

# Using Phytoplankton as Tools to Assess the Health of Aquatic Bodies and Ecosystems

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## Abstract

The cost of exponential development and population booms around the world is an increase in the level of anthropogenic pollution of natural resources, and this calls for scientists and government agencies to find resourceful and cost-effective methods of monitoring the health of these resources and their ecosystems. Given their unique, yet fundamental position in the food web and the biogeochemical cycles in nature, this paper proposes that phytoplankton can be effective bioindicators and biomonitorers of ecological and environmental health. Plankton play an enormous role in the biological processing of organic matter and therefore act as an early warning signs of change in water quality. They can also predict the cumulative effect of pollutants on living organisms and can detect early on the bioaccumulation of toxicants along the food chain. In this study, we compare the advantages of integrating biological tools with the traditional suite of ecological health assessment tools, i.e., chemical and physical assays, and discuss the features and limitations of the current applications of phytoplankton in bioindication. While phytoplankton add important dimensions like bioavailability and biotoxicity to environmental risk assessment, further research is needed to better correlate the physiological and community responses with the environmental stressors causing them in the first place.

## 1 Introduction

The oceanic biosphere is an integral component of the climate system, absorbing 30% of anthropogenic carbon emissions and storing 45x more carbon than the atmosphere [FOJ<sup>+</sup>20]. More than two-thirds of our planet is covered in water, and for the last 112 years, as the atmosphere warmed from the building up of greenhouse gases, so did the oceans [Fis13]. Apart from deep heating, excess carbon dioxide in the atmosphere is quickly dissolving into the oceans at a pace and in an amount incapable of being stabilized by the natural buffering

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processes, leading to acidification of the oceans. These multiple drivers of climate change lead to extreme temperatures, severe droughts, heavy storms, and heavy flooding [ZWVDH<sup>+</sup>18]. Between 1994 and 2007, the oceans absorbed 34 gigatons of CO<sub>2</sub>, or 31 percent of the gas anthropogenically introduced into the atmosphere at that time [Ros19]. Another offshoot of anthropogenic emission of traces gases, primarily chlorofluorocarbons (CFCs) and other halogenated species containing bromine and chlorine, is stratospheric ozone depletion. The spatial scale of ozone loss, especially over the Antarctic, is immense and exposes oceanic ecosystems to higher UV-B radiation [PP14].

On the other hand, pollutants and effluents have altered nutrient compositions and pH levels in major water bodies. Human and veterinary pharmaceuticals are emerging as major environmental contaminants, having been percolated to as far as into groundwater and near sea coasts [BCC10]. Antimicrobial compounds are likely to escape into the natural aquatic environment and have an unintentional inhibitory effect on bacterial microflora [Aus85]. The risk of exposure to other common-place pharmaceuticals like ibuprofen, paracetamol, amoxicillin, oxytetracycline, and mefenamic acid are present at risk levels non-negligible for water biota [BCC10], and run the risk of disrupting food chains and growth in aquatic ecosystems. Indiscriminate discharge of heavy metals, pesticides, persistent organic pollutants (POP), and accidental oil spills of crude oil products are other anthropogenic contributors to changes in aquatic food chains and ecosystems [HG15].

The various factors listed above greatly impact marine systems and change oceanic habitats irreversibly. Interactions between various external factors- for example, pH, nutrient concentration, CO<sub>2</sub> concentration, temperature, and exposure to light and UV radiations- influence the productivity of these systems [BOS<sup>+</sup>06]. The spatial and temporal variability of these factors calls for rigorous, reproducible, and affordable monitoring of water bodies across the world. This paper discusses bioindication as a method to monitor water bodies. Since the listed external factors influence the growth and productivity of aquatic biota to a large extent, the method of bioindication makes use of biological processes, species, or communities to assess the quality of an environment and how it changes over time. Populations adapt to their niche in their local ecosystems and evolve to maximize growth and reproduction within a specific range of environmental variables [HM11]. Outside their respective developed environmental optima, the physiology and behavior of the individuals, or the size of a population may alter drastically, disrupting the population dynamics and the net productivity of the ecosystem at large. Even though every organism in an ecosystem can report the health of its environment [Sam19], to choose a species or a species assemblage to effectively indicate the health of an ecosystem, they need to display a moderate tolerance to environmental variability, i.e, tolerance to be sensitive to variability, yet the endurance to withstand the extremes so as to reflect a general biotic response that can be monitored [CV02].

The following study explores the capacity and capability of phytoplankton as strong and effective bioindicators to map local environmental stress and global anthropogenic climate change, given their abundance and tolerance to variability, and their unique position in both the aquatic food web and the global carbon cycle. Phytoplankton is a group of autotrophic microalgae, which use chlorophyll and sunlight to assimilate nutrients and grow, and are mainly classified as either dinoflagellates or diatoms [NOA]. There are primarily two classes of phytoplankton: (1) the non-motile, fast-growing diatoms; and (2) motile flagellates and dinoflagellates, which migrate vertically in the water column in response to light. They are the foundation of the aquatic food web, indirectly fixing nutrients for organisms from zooplankton to massive whales, and also sequester 26 million tons of anthropogenically released per day, regulating atmospheric CO<sub>2</sub> and temperature, and contribute to more than 90% of photosynthetic carbon fixation [HG15]. They are the primary producers in oceans and assimilate nitrates, phosphates, silicates, and calcium to varying degrees [LSS10], and can potentially be used to monitor inorganic pollutants like fertilizer and pharmaceuticals in water.

## 2 Results

### 2.1 The Role of Bio-Environmental Monitoring in Pollution Science

In the post-industrial period, environmental health has deteriorated as a result of rapid, unchecked industrialization and increasing population pressure, and is a major concern for developing countries [CHR18]. The recent disturbances in the natural cycles of elements like carbon [PRRC09] and nitrogen [FSK09] are exacerbating global climate change and making it more difficult for carbon-sequestering organisms to fight global warming. Environmental water quality monitoring and analysis tackle the extension of pollution, climate change, and global warming to oceans, seas, and other water bodies, but despite advances in pollution control methods, the continuous rise in pollution levels remains inevitable [AB19]. In recent years, the case of “emerging pollutants” has come to light, and these synthetic or naturally occurring chemicals, and their metabolites and transformation products can enter water systems and cause unsuspected, adverse ecological or human health effects [GMK<sup>+</sup>15]. While chemical assays remain the most prevalent and researched upon tools of environmental monitoring [DEC<sup>+</sup>20], they fail to reflect the response of the local biota to pollution and are inefficient at analyzing the synergy of climate change and organic & inorganic contamination.

On the other hand, environmental monitoring using biological elements, and their communities, physiology, and behavior, can be used to detect changes in the health of an ecosystem, free of the listed shortcomings of physiochemical assays. Bioindicators can assess the cumulative effects of different pollutants

in an ecosystem and organisms like phytoplankton, which are essential components of biogeochemical cycles of carbon and nitrogen, allow for better tracking of climate change. Any changes in their productivity can also predict the yield of the whole local ecosystem. Phytoplankton are a particularly responsive and are a widespread category of organisms, which form the foundation of aquatic food webs, and have been employed to track numerous contaminants, which cause changes in factors important for the plankton’s growth: oxygen availability, temperature, salinity, light availability, and nutrient concentration. Their capacity to cumulate the changes in any of these variables, makes them a powerful tool of biological monitoring and biochemical assays: an integrated method that takes into consideration both biological and physiochemical aspects. As listed in (Fig. 1), the paper will focus on select drivers of climate change and pollutants, which cause significant changes in the biologically relevant variables mentioned above.

