000.00
3							
5	132591.27	137776.40	124072.83	74814.07	34444.10	32221.90	173331.60
7	433329.00	387033.17	386292.43	77406.63	29999.70	27407.13	334811.47
9	795183.20	736666.67	676666.67	490000.00	110000.00	106666.67	946666.67
11	806666.67	630000.00	560000.00	576666.67	496666.67	103333.33	583333.33
13	473333.33	396666.67	350000.00	526666.67	693333.33	523333.33	793333.33
15	376666.67	240000.00	243333.33	573333.33	800000.00	550000.00	333333.33

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3							
5	3394.47	3333.30	3394.47	3394.47	2939.69	2222.20	2939.69
7	2222.20	5592.41	5480.93	3394.47	2222.20	2796.21	4490.46
9	181425.41	25166.11	25166.11	79372.54	20000.00	30550.50	25166.11
11	45092.50	26457.51	20000.00	11547.01	25166.11	15275.25	35118.85
13	35118.85	25166.11	30000.00	20816.66	20816.66	41633.32	35118.85
15	30550.50	45825.76	30550.50	23094.01	20000.00	20000.00	70237.69

10 nM Fe

10	50	100	500	2500	10000	Control
8000.00	8000.00	8000.00	8000.00	8000.00	8000.00	8000.00
137035.67	102221.20	91110.20	25925.67	17036.87	15555.40	110739.63
454439.90	424810.57	311108.00	38888.50	30370.07	22592.37	329996.70
713333.33	540000.00	513333.33	183333.33	146666.67	93333.33	716666.67
776666.67	716666.67	763333.33	510000.00	236666.67	103333.33	783333.33

543333.33	456666.67	590000.00	776666.67	566666.67	493333.33	530000.00
423333.33	326666.67	313333.33	383333.33	723333.33	783333.33	446666.67

Standard deviation

10	50	100	500	2500	10000	Control
0.00	0.00	0.00	0.00	0.00	0.00	0.00
1697.23	1111.10	2222.20	3902.05	2796.21	1924.48	1697.23
7285.97	1697.23	4444.40	2939.69	3902.05	2796.21	4006.13
47258.16	36055.51	25166.11	15275.25	15275.25	11547.01	20816.66
25166.11	25166.11	20816.66	20000.00	15275.25	15275.25	20816.66
15275.25	30550.50	40000.00	35118.85	45092.50	20816.66	30000.00
41633.32	50332.23	60277.14	41633.32	40414.52	40414.52	41633.32

ALEXANDRIUM

Alexandrium

1 nM Fe

Day/Sid	10	50	100	500	2500	10000	Control
1	180.00	180.00	180.00	180.00	180.00	180.00	180.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	860.00	633.33	733.33	326.67	426.67	413.33	926.67
7	1306.67	1053.33	953.33	726.67	546.67	620.00	1086.67
9	1213.33	946.67	773.33	686.67	593.33	753.33	1640.00
11	1373.33	2086.67	2220.00	1573.33	1280.00	1126.67	3313.33
13	1973.33	2466.67	1633.33	1833.33	1533.33	613.33	1760.00
15	2960.00	2420.00	2260.00	2886.67	1153.33	1013.33	3553.33
17	3433.33	3006.67	3786.67	3133.33	1920.00	1726.67	2946.67
19	4233.33	3706.67	3913.33	3053.33	2426.67	1553.33	3613.33
21	5806.67	3573.33	3473.33	4060.00	2193.33	2466.67	5233.33
23	4386.67	4580.00	2953.33	5860.00	3120.00	3420.00	3846.67
27	4520.00	6360.00	3500.00	6360.00	5446.67	4573.33	4313.33

Standard deviaton

Dia / Sid	10	50	100	500	2500	10000	Control
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3							
5	40.00	23.09	41.63	30.55	30.55	30.55	30.55
7	50.33	50.33	30.55	61.10	41.63	91.65	50.33
9	70.24	100.66	61.10	61.10	50.33	50.33	52.92

11	30.55	120.55	124.90	50.33	52.92	61.10	80.83
13	50.33	61.10	50.33	83.27	64.29	83.27	80.00
15	52.92	72.11	80.00	117.19	94.52	70.24	30.55
17	70.24	61.10	100.66	90.18	52.92	41.63	113.72
19	75.72	61.10	64.29	50.33	83.27	70.24	61.10
21	94.52	110.15	90.18	91.65	90.18	83.27	94.52
23	50.33	72.11	90.18	91.65	72.11	60.00	90.18
27	111.36	60.00	105.83	72.11	110.15	50.33	100.66