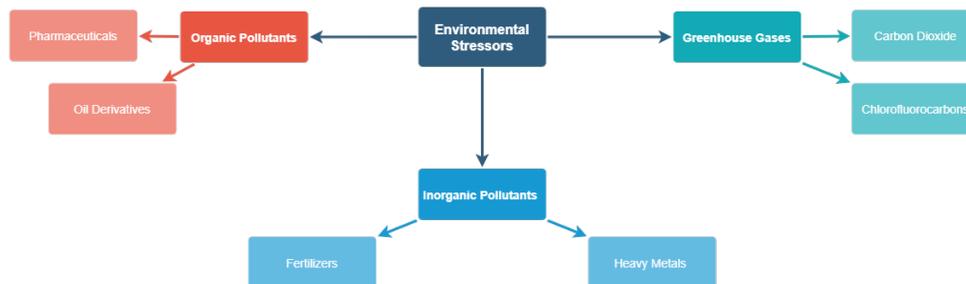


Figure 1: Environmental stressors can be categorised as organic pollutants, inorganic pollutants, or greenhouse gases. For this study, we take into consideration limited examples from each category, and explore if any responses of phytoplankton organisms or communities can be used to monitor the stress these factors induce in the local ecosystems.

## 2.2 Functional Importance of Phytoplankton in Aquatic Ecosystems

Being the main primary producers in aquatic ecosystems, phytoplankton are extremely important components in the functioning of ecosystems and regulation of climate [TGS18]. Their biomass production and CO<sub>2</sub> sequestration are approximately equal to those of all terrestrial plants taken together [FSB<sup>+</sup>00]. Phytoplankton are “photoautotrophs” that harvest light to convert inorganic carbon into organic carbon, which is passed up the food chain to other “heterotrophs” [SH12]. There are many nested cycles of carbon associated with the concept of ocean productivity:

$$GPP, \text{ or Gross Primary Production} = \text{total rate of organic carbon production by autotrophs} \quad (1)$$

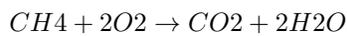
### *NPP, or Net Primary Productivity = GPP–Respiration*

Therefore, net primary productivity is defined as the amount of photosynthetically fixed carbon available to the heterotrophic level in an ecosystem [FBRF98], or as illustrated in the equation above, the difference between autotrophic photosynthesis and respiration. Net primary productivity, as we will read further on in the study, is an important bioindicator of anomalies in the biogeochemical cycles.

The unique position of phytoplankton in both the nutrient and the carbon cycles posits them to be useful tools of bioassessment. To understand how these bioassessments make use of phytoplankton we must first explore the role of phytoplankton in more depth.

#### **2.2.1 Prehistoric Significance**

Phytoplankton have been the key to the origin of Earth’s first breathable atmosphere. In what is now called the Great Oxidation Event [Hol06], cyanobacteria, or the blue-green algae appeared to pump free oxygen into the Earth’s atmosphere, paving the way for aerobic life to develop on the planet. In addition, this free oxygen reacted with methane to form carbon dioxide:



Carbon dioxide is not as insulating as methane, and therefore, the introduction of cyanobacteria also enabled the planet to cool off. [SdVAB13] points out a link between the oxygenation of the atmosphere and the rise of multicellular life forms. Later, during the late Cambrian period, over 500 million years ago, in a Phytoplankton Bloom, the atmosphere’s oxygen first approached modern levels ( 21%), setting the stage for the Great Ordovician Biodiversification Event [SPD<sup>+</sup>16], which saw rapid diversification of marine organisms. During the Jurassic Period, the evolution of calcifying marine phytoplankton – coccolithophores – balanced ocean chemistry by nullifying the pH alterations caused by events like volcanic eruptions, as the layers of dead carbonate coccolithophores on the ocean floor released carbonate ions to act as a buffer to runaway acidity [MBB<sup>+</sup>16]. As we will further explore in this study, both these attributes, i.e., photosynthesis and pH moderation, make phytoplankton suitable for climate change bioindication.

#### **2.2.2 Phytoplankton as Drivers of the Global Carbon Cycle**

Phytoplankton were early regulators of ocean’s chemistry- and consequently, the climate- and they play a huge role in the cycling of carbon dioxide (Fig. 4) from the atmosphere to the biosphere, and back to the atmosphere, annually fixing between 30 to 50 billion metric tons of carbon [Fal94].



Figure 2: Fossilised Cyanobacteria [oP21]



Figure 3: Cyanobacteria (blue-green algae) [cya]

The carbon dioxide they absorb would have otherwise dissolved in the ocean and seawater, reducing its pH and making it more acidic [DBCK20], or would have remained undissolved, contributing to global warming [SPKF09]. The role of phytoplankton in the global carbon cycle highlights their regulatory role in the environment and establishes their close interaction with greenhouse gases, and how an anomaly in their quantity can affect the physiology of these organism

From phytoplankton, carbon can be recycled to the deep oceans in either of the two following ways [Fal12]:

1. Heterotrophs consuming the phytoplankton in the top 100 meters of the ocean use the organic matter to obtain energy and nutrients to build their tissues, and in turn produce carbon dioxide, or
2. Dead phytoplankton and fecal matter & bodies of heterotrophs sink to the bottom of the ocean, where microorganisms break down the organic matter into its chemical constituents. Due to water stratification, carbon dioxide and other nutrients at the bottom do not mix with the upper layers of the ocean water.

Carbon Dioxide and other nutrients only return to the surface after a slow cycle of deep ocean circulation.

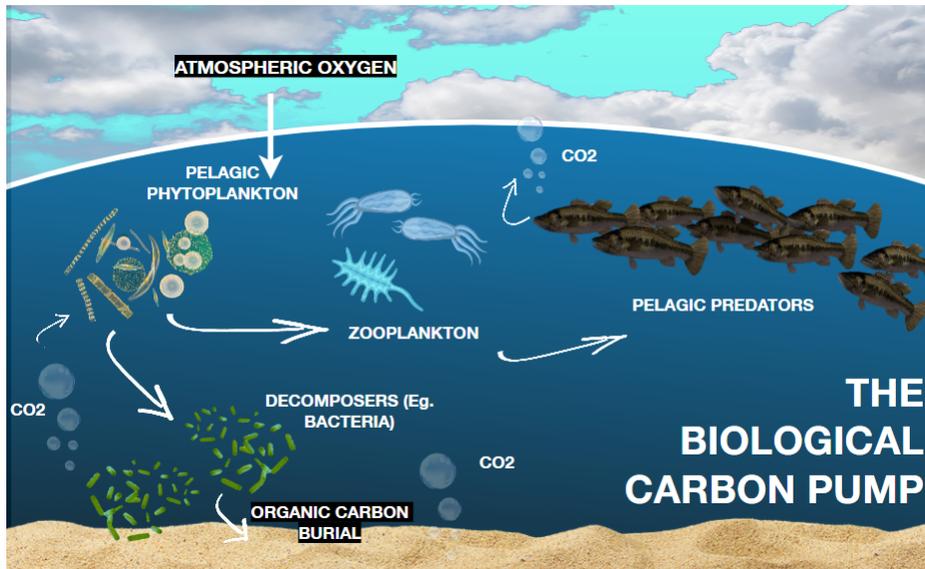


Figure 4: Biological Carbon Pump

### 2.2.3 Phytoplankton as Primary Producers

Just like with carbon dioxide, phytoplankton provide organic matter for the majority of marine life. Phytoplankton are the foundation of the aquatic food web, feeding everything from microscopic, animal-like zooplankton to larger fishes. They are called primary producers as they make food by carrying out photosynthesis and assimilating nutrients like nitrogen and phosphorus directly from their cycles to put them into biologically usable elements in the food cycle.

In 1934, Redfield described how the proportion of nutrients in the ocean are not random or haphazard. In every region of ocean sampled, the ratio of nitrogen atoms to phosphorus atoms was fixed at 16:1. Furthermore, the ratio of carbon atoms to nitrogen atoms to phosphorus atoms (C:N:P) came out to be 106:16:1 [MVL14]. This consistent atomic ratio of dissolved nutrients came to be known as the Redfield Ratio, and the same ratio of nutrients is found within phytoplankton [Red58]. The Ratio also suggests that not only do the phytoplankton reflect the chemical composition of the deep oceans but as they and the heterotrophs who consume them sink, microorganisms biodegrade to release the nutrients in the Redfield Ratio into the deep sea, hence influencing the chemical composition of the oceans.