10 nM Fe

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
180.00	180.00	180.00	180.00	180.00	180.00	180.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00
666.67	720.00	353.33	433.33	393.33	466.67	1033.33
1306.67	940.00	1300.00	893.33	580.00	533.33	773.33
2166.67	1213.33	1106.67	1033.33	860.00	726.67	1840.00
2493.33	1820.00	1333.33	1186.67	1040.00	793.33	2640.00
2360.00	2126.67	2140.00	1293.33	1073.33	1380.00	1753.33
3380.00	2100.00	1866.67	2120.00	2226.67	1626.67	2786.67
4340.00	3630.00	2200.00	2986.67	2613.33	2126.67	2446.67
6280.00	3320.00	2420.00	3480.00	3866.67	2640.00	4226.67
6553.33	4610.00	4346.67	4960.00	4013.33	3213.33	4720.00
6426.67	4230.00	4093.33	3606.67	2980.00	3000.00	5533.33
7260.00	6220.00	4406.67	4653.33	4353.33	4066.67	6540.00

Standard deviation

10	**50**	**100**	**500**	**2500**	**10000**	**Control**
0.00	0.00	0.00	0.00	0.00	0.00	0.00
30.55	40.00	41.63	23.09	11.55	30.55	30.55
50.33	72.11	60.00	41.63	40.00	30.55	83.27
141.89	147.42	41.63	30.55	72.11	61.10	60.00
90.18	84.85	41.63	64.29	91.65	90.18	72.11
60.00	113.72	60.00	41.63	90.18	87.18	61.10
40.00	113.14	70.24	91.65	80.83	30.55	150.11
60.00	70.71	60.00	161.66	61.10	70.24	83.27
105.83	84.85	72.11	72.11	80.83	91.65	80.83
61.10	98.99	61.10	87.18	70.24	61.10	111.36
64.29	42.43	90.18	90.18	40.00	40.00	61.10
91.65	56.57	70.24	120.55	83.27	120.55	131.15

APPENDIX 3 CHELEX BLANKS AND SAMPLES

A.3.1 CHELEX BLANK

Table A3.1 The total concentration of Iron (Fe) in blanks of Chelex-100 technique samples analyzed by ICP-MS before the start of the culture experiment

	Sample received	Project-lnr	ID-1	Preservation
Start formulas				
	13/02/2015	424	Blank 6	5 ml preconcentrated sol. in 0.6 M HNO3
	13/02/2015	425	Blank 7	5 ml preconcentrated sol. in 0.6 M HNO3
	13/02/2015	426	Blank 8	5 ml preconcentrated sol. in 0.6 M HNO3
	13/02/2015	427	Blank 9	5 ml preconcentrated sol. in 0.6 M HNO3
	13/02/2015	428	Blank 10	5 ml preconcentrated sol. in 0.6 M HNO3
Stop formulas				
number		5	5	5

Date of analyses: 24.02.15 sekvens 15

Counting digits = 3

Remarks	Sample ID	Isotope Parameteres	Fe56(MR) Conc. µg/L	RSD, %	
	Start statistical calculations				
Chelex	Nico-420-428-424-blank		1.25	3.8	
Chelex	Nico-420-428-425-blank		0.76	4.0	
Chelex	Nico-420-428-426-blank		1.14	1.0	
Chelex	Nico-420-428-427-blank		2.03	1.6	
Chelex	Nico-420-428-428-blank		1.51	2.7	Total amount blank calculated (nmol)
	Stop statistical calculations				nmol sd
5	Average		1.34	2.6	0.11
	Min		0.76	1.0	0.07
	Max		2.03	4.0	0.10
	Std		0.47	1.3	0.18

Rsd % <5, 5-10, >10	35.2		0.13		
Confidence interval 95%	0.47	1.3			
Confidence interval 95% (%) <5, 5-10, >10	35.2		0.1	#DIV/0!	
Number	5	5			

A.3.2 CHELEX SAMPLES

Table A3.2 The final concentration of Iron (Fe) remaining in the different phytoplankton species (Skeletonema costatum and Alexandrium catanella) cultures with their various DFB siderophore additions after the culture experiment using the Chelex-100 preconcentration technique.