Anthropogenic activity, especially agricultural and urban development has dramatically affected the biogeochemical cycles of Nitrogen (N) [Bak92] and Phosphorus (P). However, while nutrient-run off is thought to be the primary

cause of Harmful Algal Blooms (HABs) , independent quantities do not favor cyanobacteria unless they are in a certain ratio of N:P. Cyanobacteria dominate in environments with a low N:P ratio (below the Redfield Ratio [Red36]), as they have a competitive advantage over other algae due to their ability to directly fix atmospheric nitrogen (N<sub>2</sub>) [Pal15].

Phytoplankton, even as primary producers in the oceans, are in an appropriate position to indicate the health of a water body via assessment of the nutrient content.

## 2.3 Biological Dimensions in Ecological Assessments

Pollutants undergo numerous processes in the environment depending on the range of their physiochemical properties. For example, hydrophilic contaminants are ionic and remain dissolved in water [Gig09], whereas, hydrophobic contaminants like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) bind strongly to the sediment, and become long-term sources of contamination.

This leads us to the primary reason why physical and chemical assays of environmental pollution should be supplemented by biological tools: Chemicals with different physiochemical properties, concentrations, and toxicity estimates may act synergistically, antagonistically, or additively with other chemicals in the environment, and react to produce a variety of adverse effects in ecosystems [WKN15]. Bioassessments are important indicators of how pollutants interact with each other, and with living organisms in the local ecosystem. Drawing upon the concept of the Redfield Ratio, apart from the individual concentration anomaly of Nitrogen and Phosphorus, alterations to the N:P ratio can also induce overgrowth of cyanobacteria. In another instance from 1976, epidemic organophosphate insecticide poisoning occurred among 7,500 field workers in a Pakistani malaria control program [BJZM<sup>+</sup>78]. They were responsible for the dispersion of a formulation of malathion , and an excess of isomalathion, an impurity in malathion preparation due to improper storage, accounted for a supra-additive, cholinergic effect, resulting in sickness [KB94].

### 2.3.1 Biotransformation

Transformation refers to the change induced in the pollutant because of its interaction with abiotic components of an environmental system, for example, ozonation or photocatalysis [Fox86]. When a xenobiotic (foreign) pollutant is enzymatically modified to chemicals that differ in their excitability, biological activity, and toxicity [RS14], as a result of an interaction with abiotic component, it is known as biotransformation.

As studied by [FFK<sup>+</sup>20], biotransformation plays a critical role in regulating the toxicity of organic compounds in organisms but is poorly understood

as an emerging contaminant. Diclofenac, a pharmaceutical was examined for biotransformation on *Gammarus pulex* and *Hyallela azteca*, on oxidizing within the said phytoplankton formed two novel and unexpected products: diclofenac turine conjugate, which is less toxic than its parent compound, and diclofenac methyl ester, which had a 430-fold increase in toxicity as compared to diclofenac.

Biotransformation, therefore, highlights another advantage of using bioassays, as they can estimate the objective toxicity of a sample organism, regardless of whether or not the toxic contaminant is known or not.

### 2.3.2 Bioavailability

A term often associated with pharmacology, bioavailability refers to the fraction of an administered drug that reaches the systemic circulation [VG15], i.e., bioavailability is a measure of the rate and fraction of the dose of a drug that successfully reaches the intended site of action or body's systemic circulation, from where it has unimpeded access to the target.

Similarly, in the case of pollutants, the physiological characteristics of a species influence the availability of chemicals, such that the exposure to the same contaminant alters from one species to another. Various studies [Sch91] focus more on the bioavailability of hydrophobic contaminants of sediment, like heavy metals. Risk assessments of the bioavailability of contaminants are essential for evaluating exposures of humans and ecological receptors to persistent compounds.

Phytoplankton, having a high metabolic rate and being the foundation of the marine food web, are used to trace and control trace metal nutrients (iron, zinc, cobalt, manganese, copper, and cadmium) [Sun12]. Of these, iron has the greatest effect on algal species diversity and phytoplankton growth, and di-nitrogen fixation rates, and given its close interactions with the trace metal, phytoplankton are often used for assessment of the bioavailability of dissolved iron (or, dFe) [HM90] [MP01]. Phytoplankton growth is limited by the low availability of dissolved iron, and therefore, they are used to evaluate seawater dFe bioavailability based on its uptake rate constant [SBMM20].

Apart from dFe, phytoplankton also form a biological feedback mechanism with other trace metals, as the interaction between the two is reciprocal [SMH<sup>+</sup>11] [Sun12]. Trace metals influence the productivity and species composition of phytoplankton, while these microorganisms profoundly impact the chemistry and cycling of these metals in the ocean.

### 2.3.3 Bioaccumulation and Biomagnification

Bioaccumulation and biomagnification are two different processes that occur in tandem with each other and are responsible for producing massive effects even

with small concentrations of toxic contaminants in water.

Bioaccumulation is a result of dynamic equilibrium between the uptake and elimination of contaminants [CM14], where the contaminant builds up in an individual organism due to a rate of absorption of a toxic substance that is higher than the rate at which it can be eliminated [MC14]. On the other hand, biomagnification refers to how toxins concentrate within a food web as they are passed from one trophic level to another [Dro08]. When looking at health hazards incurred by bioaccumulation, primary chemicals of concern include Persistent Organic Pollutants (POPs), who do not degrade in the environment and build up in the fatty tissues of organisms [IKK<sup>+</sup>18].

Bioaccumulation occurs at the base of a food web, i.e., within phytoplankton. Phytoplankton absorb POPs directly from the seawater and accumulate them over time, as they are being absorbed from the water at a rate faster than they can metabolize. When predating zooplankton feeds on contaminated phytoplankton, they, in turn, absorb the POPs into their own tissues.

Bioaccumulation and biomagnification of mercury in form of Methylmercury (MeHg) have been a focus of bioaccumulation research [HDV18] [MKA98] [SQD<sup>+</sup>18]. Mercury is known to be fatally neurotoxic in humans [MC06], and MeHg, which is highly bioaccumulative and toxic, negatively impacts the health of people consuming seafood exposed to the compound. As with any other toxic contaminant, mercury enters the food chain through phytoplankton absorption, whose size ( 0.001 -0.1 mm) greatly facilitates the uptake of MeHg. Smaller phytoplankton end up with larger concentrations of MeHg due to an increased surface area, which is approximately 500 to 500,000 times higher concentrations than the surrounding water [SQD<sup>+</sup>18].

This brings us to another feature of biomonitoring, especially using phytoplankton. Given their large surface areas and their ability to bioaccumulate toxic substances, phytoplankton make excellent tools for pollution assessment. Being at the first step of the marine food chain, they have an added advantage of early detection.

## 2.4 Bioanalytical Tools

Biological analysis is based on the principle that environmental pollution should be approached comprehensively while taking naturally occurring physical, chemical, and biological phenomenon into consideration, and that bioindicators and biomonitors with a narrow range of ecological tolerance for foreign substances can indicate stress employing a change in function, behavior or whole population of bioindicators [WNK16]. Several tools and techniques can be used to perform biological environmental monitoring, which are primarily divided into (i) biomonitoring tools, and (ii) bioanalytical tools (Fig. 5), which will further be analyzed to how they make use of phytoplankton roughly based on the

classification given in [WNK16].

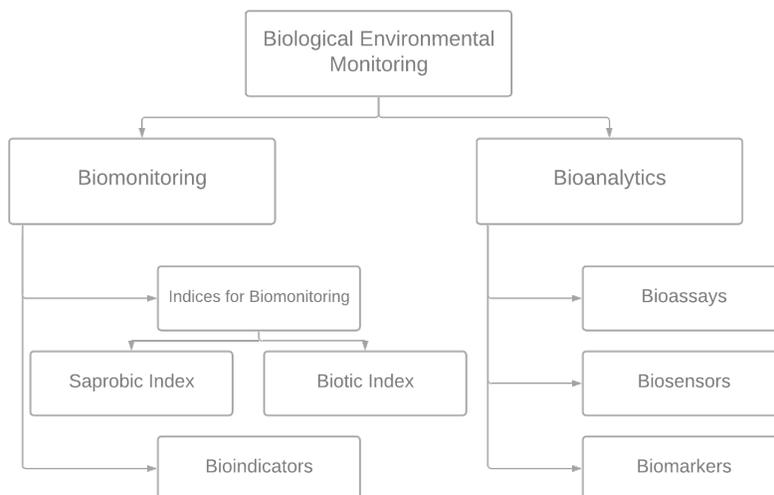


Figure 5: Adapted from [WNK16], this chart categorises the various types of tools used in health assessment of water bodies.