Date of analyses: 24.04.15
sekvens 33
Counting digits = 3

Fe	Siderophore	bottle	Project-Inr	Isotope / Parameteres / Sample ID: Siderophore	Fe56(MR) Conc. µg/L	Fe56(MR) RSD, %
1	50	4	1	Skeletonema	5.54	1.4
1	50	6	2	Skeletonema	3.03	1.9
1	500	10	3	Skeletonema	4.73	2.7
1	500	12	4	Skeletonema	4.34	1.5
1	10000	16	5	Skeletonema	4.29	2.7
1	10000	18	6	Skeletonema	1.42	1.6
1	Control	19	7	Skeletonema	1.58	1.7
1	Control	21	8	Skeletonema	1.89	1.0
10	50	25	9	Skeletonema	2.89	2.9
10	50	26	10	Skeletonema	1.24	1.3
10	500	31	11	Skeletonema	2.78	2.1
10	500	33	12	Skeletonema	2.97	3.9
10	10000	37	13	Skeletonema	1.35	1.6
10	10000	39	14	Skeletonema	2.66	0.5
10	Control	40	15	Skeletonema	0.91	2.4

10	Control	42	16	Skeletonema	1.93	2.7
1	50	46	17	Alexandrium	10.24	1.4
1	50	48	18	Alexandrium	10.23	0.2
1	500	52	19	Alexandrium	6.72	1.8
1	500	54	20	Alexandrium	6.76	0.6
1	10000	58	21	Alexandrium	5.44	2.0
1	10000	60	22	Alexandrium	4.90	0.2
1	Control	61	23	Alexandrium	4.09	2.2
1	Control	63	24	Alexandrium	1.90	0.5
10	50	67	25	Alexandrium	6.12	2.6
10	50	69	26	Alexandrium	2.87	1.3
10	500	73	27	Alexandrium	4.16	3.2
10	500	75	28	Alexandrium	4.35	0.5
10	10000	79	29	Alexandrium	2.06	3.7
10	10000	81	30	Alexandrium	4.22	1.1
10	Control	82	31	Alexandrium	3.33	2.0
10	Control	84	32	Alexandrium	2.23	0.2

Stop statistical calculations

Average	3.85	1.7
Min	0.91	0.2
Max	10.24	3.9
Std	2.33	1.0
Rsd % <5, 5-10, >10	60.4	
Confidence interval 95%	0.84	0.4
Confidence interval 95% (%) <5, 5-10, >10	21.7	
Number	32	32

Corrected						
µg.L^{-1}	Mol.L^{-1}	nM	avg	std	%std	vol smpl (ml)
0.184635	3.30E-09	3.30				149.96
0.081711	1.46E-09	1.46	2.38	1.3	54.65	185.12
0.140214	2.51E-09	2.51				168.72
0.155491	2.78E-09	2.78	2.64	0.2	7.31	139.4
0.163756	2.93E-09	2.93				131.1
0.054763	9.80E-10	0.98	1.95	1.4	70.54	129.77
0.058102	1.04E-09	1.04				136.02
0.072987	1.31E-09	1.31	1.17	0.2	16.06	129.36
0.123221	2.20E-09	2.20				117.26
0.048413	8.66E-10	0.87	1.54	0.9	61.64	127.72
0.086266	1.54E-09	1.54				161.23
0.092237	1.65E-09	1.65	1.60	0.1	4.73	161.13
0.042734	7.64E-10	0.76				158.16
0.098475	1.76E-09	1.76	1.26	0.7	55.82	135.1
0.036512	6.53E-10	0.65				124.64
0.081484	1.46E-09	1.46	1.06	0.6	53.90	118.7
0.327618	5.86E-09	5.86				156.31
0.376242	6.73E-09	6.73	6.30	0.6	9.77	135.92
0.208561	3.73E-09	3.73				161.03
0.272433	4.87E-09	4.87	4.30	0.8	18.78	124.13
0.17492	3.13E-09	3.13				155.39
0.266074	4.76E-09	4.76	3.94	1.2	29.23	92.045
0.183203	3.28E-09	3.28				111.52
0.084487	1.51E-09	1.51	2.39	1.2	52.15	112.34
0.304251	5.44E-09	5.44				100.66
0.096693	1.73E-09	1.73	3.59	2.6	73.21	148.32
0.137756	2.46E-09	2.46				150.88
0.187975	3.36E-09	3.36	2.91	0.6	21.80	115.72
0.078594	1.41E-09	1.41				130.79
0.141372	2.53E-09	2.53	1.97	0.8	40.36	149.14
0.144579	2.59E-09	2.59				115.11
0.090621	1.62E-09	1.62	2.10	0.7	32.44	123.31

A.3.2 DGT BLANKS AND SAMPLES

A3.2.1 DGT BLANKS

Table A3.3 The total concentration of Iron (Fe) in blanks of Chelex-100 technique samples analyzed by ICP-MS before the start of the culture experiment.