### 2.4.1 Biomonitoring

The value of phytoplankton as bioindicators was recognized as early as the 19th Century [WSF14], and the first system of bioindication developed was the system of saprobity, which was mainly designed for organic pollution of stream and rivers.

Using indices to monitor changes in the biota is a common practice. Many biotic indices are based on species-specific sensitivities and tolerances [LZL10], and are indicators of organic pollution [WK95]. Phytoplankton have ecological niches that are constrained by their ecological properties [MLP<sup>+</sup>15]. Any change in the physiochemistry of these properties can be used to monitor an ecosystem. Biotic indices rely on the fact that biological communities are a product of their environment, and that different organisms have different habitat preferences and different pollution tolerances [SL09]. In presence of organic effluents in the stream, intolerant organisms will diminish in number, while competing species will thrive. The most famous biotic index making use of phytoplankton is the Palmer’s Pollution Index [JG06] [NR15] [SGEN17].

The primary principle behind the index is that the presence and predominance of a certain kind of phytoplankton depend on the climate, physicochemical characteristics, and nutrient content of a water body. Palmer’s Index is a list of

60 genera and 80 species of algae tolerant to organic pollution and is complemented by the Palmer Algal Index. A pollution factor is assigned to each genus depending on their relative tolerance to organic pollution as per the following sample table [VAWH19]:

Genus	Index Pollution	Genus	Index Pollution
<i>Anacystis</i>	1	<i>Micractinium</i>	1
<i>Ankistrodesmus</i>	2	<i>Navicula</i>	3
<i>Chlamydomonas</i>	4	<i>Nitzschia</i>	5
<i>Chlorella</i>	3	<i>Oscillatoria</i>	5
<i>Closterium</i>	1	<i>Pandorina</i>	1
<i>Cyclotella</i>	1	<i>Phacus</i>	2
<i>Euglena</i>	5	<i>Phormidium</i>	1
<i>Gomphonema</i>	1	<i>Scenedesmus</i>	4
<i>Lepocinlis</i>	1	<i>Stigeoclonium</i>	2
<i>Melosira</i>	1	<i>Synedra</i>	2

Depending on its tolerance for organic pollutants, every genus is assigned a single Pollution Index Factor. The higher the factor, the more likely it is for the Genus to be present in highly-polluted waters. The assigned numbers of all genera present in the sample obtained from the polluted water body are totaled. The following index scale is used to assess the health of water:

Pollution Index (Sum)	Status of Pollution
Lesser than 15	Very Light Organic Pollution
15 - 20	Moderate Organic Pollution
Greater than 20	High Organic Pollution

These indices have been in use for a very long time and are inexpensive methods of bioindication, finding usage in many developing countries.

#### 2.4.2 Bioanalytics

Bioanalysis builds heavily on the concept of chemical analysis and relies on the receptors of contaminants within the organism. Bioanalytics tests not only the biological activity of chemical pollutants but also measures the response of the organism to the pollutant on a cellular level. A major component of bioanalytics is the practice of using biosensors, which are analytical devices that measure biological reactions by generating signals proportional to the concentration of the analyte in the reaction [NPNP16].

Typically, the model of whole-cell biosensors is dependent on the reporter gene and the associated regulatory protein [GLS<sup>+</sup>17]. Whole cell-based biosensing using cyanobacteria can detect the presence of heavy metals (Cu, Pb, and Cd), herbicides (2,4-dichlorophenoxyacetate (2,4-D)), and pesticides (Chlorpyrifos) [WLS13].

Photosynthetic microorganisms, like phytoplankton, are known to be effective in fluorescence-transduced biosensors [GKM01] [WLS13], as they contain the light-absorbing pigments, chlorophylls, which re-emit a small amount of energy back as fluorescent emission. A fluctuation in the rate of these emissions can be used to detect pollutants, and this process is called chlorophyll fluorometry. There is a known inverse correlation between chlorophyll fluorescence and photosynthetic efficiency [KBO<sup>+</sup>07]. On exposure to toxic pollutants, electron transport pathways in the photosynthetic apparatus are inhibited [ASK12], and as a result, the photosynthetic efficiency to absorb light decreases. Conversely, fluorescence increases. Therefore, biosensing practices using chlorophyll can produce fast and accurate data about environmental stresses based on the fluorescence emission patterns and their anomalies [NSG21].

Biomarkers are another set of tools used in bio-analytics and are an indicator for detecting pathogenic or toxic factors and assessing the biological status of an organism [SK18]. Usually used in the context of disease in humans, biomarkers, or biological markers are molecules found in an organism's tissues and are signs of the health status of the organism.

## 2.5 Pollutant-wise Health Assessment

### 2.5.1 Oil Derivatives (Polycyclic Aromatic Hydrocarbons)

Polycyclic Aromatic Hydrocarbons (PAHs) are toxic pollutants released by various urban and industrial combustion sources. They occur naturally in coal, crude oil, and gasoline, and are produced when these fuels are burned. For large-scale pollution, however, the biggest source of PAH pollution is petroleum combustion, and due to their hydrophobic nature, PAHs have been readily found in sediments of water bodies surrounding oil rigs and major industrial ports [VMC<sup>+</sup>19]. Many PAHs have toxic, mutagenic, and/or carcinogenic properties [ASM16], which makes their detection and monitoring of utmost importance.

The most popular marine diatom to be used in the case of PAH pollution is *Thalassiosira pseudonana*, one of the few diatom species that have had their complete genome sequenced [Pen04]. The availability of extensive molecular information has made studying the species' interaction with pollutants easier. Additionally, in the case of diatoms, their biosilica formation can also be studied for their enzymatic requirements and nanopatterns to assess the state of the diatom population's habitat.

In [CBS<sup>+</sup>11], Carvalho and her colleagues exposed a culture of *T. pseudonana* (Fig. 6) to a complex mix of PAHs extracted from the surface sediments containing high PAH concentration and monitored the gene expression and silica uptake in the sample. They observed an up-regulation of several heat shock proteins (HSP) and heat-shock transcription factors (HSF). Additionally, they

observed the regulation of *sil1* and *sil3* genes involved in the biosilification process in the diatom cell wall by PAH contamination. A down-regulation of the *sil1* gene was observed by both a DNA microarray and qRT-PCR, followed by a down-regulation of silification proteins, and finally resulting in a decreased uptake of silica in exposed cells. This is an example of how gene biomarkers can be employed to study and measure the quantity, bioavailability, and accumulation of PAHs in a local environment.

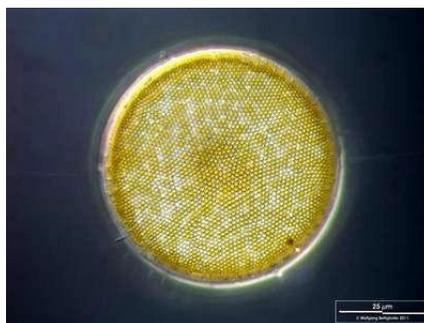


Figure 6: Diatom *Thalassiosira*, a genus of centric diatom predominantly found in marine water [Lee]

Cyanobacteria are characteristically found in association with oil seeps and wet areas and contribute to both biosynthesis and degradation of oil [RAH00]. Cyanobacteria have ubiquitous bioremediation processes to clean up hydrocarbon-contaminated environments [HSY16]. It is for the same association with the presence of petroleum, be it from natural or anthropogenic sources, that cyanobacterial mat formations can be observed, comprising cyanobacteria, predominantly among other hydrocarbon-degrading microbes. [CGS<sup>+</sup>06] defines cyanobacterial mats as “microbial consortia composed of photosynthetic and non-photosynthetic bacteria and fungi embedded in a cyanobacterial mucilage” with a high-efficiency hydrocarbon degradation capacity.

### 2.5.2 Pharmaceuticals

Pharmaceuticals are a class of emerging environmental contaminants [VCARH<sup>+</sup>20], that can be traced back to human and veterinary medicine and pesticides and herbicides used in agriculture. What makes pharmaceutical pollution so threatening to ecosystems exposed to their run-offs is (1) their persistence outside the body and off the fields [FWC06], and (2) the non-specificity of pharmaceuticals like pesticides and antibiotics, which can disrupt bacterial communities outside their target microbes. Additionally, monitoring wastewater or agricultural run-offs for pharmaceuticals is a difficult process, with limited options and no assessment of biotoxicity and bioavailability.

For many pharmaceuticals, urine and feces from consumers contain a considerable amount of active residues [Lar14]. The most harmful of these residues are endocrine disruptor chemicals (EDCs), which interfere with the hormonal balance and homeostasis of marine animals. As studied in [CSK<sup>+</sup>19a], cyanobacteria *Microcystis aeruginosa* was a good indicator of biotoxicity of synthetic hormones like estrone, 17 $\beta$ -estradiol, and estriol, which are active EDCs used extensively in oral contraceptives. Biomass and chlorophyll-a were used as parameters to measure the response of the cyanobacteria, and it was concluded that these particular hormones were more biotoxic when combined than they are individually.

Another study by Czarny- [CSK<sup>+</sup>19b] - developed a model relating inhibition of growth in phytoplankton population to single & mixed steroid pollution. This time, the study used *Anabaena variabilis* to examine the effects of estrogens, progestagens, and androgens on the cyanobacteria and commented on how to use this correlation for ecological risk evaluation. As with the previous study, The research reports that steroid hormone mixtures are more toxic than single hormones. Steroid hormones, as the study concludes, variably inhibit the growth in *A. variabilis*.

In [DCC17], Dell Aglio and his colleagues exposed *Scenedesmus* microalgae with four common active pharmaceutical ingredients Ofloxacin, Cloxacillin, Chloramphenicol, and Propranolol; a majority of these drugs are antibiotics. While molecules with antibiotic properties have always been around, the discovery and mass production of synthetic antibiotics and antimicrobials in the last century has exposed bacterial communities to unprecedented antibiotic selection pressures and even disrupt phytoplankton communities.

The researchers in [DCC17] study the functional perturbations in biochemical metabolism processes, namely respiration and photosynthesis, in *Scenedesmus*. As with gene expression in the previous section, the production of metabolites is altered by exposure to environmental stresses. These processes can either be inhibited or activated. Under normal conditions, in both photosynthesis and respiration, oxygen and carbon dioxide are consumed and produced in a stoichiometric ratio, but under exposure to biotoxic pharmaceuticals, the ratio of the metabolites deviates from their ideal stoichiometric values. This research uses this very deviation in phytoplankton to be good bioindicators, reacting differently to different drugs, and producing distinct quantifiable deviations.

In another attempt to better biomonitoring practices making use of diatoms, in [LHM<sup>+</sup>17] Lavoie and her colleagues discuss diatom teratologies (Fig. 7) as a meaningful metric in bioassessments. Diatomic teratologies are aberrations in their valves due to ecological stress and reflect sub-lethal responses to environmental stressors. While the mechanism of teratologies is still unclear, organic pollutants like anti-microtubular agents or pesticides can sabotage the diatom's microtubular and microfilamentous systems.

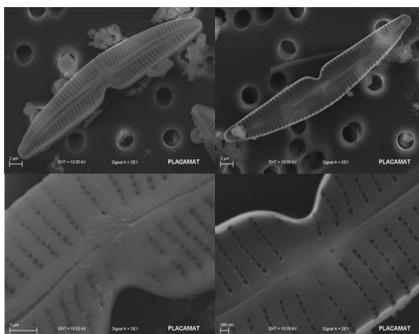


Figure 7: Polymorphism in a sample of *Gomphonema gracile* [CM19]

### 2.5.3 Fertilizers

Non-organic fertilizers mainly contain nitrate, ammonium, and phosphate salts. The fertilizer industry is also known to be the source of xenobiotic heavy metals [Sav12]. The overuse of fertilizers in recent decades has resulted in frequent water eutrophication, disturbance of the natural nitrogen cycle, and heavy metal accumulation in the water bodies, which are all serious environmental problems.

While nitrogen is the most abundant chemical element in the Earth’s atmosphere, and also an essential component of many key biomolecules (e.g. amino acids), natural oceanic ecosystems are often not nitrogen saturated, and therefore, aquatic organisms are better adapted to and function optimally in low levels of available nitrogen [Bra05]. However, anthropogenic alterations of the nitrogen cycle have doubled the rate of nitrogen input into the nitrogen cycle [VAH<sup>+</sup>97]. High levels of ammonia, nitrite, and nitrate from human activities, like agricultural or urban run-offs, can impair the ability of organisms to survive, and drastically restructure aquatic communities.

An increase in environmental availability of inorganic nitrogen and phosphorus favors primary producers like phytoplankton and leads to eutrophication, characterized by nutrient enrichment in the water body [CCC<sup>+</sup>98]. Eutrophication causes oxygen content in the water body to deplete and the consequent loss of habitat.

However, numerous studies make use of the composition of phytoplankton assemblages to monitor nitrogen pollution before it escalates into an algal bloom. In [PR14], researchers analyse the water quality in Sefid Rud River, Iran, using phytoplankton groups, and identified an abundance of Genus *Oscillatoria*, which is a direct indicator of agricultural run-off induced pollution.

[SES<sup>+</sup>14] makes use of diatoms from three national parks in Washington State to calculate the “critical load”, or “the deposition of a pollutant below

which no detrimental ecological effect occurs over the long term based on present knowledge”. They use critical load as a parameter to determine the impact of nitrogen deposition.

#### 2.5.4 Heavy Metals

Heavy metals are a category of naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water [TYP12]. Their application in multiple industries ranging from medicine, technology, and agriculture has led to their wide and unnatural distribution in the environment. Biologically, heavy metals are classified into essential heavy metals (e.g., iron, nickel, copper, and zinc) which are essential for marine metabolism, and non-essential heavy metals (e.g., lead, mercury, and cadmium), which are toxic to ecosystems even in trace amounts. Cadmium, for example, is used in TV screens, lasers, batteries, and paint pigments, and it is also known to be toxic to several human organs in variable quantities: it can injure tissues through oxidative stress, especially those in kidneys [Ber13]. Arsenic, another heavy metal, is found in different chemical forms & oxidation states, and its toxicity can cause acute and chronic health effects in humans, including cancers [Hug02].

Regardless, heavy metals are present in the oceans at a very low concentration and help stabilize protein structures, catalyze enzymatic reactions, and facilitate electron transfer reactions in living organisms [Met]. Earlier in the paper, we employed the example of methylmercury to understand bioaccumulation. Heavy metals, like mercury in this example, are especially hazardous as pollutants since, unlike their organic counterparts, they cannot be removed from water through biodegradation. They persist in the aquatic environment, either in sediment or in food chains. As [TBC<sup>+</sup>08] observes, several of these metals can displace or substitute essential heavy metals and interfere with the proper functioning of enzymes and associated co-factors.

In [WLS13], cyanobacteria *Anabaena torulosa* has been used to construct a fluorometric whole cell-based biosensor sensitive to copper, cadmium, and lead. Being a photosynthetic bacteria, *Anabaena torulosa* contains chlorophylls which re-emit a small portion of the light absorbed during photosynthesis, as fluorescence emission. The photosynthetic yield is dependent on the overall state of the photosynthetic apparatus and pigments, and when a heavy metal toxicant inhibits the photosynthetic electron transport pathway, fluorescence emissions begin to fluctuate. This fluctuation is used as a detection parameter in the biosensor.