Sample	Columns for specification of sample-ID, choose your oven heading		
received	Dato	Type of sample	Preservation
Start formulas			
16/02/2015	Blank-1	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-2	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-3	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-4	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-6	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-7	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-8	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-9	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-10	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-11	DGT	5 ml preconcentrated sol. in 0.6 M HNO3
16/02/2015	Blank-12	DGT	5 ml preconcentrated sol. in 0.6 M HNO3

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Counting digits = 3

		Isotope	Fe56(MR)	
Project-lnr		Parameteres	Conc.	
	Sample ID		µg/L	RSD, %
	Start statistical calculations			
1	Cintera-1-156-1-blank		u0,491595775547848	1.2
2	Cintera-1-156-2-blank		0.42	4.1
3	Cintera-1-156-3-blank		0.26	11.3
4	Cintera-1-156-4-blank		0.35	2.4
98	Cintera-1-156-98-blank		u0,608428331740494	5.5
99	Cintera-1-156-99-blank		0.26	5.0
152	Murat-Cintera-149-156-152		0.32	5.4
153	Murat-Cintera-149-156-153		u0,802561746682815	4.3
154	Murat-Cintera-149-156-154		0.25	2.1
155	Murat-Cintera-149-156-155		0.23	2.8

156	Murat-Cintera-149-156-156	0.21	3.2
	Stop statistical calculations		
	Average	0.29	4.3
		0.29	**4.3**
11	Min	0.21	1.2
	Max	0.42	11.3
	Std	0.07	2.7
	Rsd % <5, 5-10, >10	24.8	
	Confidence interval 95%	0.05	1.7
	Confidence interval 95% (%) <5, 5-10, >10	18.7	
	Number	8	11

A.3.2.2 DGT SAMPLES

Table A3.4 The final concentration of Iron (Fe) present in the different phytoplankton cultures with various siderophore and iron treatments after the culture experiment using the DGT technique.

Date of analyses:
24.04.15 sekvens 33

Counting digits = 3

					Isotope	**Fe56(MR)**	
Fe	**Siderophore**	**bottle**	**Remarks**	**Project-Inr**	**Parameteres**	**Conc.**	
					Sample ID	**µg/L**	**RSD, %**
					Start statistical calculations		
1	10000	16,17, 18	DGT	33	Skeletonema	1.19	2.7
1	10000	16,17, 18	DGT	34	Skeletonema	1.20	4.0
1	10000	16,17, 18	DGT	35	Skeletonema	1.40	1.9
1	Control	19,21, 22	DGT	36	Skeletonema	1.57	2.1
1	Control	19,21, 22	DGT	37	Skeletonema	1.93	2.0
1	Control	19,21, 22	DGT	38	Skeletonema	u1,00498540280587	3.9
10	10000	37,38, 39	DGT	39	Skeletonema	3.11	1.1

10	10000	37,38, 39	DGT	40	Skeletonema	3.20	1.9
10	10000	37,38, 39	DGT	41	Skeletonema	3.33	0.5
10	Control	40,41, 42	DGT	42	Skeletonema	1.80	2.5
10	Control	40,41, 42	DGT	43	Skeletonema	2.00	1.7
10	Control	40,41, 42	DGT	44	Skeletonema	1.99	1.0
1	10000	58,59, 60	DGT	45	Alexandrium	0.49	4.5
1	10000	58,59, 60	DGT	46	Alexandrium	0.64	2.8
1	10000	58,59, 60	DGT	47	Alexandrium	u1,07630389522 661	3.5
1	Control	61,62, 63	DGT	48	Alexandrium	u2,93738583014 131	1.0
1	Control	61,62, 63	DGT	49	Alexandrium	0.79	2.6
1	Control	61,62, 63	DGT	50	Alexandrium	0.52	4.2
10	10000	79,80, 81	DGT	51	Alexandrium	0.43	3.7
10	10000	79,80, 81	DGT	52	Alexandrium	0.56	1.6
10	10000	79,80, 81	DGT	53	Alexandrium	0.49	2.4
10	Control	82,83, 84	DGT	54	Alexandrium	u1,88265348315 53	2.2
10	Control	82,83, 84	DGT	55	Alexandrium	0.74	0.9
10	Control	82,83, 84	DGT	56	Alexandrium	0.69	3.2
	INITIAL		DGT	57	INITIAL	0.77	3.3
	INITIAL		DGT	58	INITIAL	1.19	2.7
	INITIAL		DGT	59	INITIAL	u2,56782709894 215	0.4
	INITIAL		DGT	60	INITIAL	u3,99416760511 874	2.6
	INITIAL		DGT	61	INITIAL	0.83	2.1
	INITIAL		DGT	62	INITIAL	0.88	2.6
					Stop statistical calculations		
					Average	1.32	2.4
24	30	24	**Number of**	30	Min	0.43	0.4