Even though iron is an essential metal, an overload of the aforementioned metal can cause severe health problems in humans, like cirrhosis of the liver, liver cancer, and infertility. Diatoms have been extensively studied under various concentrations of iron, and also with different concentrations of the metal

couple with a diverse range of environmental variables. One in many studies on the subject is [CEL<sup>+</sup>17], in which diatomic transcriptional and physiological responses to changes in iron bioavailability are recorded. Iron availability is a major limiting factor in phytoplankton growth, and since it is predicted to change with rising temperatures and acidification of surface seawater, this study is a quintessential example genomics and proteomics can find extensive application in enhancing bioindicator performance.

One of the advantages of using phytoplankton as bioindicators is their capacity to acclimate to sudden changes in their environment. They have developed mechanisms of protection against stress-induced damages, an important example of which are specific membrane lipids and stress proteins which play a role in the pollutant uptake processes [Šaj97]. As discussed by [VGM01], such responses are often used as biomarkers of heavy metal pollution. Under the influence of heavy metals, teratologies in diatoms are also observed.

### 2.5.5 Carbon Dioxide

One of the major greenhouse gases that contribute to global warming whereby the dramatic increase in global temperature anomalies are changing the health of our environment [WGI17], carbon dioxide (CO<sub>2</sub>) has become a commonplace by-product of almost all major industrial and mechanical processes, ranging from the combustion of hydrocarbon fuel in automobiles to factories (Kent, 2021) [Thu17]. One of the major mechanisms which sequester CO<sub>2</sub> from the atmosphere is the oceanic uptake of the gas through dissolution. As the CO<sub>2</sub> in the atmosphere grows, the oceanic uptake steadily rockets, causing pH reduction and alterations in the fundamental chemical balance that supports life in the ocean [DFFK09].

As discussed in earlier sections, phytoplankton and their primary productivity are deeply tied to the global carbon cycle. By consuming CO<sub>2</sub> and pumping it to the bottom of the ocean, phytoplankton not only remove the greenhouse gas from the atmosphere but also prevent it from dissolving in the ocean water in disproportionate quantities sufficient to alter sea-water chemistry.

In [Fal12], Falkowski discusses in depth the relevance of phytoplankton in understanding how carbon moves through the atmosphere and the oceans. Given their capacity to sequester carbon from the air, they are used to track the environmental off-shoots of excess carbon dioxide in the atmosphere, and this is primarily achieved through analyzing the change in chlorophyll-a concentration and primary productivity.

Coastal Zone and Colour Scanner (CZCS) was the first satellite ocean color sensor that allowed for the estimation of phytoplankton pigment concentration in the global ocean [Fuk97]. In particular, chlorophyll-a (Chl-a), a necessity for an organism to perform oxygenic photosynthesis [MMD18], is used to monitor

biological activity. Chl-a enables phytoplankton to absorb blue light, which would have otherwise been scattered by the seawater. With the bio-optical screening technology aboard the CZCS, it was observed that the more the concentration of Chl-a in an area, the darker it appeared on the screen. While CZCS was seen as a “proof of concept” mission [AWC<sup>+</sup>14], another method to measure upper-ocean chlorophyll concentration was developed soon after- the Sea-viewing Wide Field-of-View Sensor (or SeaWiFS).

The concentration of chlorophyll in the oceans is often expressed as depth-integrated chlorophyll concentration, a fundamental indicator of change in primary production [FRC12]. As discussed in [BOS<sup>+</sup>06], Chl-a concentrations have been on a low since 1999. Warmer environmental conditions reduce the Chl-a and the primary productivity because surface warming leads to a phenomenon called water column stratification, i.e., the division of water column into layers with different densities, caused by a difference in temperature [Rab09]. The two layers do not mix, and this is particularly harmful to pelagic phytoplankton, which rely on vertical nutrient transport to sustain their photosynthetic productivity.

On the other hand, ocean acidification in response to rising atmospheric CO<sub>2</sub> partial pressure is expected to reduce overall calcification by marine organisms [IRHR<sup>+</sup>08]. Previous research looked to coccolithophores (Fig. 8), which are pelagic phytoplankton that consumed dissolved carbon dioxide as carbonate ions to form protective calcite plates (coccoliths) on their exterior, to increase their efficiency of carbon sequestration in face of global warming. Being the most prolific oceanic producers of calcium carbonate since prehistory, coccolithophores dually fix carbon, both through photosynthesis and through coccolith formation [MWB12].

They are intricately related to ocean chemistry, having formed one of the first pH regulation mechanisms of the hydrosphere. Carbon dioxide undergoes hydrolysis in seawater, increasing the hydrogen ion concentration [H<sup>+</sup>], and as reported by the United States Environmental Protection Agency (US EPA), surface pH is already 0.1 unit lower than preindustrial values. Their coccoliths are made up of calcium carbonate, which, in the event of extreme acidification, dissolves to balance the pH of the waters, hence acting as a buffer [EBS<sup>+</sup>19].

Several studies discuss the effect of ocean acidification on the calcification of calcifying phytoplankton, i.e., coccolithophores. Coccolithophore calcification decreases under high CO<sub>2</sub> concentrations ([CO<sub>2</sub>(aq)]). As Bach and his colleagues discuss in their study on coccolithophore sensitivity to anomalies in ocean carbonate chemistry [BRG<sup>+</sup>15], calcification rates can depend on the radicals involved in the process. The study observes that calcification rates increase positively with bicarbonate (HCO<sub>3</sub><sup>-</sup>) and CO<sub>2</sub>, while it is inhibited by protons (H<sup>+</sup>).

In 2021, [STB<sup>+</sup>21] studied the composition and succession patterns in post-



Figure 8: *Emiliana huxleyi*, one of the most prolific species among coccolithophores [Pou]

bloom plankton communities and how the restructuring of the coastal plankton community acts as an indicator of extreme acidification events. Extreme ocean acidification led to lower chlorophyll-a, decreased primary production, and decreased concentration of particulate matter. In another study, [THB<sup>+</sup>17] employed an imaging approach to analyze plankton community structure under the influence of ocean acidification. The study examined the community structure by investigating its food web structure, by using data on the size distribution of plankton. Body size majorly influences the position of an organism in the marine food web, and as per the concept of the size spectrum, there is a relationship between the abundance of organisms and their body size. Their inverse relationship is linear on a logarithmic scale, with steeper slopes indicating a higher proportion of smaller organisms and shallower slopes indicating a higher proportion of larger organisms. Variation in these slopes can be linked with disturbances in the food web.

(Fig. 9) maps the change in phytoplankton density against datasets on atmospheric carbon dioxide concentration and global temperature anomaly, as obtained from OurWorldInData [RR20]. The data sets have been portrayed on a three-axis graph, and have been mapped over 50 years. Beginning in 1960, the global temperature anomaly and the atmospheric concentration of CO<sub>2</sub> has rocketed steadily and constantly, and at the same time, the density of phytoplankton in the Arctic has plummeted. This graph shows a strong correlation between CO<sub>2</sub> -climate change and the rise in global temperatures, and also affirms the previously described alterations in the phytoplankton communities.

### 2.5.6 Chlorofluorocarbons

Chlorofluorocarbons, or CFCs, are a family of inert, non-flammable, and easily produced liquefied chemicals [RRvR04]. First patented in 1928 by General Motors as a non-toxic refrigerant [Elk99], today, CFCs are widely used and popular as commercial refrigerants, aerosol sprays, and blowing agents. Extensive

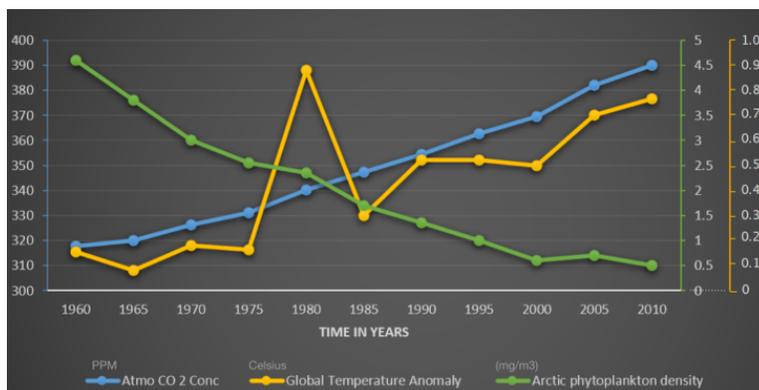


Figure 9: The graph shows the correlation between atmospheric carbon dioxide concentration and global temperature anomaly, and phytoplankton density as sampled in the Arctic.

usage of CFCs in the 20th and the early 21st Century resulted in a climatic catastrophe, as CFCs can cause significant damage to the stratospheric ozone layer [Tsa14]. The ozone layer protects us from ultraviolet (UV) radiation, especially from ultraviolet B (UVB) radiation, which is the main cause of skin damage and cancers due to over-exposure to UV rays [Ker19].

The reason for the ozone-depletion capability of CFCs is their inability to react and degrade in the atmosphere, apart from through ultraviolet photolysis. UV Photolysis releases a free chlorine radical from the CFC gas. This radical reacts with stratospheric ozone to form another free radical of chlorine oxide, which reacts further to regenerate atomic chlorine. This chain reaction can cause the removal of 100, 000 molecules of ozone per Cl atom [Row90].

With the consumption of the ozone in the stratosphere, land on earth is exposed to ultraviolet radiation, which is known to cause skin cancer [LYY<sup>+</sup>19]. Lesser-known offshoots of UV radiations are found among microscopic phytoplankton. Being the primary producers in the marine food web, phytoplankton dwell in the photic zone to harvest light for photosynthesis [HG15], where they are abundantly also exposed to UV radiation. In clear waters, UV radiation penetrates up to tens of meters [WdMD00].

In response to exposure to UV radiations, cyanobacteria and several diatoms and dinoflagellates have evolved a defense mechanism against the potential damage. Mycosporine-like amino acids (MAAs) are low-molecular-weight water-soluble molecules [OGC07] absorbing UV radiation in the wavelength range 310 - 365 nm, and they act as UV-sunscreens in phytoplankton [HHWW11]. A correlation between mycosporine content and in situ irradiance levels has been reported, such as in [LH03], a study which noted MAA accumulation due to

high light exposure in *Guinardia striata* and *Phaeocystis pouchetii* found in the English Channel.

At a molecular level in photosynthetic organisms, UV-B radiations cause detrimental effects like [SCA94]:

1. Photoinhibition, or inhibition of photosynthesis due to damage in the photosystem II complex),
2. DNA damage, and
3. Lipid peroxidation.

UV radiations mainly target and degrade the D1 protein in the electron transport chain (ETC) of Photosystem II, disrupting photosynthesis. Higher water column stratification due to rising global temperature adds an additional layer of complexity to the problem, causing the upper layer of water to warm up, further impairing the ETC repair mechanism which is hindered by heat [EADDD01]. Conversely, low temperatures and high exposure to UV radiation alter the morphology of cyanobacteria *Arthrospira platensis*, which loses its spiral structure under their combined effect [GLWWH08]. This morphological alteration, much like teratologies in diatoms, is a tell-tale biomarker of harmful exposure to UV radiation. Phytoplankton are also threatened by the formation of cyclobutane pyrimidine dimers (CPDs), i.e, a category of DNA damage that accumulates under high UV exposure, and is seen as a predictive biomarker of the same exposure.

The primary mechanism of damage caused by UV radiations, both A and B, is the formation of reactive oxygen species (ROS). In another study using *Thalassiosira pseudonana*, [Rij02] assesses the oxidative stress in the diatom in response to UVA and UVB radiation. The study used photosynthetic efficiency ( $F_v/F_m$ ) as a metric. In presence of UVB, this efficiency decreased greatly. While they observed a minor increase in ROS production associated with UVA, UVB incurred a 100% increase. Biomarkers of oxidative stress are well-researched across a range of organisms and are, therefore, subject to constant innovation and progress [GÅ20]. In phytoplankton, for example, malondialdehyde, an antioxidant, is an important potential biomarker doubled under UVA and tripled under UVB. Other antioxidants like superoxide dismutase and glutathione reductase also showed an increase in exposure to UVB.

### 3 Discussion

Every organism can report the health of its environment and ecosystem, and the mark of a developed and functional bioassessment practice is having a clear link between biotic components of a system and abiotic pollution exposure, to make early and realistic environmental risk assessments.

Phytoplankton can radically boost our capacity to evaluate the environmental risk due to pollutants and other drivers of climate change, as their productivity is directly associated with the components making up the aquatic environment. They are at the base of the aquatic food web, which means they directly intake carbon dioxide and light as a means to produce. Therefore, any fluctuations in the level of carbon dioxide or the availability of light have the potential to impact their growth, productivity, reproduction, and community structure. Furthermore, they obtain nutrients for their growth directly from nutrient cycles from deep in the water bodies, and when global temperature and salinity cause water to stratify, a change in the pattern of phytoplankton growth or productivity responds accordingly. Their association with raw nutrients like nitrogen, phosphorus, and silica helps them detect changes in their availability. Phytoplankton tie a multitude of factors contributing to climate change together, and therefore, in recent years, have emerged as strong candidates for biomonitoring.

Bioanalytics and biomonitoring provide for rapid detection of not only the presence of xenobiotic stressors or toxins but also test for bioavailability, toxicity, and genotoxicity of the pollutants quantitatively. As an example, traditional high-performance analytical equipment used for pesticide or heavy metal detection, like chromatography or atomic absorption spectrometer, can distinguish bioavailable pollutants from non-bioavailable pollutants. To perform the same detection, cyanobacteria, as discussed in [WLS13]) using the example of *Anabaena torulosa* in fluorescence-transduced biosensors, can serve as effective biosensors, on account of (1) the widespread presence of cyanobacteria in the environment, and (2) how well-understood the mechanism of chlorophyll fluorescence is. Similarly, biomarkers are used to measure the exposure, effect, and susceptibility of phytoplankton to a particular toxicant in the environment, and they can be seen as indirect signals of a toxic pollutant in the immediate environment of the organism. Recent developments in molecular genomics and proteomics are improving the accuracy of bioanalytics practices, as we better correlate the pollutants with the stress-induced and response in phytoplankton.

In line with the requirement of biological identification and analytics as discussed in (McCarty & Munkittrick, 1996) [MM96], we can conclude that based on their relationship with the environmental stressor, phytoplankton can be constructively used as biomarkers for the following reasons:

1. A deeper understanding of biomarkers can help us understand the fundamental mechanisms of toxicity much better, and better the ecological risk evaluation process at the level of product or clinical development of heavy metal products and pharmaceuticals fertilizers respectively,
2. Given their high rate of reproduction and accumulation, specific, sensitive, and practical biochemical tests can be developed to identify molecular response to biotoxicity at the earliest, and

3. Biomarkers in phytoplankton can be linked to changes in population, communities, and ecosystems, and even trace the impact of the environmental stressor to higher levels in the food web, as is explored in the next paragraph.

The first major advantage of biological assessment lies in its ability to assess the biotoxicity and bioavailability. As explored in [CSK<sup>+</sup>19b], cyanobacteria *Microcystis aeruginosa* not only indicated the biotoxicity of active ingredients found in oral contraceptives, but also, how a combination of those synthetic hormones would add up to result in heightened biotoxicity. Bioavailability in itself is often a potential indicator of bioaccumulation in the higher levels of the food web. Estrogenic compounds, which formed a majority of the compounds tested for in the above study, are widely known to negatively affect fish populations. These compounds are endocrine disruptors, and as they accumulate in fish populations, they hamper with their reproductive development, increasing the ratio of intersex male fishes, which further disrupts the breeding patterns in fish populations [KBM<sup>+</sup>07]. Estrogenic compounds have been well-researched and therefore, using their example we can affirm that phytoplankton populations can be reliable predictors of pollutant bioaccumulation.