			samples				
			Total number of analyses		Max	3.33	4.5
					Std	0.89	1.1
					Rsd % <5, 5-10, >10	66.9	
					Confidence interval 95%	0.37	0.4
					Confidence interval 95% (%) <5, 5-10, >10	27.9	
					Number	24	30

Vol (ml)	Start time shaker (Date)	(Hrs)	Stop time shaker (Date)	(Hrs)	Days	Hrs	Total time (Hrs:mm)	Total time (sec)
770	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
770	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
770	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
750	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
750	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
750	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
760	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
760	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
760	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
790	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
790	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
790	3/13/2015	11:01	3/16/2015	13:01	3	2:00	74:00:00	266400:00:00
685	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
685	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
685	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
695	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
695	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
695	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
690	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
690	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
690	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
720	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
720	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
720	3/23/2015	16:51	3/26/2015	17:02	3	0:11	72:11:00	259860:00:00
1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00
1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00
1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00

1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00
1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00
1850	2/3/2015	17:26	2/6/2015	18:10	3	0:44	72:44:00	261840:00:00

						M (ng)	Values	Ce (µgr/L)	
						Vg (mL)	0.16	Δg (cm)	0.093
						Ve (mL)	5.00	A (cm^2)	3.14
[ ] Uncorrected				[ ] Corrected for diffusion		*fe* (Fe)	0.70	D (10°C) Fe	3.91E-06
µg.L^{-1}	RSD %		µg.L^{-1}	Ce (µg/L)	Mol.L^{-1}	nM	avg	std	%std
0.9	2.7		6.708	1.91E-01	3.41E-09	3.41			
0.9	4.0		6.696	1.90E-01	3.41E-09	3.41			
1.1	1.9		8.167	2.32E-01	4.15E-09	4.15	3.66	0.4	11.76
1.3	2.1		9.445	2.69E-01	4.80E-09	4.80			
1.6	2.0		12.112	3.44E-01	6.16E-09	6.16			
#VALUE!	3.9						5.48	1.0	17.49
2.8	1.1		20.778	5.91E-01	1.06E-08	10.57			
2.9	1.9		21.446	6.10E-01	1.09E-08	10.91			
3.0	0.5		22.436	6.38E-01	1.14E-08	11.41	10.96	0.4	3.87
1.5	2.5		11.156	3.17E-01	5.67E-09	5.67			
1.7	1.7		12.580	3.58E-01	6.40E-09	6.40			
1.7	1.0		12.528	3.56E-01	6.37E-09	6.37	6.15	0.4	6.68
0.2	4.5		1.474	4.30E-02	7.69E-10	0.77			
0.4	2.8		2.593	7.56E-02	1.35E-09	1.35			
#VALUE!	3.5						1.06	0.4	38.90
#VALUE!	1.0								
0.5	2.6		3.684	1.07E-01	1.92E-09	1.92			
0.2	4.2		1.689	4.92E-02	8.81E-10	0.88	1.40	0.7	52.49
0.1	3.7		1.026	2.99E-02	5.35E-10	0.53			
0.3	1.6		2.006	5.85E-02	1.05E-09	1.05			
0.2	2.4		1.444	4.21E-02	7.53E-10	0.75	0.78	0.3	32.97
#VALUE!	2.2								
0.4	0.9		3.298	9.61E-02	1.72E-09	1.72			
0.4	3.2		2.963	8.64E-02	1.55E-09	1.55	1.63	0.1	7.56
0.5	3.3		3.566	1.03E-01	1.85E-09	1.85			
0.9	2.7		6.606	1.91E-01	3.42E-09	3.42			
#VALUE!	0.4								
#VALUE!	2.6								

0.5	2.1		4.002	1.16E-01	2.07E-09	2.07			
0.6	2.6		4.340	1.26E-01	2.25E-09	2.25	2.40	0.7	29.29