The second advantage of a system of biological assessment of environmental risks over traditional chemical and physical assays is its ability to not only tolerate, but also cumulate the effects of the complex interactions between abiotic pollution exposure. By tracking Chlorophyll-a and the primary productivity of pelagic phytoplankton, we can analyze a complex chain of events set in motion by climate change. Chlorophyll receives signals from numerous factors, ranging from light availability and warming of oceans to salinity and pH of the upper layers of the ocean. Additionally, while it is commonly believed that global warming causes an increase in chlorophyll-a, real-world data on global warming suggests that due to water stratification, the overall effectiveness of chlorophyll is declining.

Similarly, as discussed in [OGC07], it has only recently been discovered that mycosporine-like amino acids (MAAs), which have traditionally been seen as just UV-sunscreens for the phytoplankton, also scavenge reactive oxygen species (ROS) such as singlet oxygen, superoxide anions, and hydroxyl radicals. Additionally, salt stress and osmotic stress all induce the production of MAAs. In response to salt stress, MAAs accumulate in the cell's cytoplasm as uncharged organic molecules to restore the osmotic pressure within the cell. This is an example of how a biomarker reacts to multiple stresses at once, and can potentially be used to estimate the cumulative biotoxicity of multiple pollutants in the water.

UV light is still the strongest inducer for the biosynthesis of MAAs, as special photoreceptors sense the need for MAA synthesis induction, and yet, there have been no prominent studies detailing their effective use as biomarkers of

high irradiance. Along the same line, numerous mechanisms and features of phytoplankton organisms and communities are not being capitalized on to develop better bioindicators.

Phytoplankton can also assess an additional dimension of complexity: biological stress in the form of the disease, competition, or predation. Such changes in physiology and community are real-world off-shoots of pollution and climate change and occur not as an after-effect, but simultaneously along with them. In events leading up to a harmful algal bloom, eutrophication, or nutrient over-enrichment gives cyanobacteria an added competitive advantage over its microbial and planktonic counterparts. Nutrient enrichment disturbs the ratio of N:P in the aquatic productivity web, and cyanobacteria, being one of the few phytoplankton with the capacity to harvest atmospheric nitrogen, grow in abundance as compared to that phytoplankton who cannot turn to nitrogen fixation to make up for the loss in N concentration. To add on, high temperatures and turbidity in the water column due to nutrient enrichment act as abiotic factors which further the advantage of cyanobacteria, which thrives in these conditions. On the other hand, diatomic teratologies due to exposure to pesticidal pharmaceuticals or heavy metals can be classified as a disease or deformity in the organism, and mark a disturbance in the diatom's silification process.

The formation of diatom teratologies also highlights the need for better categorization of disease, competition, or other cell responses like gene expression. As discussed in [FBB<sup>+</sup>09], while we know the root cause of diatom teratologies, there is hardly a clear threshold between normal cells and teratological cells. Two ways to tackle the problem of categorization to obtain better results would be to work towards a well-analyzed and researched framework correlating type and degree of deformity to an abiotic stress factor, and to eliminate species showing ambiguous deformities in a random response to a unique cause or stress. Similarly, the collapse of its spiral structure under the combined impact of low temperature and high UV irradiance allows us to use *Arthrospira platensis* to track a multitude of combinations of environmental stressors. For example, in a place with high irradiance, a positive anomaly in temperature big enough to induce changes in the aquatic ecosystem can be detected by observing lower rates of morphological change in *Arthrospira platensis*. Conversely, in a location characterized by a low-temperature environment, an increase in UV irradiance can be detected by observing a higher rate of morphological alterations in the said cyanobacteria.

By analyzing complex phytoplankton communities, we can observe that environmental settings and the changes induced in them by climate change and pollution drive up inter-species competition among the different phytoplankton groups and species present locally. Every species has a different, species-specific physiological response to stressors, and most organic pollution indices, like Palmer's pollution index make use of the new community composition to estimate the intensity and type of pollution in a water body.

Enhanced knowledge of how multiple stressors work to subject phytoplankton to stress and induce competition, disease, or any other cellular response would be useful in obtaining an indicator with clear definitions and parameters, which reveals the synergistic effect of pollutants and climate change stressors. The accuracy, precision, and validation of biomarkers from phytoplankton, like antioxidant enzymes or composition of microbial assemblages, will improve as we better understand the underlying mechanism and the causal relationship between stressors and organisms.

The practice of classification also enables phytoplankton to give us a heads up on newly emerging pollutants. The size and surface area of algae gives it the advantage of being sensitive to pollutants even in very low concentration. These features enable us to detect uncommon pollutants that may be susceptible to huge disruptions in the future. Phytoplankton are also frequent reproducers, and their short lifespan coupled with their size, allows them to give an early-stage diagnosis of potentially harmful pollutants that may magnify along with the food web.

It is also worth noting how the use of biomarkers derived from phytoplankton can improve exponentially given the consistent progress and innovation in the field of biomarkers overall. For example, malondialdehyde, which is used as an antioxidant biomarker of oxidative stress derived from phytoplankton, induced due to UV radiation, is also one of the most frequently used biomarkers in many health problems in humans, like cancer, psychiatry, pulmonary diseases, and asthma [KAJ15]. There is sufficient research already done on this particular biomarker, as well as on methods of imaging and detecting it. Using phytoplankton for their stress-induced biomarkers holds enormous potential on this account.

## 4 FINAL RECOMMENDATIONS

The crucial place of phytoplankton in natural food webs and element cycles cements their role as primary indicators of both pollution and climate change. To better utilize this resource, the following is a list of recommendations in the field of bioindication through phytoplankton:

1. Biological indicators have their advantages given their niche in the environmental cycles and must be integrated with physiochemical assays to produce better, more comprehensive assessments of the environment's health.
2. We need to develop a better, more robust framework to classify and employ phytoplankton as biomonitors and bioindicators.

3. Cells are prone to providing a more unique response to a stressor, through gene- and/or protein expression, and therefore, we must employ genomics and proteomics to further develop biological assays monitoring the same.
4. To identify variability or deformities due to pollutants or climate stressors, from natural variability in the organism, we must look to automation of the classification process, and use computer vision-based imaging to identify the same.
5. The consistent progress in biomarker science makes the use of phytoplankton one of the most promising fields in bioindication, and research needs to extend the readily available knowledge on biomarkers to phytoplankton to enhance the quality of environmental health assessment.
6. Biomonitoring is the need of the hour and should be integrated into national and regional environmental monitoring frameworks.

## 5 CONCLUSION

In this paper, we established that lifeforms and ecosystems are sensitive to change in their local and global environment, however, the response of individual phytoplankton species and entire phytoplankton communities remains largely untapped as tell-tale signs of degrading environmental health. Given their fundamental role in the marine food webs and biogeochemical cycles, they directly interact with several pollutants and climate change induced-environmental stressors. Overall, the environmental changes induced by pollution and climate change are leading to pronounced local and regional changes in phytoplankton communities, overlying a global trend of a decline in primary production, weaker sinking flux of particulate carbon and weaker rising flux of nutrients, and structural and chemical alterations, signaling the damage caused to the whole of the aquatic biosphere.

To fully utilize the bioindication potential in phytoplankton, there must be more research invested in correlating the physiological and community responses with the environmental stressors causing them in the first place. Till then, however, biological indication and assays can usefully complement chemical and physical assays already in use, and add a dimension of biotoxicity and bioavailability testing to the health assessment of water bodies. They can also predict the cumulative effect of pollutants on living organisms and can detail early on the bioaccumulation of toxicants along the food chain.

Overall, with better framework development and investment, the process of assessing the health of aquatic environments can be enhanced if we successfully integrate phytoplankton into the routine monitoring of water bodies, and gain a deeper perspective on the mechanism behind the damage incurred to ecosystems by pollution and climate change, and over time, minimize the loss of aquatic

diversity and irreversible damage to the well-established biogeochemical and life-cycles in nature.

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